Association between Follicular Fluid Estradiol and Clinical Pregnancy Outcome in Intracytoplasmic Sperm Injection Cycles

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Abstract

Background: The outcome of in vitro fertilization (IVF) is influenced by a number of factors. One of the most important factors is oocyte quality. The microenvironment of the follicular fluid is important for oocyte development.

Objectives: Assessment of the accuracy of follicular fluid estradiol level in predicting clinical pregnancy outcome, oocyte quality and fertilization rate in women undergoing ICSI.

Methodology: The current study was prospective study that included 180 women. Follicular fluid concentrations of 17β-estradiol were determined using the enzyme immunosorbent assays. Upon retrieval, oocytes were analyzed for hallmarks of maturity and classified as GV, MI, or MII based on appearance. Fertilization status observed at 24 h and the nutrient solution renewed, morphology of the dividing embryo was observed and 'embryo grading' done.

Results: Serum E2 concentration ranged from 2361±1583 (100 to 7589) pg\ml. The mean of total number oocytes was 10 with 53% of MII of good quality. And (47%) were of bad quality. All cases had normal fertilization. Number of transferred Embryos ranged from one to three embryos (good quality was of 63.9%), (bad quality was of 36.1) and 103 of cases had embryo transfer on day 5 ,77 had transfer on day 3.

Conclusion: Follicular fluid E2 concentration had fair predictive value in oocyte maturation, fertilization, embryo quality, chemical and clinical pregnancy. But it was an independent predictor of MII-grade oocytes production.

Keywords: Follicular Fluid Estradiol; clinical pregnancy; Intracytoplasmic Sperm Injection

INTRODUCTION

Artificial reproductive technologies (ARTs), which have been utilized since 1978, and in vitro fertilization (IVF) techniques are frequently employed to treat infertility in humans [1].

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In the physiology of follicular development, oocyte maturation, and ovulation, follicular fluid (FF) is crucial [2]. Numerous factors that mediate the formation of follicles and oocytes are present in follicular fluid [3].

In order to prevent the overproduction of embryos during human in vitro fertilization, oocyte quality evaluation is a key objective. Oocyte morphology was the first step in the process of determining the equality of oocytes, which then moved on to the identification of genetic and biochemical components [4].

Controlled ovarian hyperstimulation that produces a multi-follicular response is essential for the success of in vitro fertilization (IVF). Granulosa cells seen in the follicles secrete the hormone estradiol (E2). E2 is crucial for the development of oocytes and follicles as well as the uterus's preparation for implantation [5].

Regular metrics used to track follicular growth and oocyte maturation include serum estradiol levels and follidular size [6].

But in order to reach the objective and induce gestation, every cycle of ovarian stimulation turns into a series of challenges that must be surmounted. Finding additional elements that contribute to the overall process's optimization is crucial in this scenario. It has been proposed that certain elements of the follicular fluid medium, in which the oocyte is submerged, are biochemical indicators of the quality of the oocyte and, consequently, of the likelihood of successful fertilization and embryonic development [7].

The development of oocytes depends critically on the milieu provided by follicular fluid (FF). FF results from both the secretory activity of granulosa and thecal cells as well as the transfer of blood plasma elements that pass across the blood follicular barrier [8].

It is commonly known that healthy follicular growth and anti-atresia effects are linked to an environment that is mostly intra-follicular and estrogenic. In addition. Through direct, non-genomic action at the plasma membrane level, E2 promotes the cytoplasmic maturation of oocytes. This, in turn, causes extracellular calcium influx into the cell and a particular pattern of Ca2+ oscillations [5].

A higher risk of becoming pregnant has consistently been linked to elevated E2 in FF, which indicates a more advanced stage of oocyte maturation. Up until the fertilization stage, the role of estradiol in in vitro fertilization (IVF) is well established; however, its continued use after that point is still debatable. While some organizations report no effect of estradiol in these processes, others link high levels of the hormone to substantial oocyte production, which is accompanied with suppression of implantation and endometrial receptivity and lower pregnancy rates [9].

Follicles from which oocytes with higher rates of fertilization were retrieved were found to contain higher levels of estradiol [10]. Elevated levels of progesterone and estradiol in follicular fluid were linked to a higher likelihood of conception in terms of pregnancy rates [7].

Because exogenous steroids could be added to the culture media to improve the conditions, it is crucial to gather comprehensive information about the oocyte environment, particularly regarding the makeup and impact of FF on the maturation process. Important factors that affect the success of in vitro fertilization are the caliber of the eggs and the embryos. A study on the influence of serum estradiol on these measures may be found in [11].

It is commonly known that healthy follicular growth and anti-atresia effects are linked to an environment that is mostly intra-follicular and estrogenic. Furthermore, by directly acting non-genomically at the plasma membrane level, E2 promotes the cytoplasmic maturation of oocytes, which in turn causes extracellular calcium to enter

the cell and a particular pattern of Ca2+ oscillations [5].

A higher risk of becoming pregnant has consistently been linked to elevated E2 and E2/P ratios in FF, which signify a more advanced stage of oocyte maturation. Up until the fertilization stage, the role of estradiol in in vitro fertilization (IVF) is well established; however, its continued use after that point is still debatable.

Patients and methods

Patients who attended the infertility outpatient clinic and were candidates for ICSI at the Ain Shams University Maternity Hospital were the subjects of this prospective observational study. 180 women participated in this trial, which lasted more than two years.

All women between the ages of 18 and 38 who met the following criteria were included in the study: they had to have a BMI between 18.5 and 25 kg/m2, be free of medical conditions, have normal cervical and uterine morphology, have tubal factors other than untreated hydrosalpnix, and have good ovarian reserve as determined by the anti-mullarian hormone level and antral follicule count.

Polycystic ovarian syndrome, patient age greater than 38 years, BMI greater than 25 kg/m2, endometriosis diagnosis, hydrosalpinx, FSH and LH levels greater than 15 IU/ml, and women exhibiting poor ovarian response (POR), which is defined by two or three of the following three factors, were among the exclusion criteria: 1) The mother is older than 40. 2) prior pregnancy loss, and 3) an abnormal ovarian reserve test (i.e., low anti-mullarian hormone (AMH) less than 0.9 or antral follicular count (AFC) <5-7 follicles), women complicated by ovarian hyperstimulation syndrome (OHSS), or women who refuse to consent to the use of their data in the study.

Methods

The study involved women who underwent a comprehensive history, including personal, menstrual, obstetric, medical, surgical, sexual, and husband histories. A physical examination was conducted, including general, abdominal, and gynecological examinations. Infertility tests were conducted, including semen analysis and hormonal profiles on the third day of menstruation. A flexible antagonist protocol was used to stimulate ovulation, starting on the second day of the menstrual cycle with recombinant human rFSH. The dose was adjusted based on BMI, age, AMH, previous induction history, and expected ovarian response. A subcutaneous daily dose of GnRH antagonist was started when the leading follicle size was 14mm or more. Intramuscular human chorionic gonadotrophin (hCG) was administered when folliculometry showed an average diameter of 3 or more preovulatory follicles approaching 18-20mm.

Oocyte retrieval and semen processing

Using a single lumen needle guided by vaginal ultrasonography, oocyte retrieval was carried out under general anesthesia (intravenous administration of Propofol) 34–36 hours after hCG administration. Selection and immobilization of sperm. A dish with an electrostatic coating is used to store the garnets for ICSI. Placing a 10-milliliter microdroplet of polyvinylpyrrolidone (PVP) in the middle of the dish [12].

During oocyte retrieval, mature follicles (>17 mm) are aspirated and collected individually. The retrived fluid is linked to each cultured oocyte, labeled with patient information and identification number. Blood-stained follicular fluid samples are discharded, and the cumulus oophorus's corona radiata is removed. Oocytes are classified as GV, MI, or MII, with MII based on oocyte morphology abnormalities such as large perivitelline space, dark zona pellucida, dark incorporations, spots, vacuoles, refractile bodies, and irregular shape. Abnormal morphological criteria are observed in sperms, including amorphous, round, large,

small, vacillated or tapered head, neck, midpiece defects, excess residual cytoplasm, and coiled, broken multi and short tail [13].

These morphologic categories correlated roughly with phases of meiotic progression. Oocytes that had not progressed through meiosis to Mil were immature and not able to be successfully fertilized [14].

Steps of FFE2 Measurement

The quantitative determination of Follicular Estradiol was performed using an ELISA kit supplied by Immunospec Corporation's Monocent Inc Estradiol. The kit was precoated with an anti-Estradiol monoclonal antibody epitope (Biotin reagent). Standard solutions, follicular fluid, and controls were dispensed into wells, followed by 50pl of Estradiol Biotin Reagent and 100pl of Estradiol Enzyme Reagent. The liquid was then washed three times with IX wash buffer and blotted on absorbance paper or paper towel. The blue color changed to yellow after adding 50pi of Stop Solution. Absorption values were read at 450 nm within 15 minutes, and the mean absorbance values were calculated for each set of reference standards, controls, and samples. A standard curve was constructed by plotting the mean absorbance against its concentration in ng/ml on a linear-linear graph paper. The mean absorbance values were used to determine the corresponding concentration of Estradiol in ng/ml from the standard curve.

Injection of oocytes and fertiliation

The oocytes were placed in a special culture (PVP) by the embryologist, who then used a microscope and a tiny needle to inject one sperm into each oocyte. The state of fertilization was monitored 16–19 hours following ICSI. The presence of two polar corpuscles and two pronuclei (2PN) indicated that fertilization was normal. Patients with no or abnormal fertilization were removed, and those who met the same inclusion and exclusion criteria were substituted.

Embryo grading and transfer

In our study we classified embryos Based on the several morphological criteria are considered in embryo classification

- Cell number: embryos should be 2 to 4 cells at 48 hours after egg retrieval and 7 to 10 cells by 72 hours [15].
- Cell regularity or degree of blastomere size equality (uneven blastomere cleavage): if individual cells are similar in size, the embryos have the best cell regularity. If they are approximately the same size, it is better to be compared with a different size.
- Degree of fragmentation: although the fragmentation phenomenon is totally common in human embryos, those with great than 25% fragmentation, have a low implantation potential.
- Presence of multinucleation: if there is more than one nucleus in each blastomere on either days 2 or 3, the embryo is multinucleated. After day 3, it is highly difficult to identify multinucleation. Additional factors to be considered for grading and selection for transfers includes the presence of vacuoles, granularity and thickness of the zona pellucida.

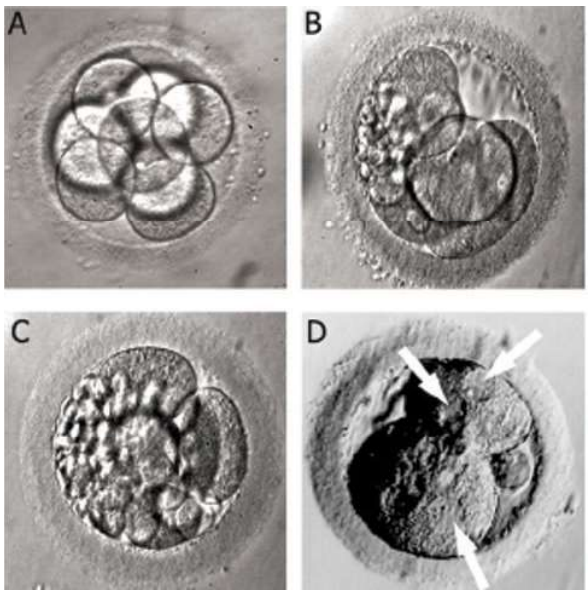

Figure (): Embryo grading by Advanced Fertility Center of Chicago

Embryo scoring based on blastocyst expansion grade according to Gardner et al, 2000 [16].

Expansion grade	Description
1	Blastocyst development and stage status
2	Blastocoel cavity more than half the volume of the embryo
3	Full blastocyst, cavity completely filling the embryo
4	Expanded blastocyst cavity larger than the embryo, with thinning of the shell
5	Hatching out of the shell
6	Hatched out of the shell

These three scores add up to the total score that is provided to each blastocyst. Consequently, the expansion score—a value between 1-6 depending on the degree of expansion and the hatching status—is the first log.

Embryo Transfer

Under ultrasound guidance, embryos were put onto a soft catheter (Labotect) and inserted into the uterine cavity through the cervix to reach the maximal implantation potential while maintaining complete bladder function. Three embryos at most were transferred during the cleavage stage (day three following oocyte retrieval) or the blastocyst stage (day five following oocyte retrieval) in our investigation. Before transfer, patients with fragile endometrium, fluid in the uterus, and high serum progesterone levels had their embryos frozen

Luteal Support

Cyclogest 400mg (Alpharma, UK) BD, acetylsalicylic acid (aspocid, CID, Egypt) 75 mg and hostacortin 5 mg (prednisolone, Sanofi Aventis, Egypt) once daily. Chemical pregnancy was defined as positive serum B HCG at 12 days post 5th day embryo transfer or 14 days post 3rd day embryo transfer. Clinical pregnancy demonstrated by embryonic cardiac pulsation by vaginal ultrasound 2 weeks after positive S. BHCG.

Study outcome:

Primary outcome was to predict association between Follicular Fluid Estradiol levels and pregnancy outcome in ICSI cycles. Secondary outcomes were to predict association between Follicular Fluid Estradiol levels and oocyte quality, fertilization rate in ICSI cycles.

Ethical Considerations:

The study was approved by the institutional review boards and ethics. Informed written consent was obtained from all the participants.

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Statistical analysis:

Data were analyzed using Statistical Program for Social Science (SPSS) version 20.0. Quantitative data were expressed as mean± standard deviation (SD). Qualitative data were expressed as frequency and percentage.

Results

The mean duration of infertility (5 years) ± 3 years (2 to 13 years). Primary infertility in 62.8 % of cases 113 cases and secondary infertility in 67 cases 37.8%. The mean cause of infertility was tubal cases 81 cases (45%) then male factor 67 of cases (37.2%), then unexplained causes 26 cases (14.4%) **(Table I).**

This table shows that Follicular fluid E2 concentration ranged from 247±199 (0 to 700) (ng\ ml). Serum E2 concentration ranged from 2361±1583 (100 to 7589) pg\ ml. Percentage of grade 1 Mil was 70.6% and grade 2,3 Mil was 29.4% with Fertilization rate 100 of cases. The mean of total number oocytes was 10 with 53% of Mil of good

quality and 47% of Mil of bad quality. Number of transferred embryos ranged from one to three embryos(good quality was 63.9% and of bad quality 36.1%) ,46 cases transferred single embryo,69 cases transferred 2 embryos and 65 cases transferred 3 embryos(57.2% of cases was transferred on day 5 and 42.8% of them on day 3) and 11 cases had frozen embryo transfer. Chemical pregnancy was positive in 90 cases (50%) and the clinical pregnancy positive in 66 cases (36.7%) **(Table II)**.

This table shows that the median follicular fluid E2 concentration was 281 ranged from (165 to 440)(ng\ml) in oocyte with good quality MII(gradel) while when the concentration of follicular fluid E2 ranged from (20 to 130) (ng\ml) median concentration 45 in oocyte with bad quality(grade 2,3) MII. The median serum E2 concentration was 2822 ranged from (1800 to 3798) (pg\ml) in oocyte with good quality MII (gradel) while when the concentration of serum E2 ranged from (390 to 1655) (pg\ml) median concentration 800 in oocyte with bad quality (grade 2,3) MII **(Table III)**.

This table shows that in follicular fluid E2 concentration ranged from (180 to 420 ng\ ml) had good embryo quality ajnd in this ranges, from (25 to 170 ng\ml) had bad embryo quality. As regard serum E2 Ranges from (1800 to 3877pg\ml) had good quality embryos and ranges from (430 to 1908pg\^nl) had bad quality embryos **(Table IV)**.

This table show's that in patient with follicular fluid E2 concentration (200 to 440 ng\ml) (median value 300) had a positive chemical pregnancy (50% of cases) and in patient with concentrations ranged from (30 to 260 ng\ml) had negative chemical pregnancy median 80 (52.8% of cases). As regard serum E2, patients with serum E2 ranging from (1898 to 3765pg\ml) had positive chemical pregnancy and in patients with serum E2 concentrations ranging from (540 to 2859pg\ ml) (median 1079) had negative chemical pregnancy **(Table V)**.

This table shows that in patient with follicular fluid E2 concentrations ranging from (220 to 476 ng\ml) (median 315) had clinical pregnancy and patients with Follicular Fluid E2 concentrations ranging from (36 to 320 ng\ml) (median 111) had no clinical pregnancy. As regard serum E2, patients with serum E2 concentrations ranging from (2388 to 3899pg\ml) (median 3241) had clinical pregnancy and patients with serum E2 concentrations ranging from (630 to 2859pg\ ml) (median 1561) had no clinical pregnancy **(Table VI)**.

Receiver-operating characteristic (ROC) curves show that follicular fluid E2 had fair predictive value in predictio^ of Mil maturity (AUC = 0.837 and 0.838, respectively). Follicular fluid E2 concentration >170 ng/ ml had sensitivity of 74.8% and specificity of 83%in prediction of Mil maturity.) Follicular fluid had good predictive value (AUC=0.880) in prediction of embryo quality. Follicular fluid E2 concentration 130ng/ ml had sensitivity of 80.9% and specificity of 72.3%. Follicular fluid E2 had fair predictive value (AUC=0.757) in prediction of chemical pregnancy. Follicular fluid E2 concentration > 144 ng/ml had sensitivity of 83.3% and specificity of 61.1%. As regard clinical pregnancy follicular fluid E2 had fair predictive value (AUC=0.749). Follicular fluid E2 concentration >160ng/ml had sensitivity of 86.4% and specificity of 57% **(Table VII)**.

Receiver-operating characteristic (ROC) curves show that serum E2 had good predictive value in prediction of Mil maturity (AUC = 0.819). Serum E2 concentration >1768 pg/ml had sensitivity of 77.2% and specificity of 81.1%in prediction of Mil maturity. Serum E2 had good predictive value (AUC=0.814) in prediction of embryo quality. Serum E2 concentration 1099pg/ ml had sensitivity of 91.3% and specificity of 64.6%. Serum E2 had fair predictive value (AUC=0.731) in prediction of chemical pregnancy. Serum E2 concentration > 1059

pg/ml had sensitivity of 94.4% and specificity of 50%. As regard clinical pregnancy serum E2 had fair predictive value (AUC=0.768). Serum E2 concentration >2134 pg/ml had sensitivity of 80.3% and specificity of 65.8% **(Table VIII)**.

Discussion

According to WHO estimates, infertility is the third most serious condition globally. Even with ICSI being used all around the world, the birth rate is still just about 30%. Exogenous gonadotropins are utilized for ovarian stimulation to produce numerous follicles in order to maximize its success. Nevertheless, it has been demonstrated that these gonadotropins negatively impact the quality of oocytes and embryos. It has been shown that exposure to gonadotropin concentrations above the normal range disrupts oocyte maturation and meiosis, resulting in chromosomal aneuploid oocytes [17].

Furthermore, since not all oocytes produce healthy embryos, in reality, retrieving more oocytes is associated with lower oocyte quality [10]. This is caused by low oocyte developmental competence, often known as "oocyte quality," which up until this point has lacked a true measurement. It is now commonly acknowledged that the quality of the oocyte influences the quality of the embryo since the oocyte provides the majority of the embryo's cytoplasm, which aids in early embryogenesis and embryonic genome activation [18]. Since the overall number of high-quality embryos has been demonstrated to be less indicative of the success of ICSI, the conventional criterion for embryo selection—which is solely focused on morphology—is really insufficient to reflect "Embryo Quality*" [19].

Predicting "oocyte quality" thus becomes essential in ICSI so that the doctor can tailor the stimulation regimen and the embryologist can choose which "best embiyo" to transfer.

Ovarian follicle development culminates in the oocyte gaining the ability to proceed with meiosis ("Nuclear maturation") and develop into an embiyo ("Cytoplasmic maturation"). A unique humoral milieu known as "The Follicular Fluid" surrounds the egg while it is in this stage of development in the antral follicle. Given that the follicular fluid is the result of blood plasma passing over the blood-follicle barrier and follicle cell secretions, a potential relationship between it and the oocyte is anticipated. Its constituents may function in a paracrine or autocrine manner, so affecting the quality of the egg and its subsequent capacity to become fertilized and grow into a healthy embryo [7].

When added to in vitro maturation media, oocytes with high follicular E2 are linked to healthy follicles that contain oocytes capable of resuming meiosis and resulting in a healthy pregnancy. These oocytes also develop to the blastocyst stage and have a direct, non-genomic effect on the oocyte surface, changing its calcium release mechanisms, which are thought to be involved in oocyte cytoplasmic maturation [20].

Follicles containing developed nucleus oocytes, typically fertilized oocytes, and oocytes resulting in pregnancy were found to have high E2 levels in their follicular fluid [20].

The 180 infertility patients who had ICSI for tubal, male, or unexplained cases of infertility provided 180 follicular fluid samples for the current prospective observational study. The induction protocol that was employed was the flexible antagonist protocol.

The objective of the research was to evaluate the relationship between the level of E2 in follicular fluid and clinical pregnancy, oocyte maturation, fertilization, and embryo quality.

The results of this study demonstrated that the development of MH-grade oocytes, fertilization, embryo quality, chemical pregnancy, and clinical pregnancy may all be independently predicted by the follicular fluid E2.

An observational study that demonstrated the relationship between the follicular fluid E2, mature and immature, fertilized and non-fertilized oocytes, and the grades of embryos formed from these oocytes was the first to challenge the theory. It demonstrates that the biochemical prediction of the ICSI outcome came from follicular fluid E2 [21].

This study discovered that follicles producing mature (Mil) oocytes had higher E2 levels in their follicular fluid than follicles producing immature oocytes, in accordance with oocyte nuclear maturation.In actuality, the follicle with the highest concentration of E2 is the one that selects other follicles by stimulating FSH receptors on granulosa cells, which promotes follicle development even in the presence of low FSH levels brought on by E2's negative feedback inhibition. Our findings show that elevated E2 may not only indicate follicular maturation but also oocyte nuclear maturation. It seems sense to assume that a healthy follicle has a big number of granulosa cells that can produce a significant amount of E2, which will result in a healthy oocyte.

Several research have reported on this finding [22–23]. On the other hand, Costa et al., 2004 discovered that follicles with mature oocytes had much lower E2 levels than follicles with immature oocytes [24].

E2 might stop premature nuclear maturation. allowing enough time for appropriate cytoplasmic maturation consequently synchronizes ovulation of a completely developmentally competent egg with meiotic maturation [25]. It has been suggested that the poorly known relationship between E2 and nuclear maturation may be due to steroid synthesis in response to LH-induced meiosis resumption. By binding on estrogen receptors (ERs) on the oocyte surface, E2 may mediate LH-induced resumption of meiosis [26–27]. This will change the oocyte's Ca2+ oscillations, which will activate the meiosis promoting factor (MPF) [28].

This study indicates that high E2 levels are associated with proper fertilization and high-quality embryos, which is relevant to oocyte developmental competence. The maturation of both the nuclear and cytoplasmic ovaries occurs during the last stage of follicle development. Since normal fertilization and blastocyst development are all dependent on the degree of cytoplasmic maturation, high E2 may be important for proper oocyte cytoplasmic maturation. During this phase, intrafollicular E2 concentrations undergo significant changes, suggesting its contribution in this crucial final stage of oocyte maturation and demonstrating that E2 in the follicular fluid is distinctly different among follicles yielding oocytes with different developmental potentials. Others have also discovered these results [10]. Gilshrest et al. [29] established a direct correlation between E2 and oocytes, observing that oocytes stimulate E2 production by cumulus cells via oocyte secreting factors (OSFs). Furthermore, they found a direct, non-genomic effect of E2 on ER on the oocyte surface, which leads to Ca2+ oscillations thought to impact cytoplasmic maturation [26]. Thus, we might conjecture that E2 may have a direct effect in the developmental potential of oocytes.

Although even MII oocytes have varying developmental potentials, exogenous gonadotropins are used in ART to enhance the number of oocytes retrieved in practice. Thus, it is possible that follicle development can continue even in the absence of oocyte maturation. Although exogenously administered gonadotropins lead to follicle development, they may interfere with steroidogenesis, which could account for variations in oocyte quality, since appropriate steroid sequence and pattern has been linked to oocyte maturation and its acquisition of die molecular programming for proper fertilization and embryo development [30].

Follicle E2 has a positive and significant relationship with oocyte developmental potential. It also plays a role in follicle

development and enhances oocyte cytoplasmic maturation [31].

Unlike a study published in 2002 by Mendoza et al., it was found that the greatest amounts of estradiol in follicular fluid may be used to identify the quality of the embryo. In all situations, the ratio of estradiol to progesterone was higher in embryos of A and B grade quality compared to those of C quality, and the ratio of estradiol to testosterone was higher in embryos of B quality compared to those of C quality [7].

However, with gestation being the ultimate objective, there were higher amounts of estradiol in follicular fluid in cases of pregnancy when looking at total pregnancy rates. This was consistent with a 2008 study by Asimakopoulos et al. that found no variations in the E2 products in the follicular fluid between follicles that produce a healthy egg and those that do not fertilize. By accounting for nuclear maturation during fertilization, we were able to precisely examine the connection between intrafollicular E2 levels and the particular fertilization result [32].

On the other hand, follicular fluid E2 levels were associated with oocyte competence, which is the capacity to carry out a typical fertilization process. It should come as no surprise that patients whose follicle produced a MII oocyte that developed into a 2PN also had higher serum E2 levels. Additionally, serum E2 levels were higher for each follicle and egg that was recovered, indicating a possible rise in granulosa cell capacity worldwide.

As this study's results showed, there was a known positive correlation between estradiol and follicular volume as well as a positive correlation between estradiol and follicular diameter in plasma during the follicular phase in both spontaneous and induced cycles [33].

The current data indicate that mature oocytes are linked to lower estradiol levels in FF fluid following hCG. It appears that the process of oocyte maturation and this inversion of the steroidogenic pattern are both impacted by the ovulatory LH surge (Moor et al., 1980). Follicle diameter and estrogen levels in FF did indeed correlate negatively. Estradiol and testosterone levels significantly decrease with oocyte maturation, and there is a positive correlation between them. As androgens, and testosterone in particular, are direct precursors of estradiol, this could not be any different [34].

Additionally, this was refuted by a study (Frederick et al., 1991) that showed concurrent observations of follicular fluid estrogen synthesis and oocyte development. The relationship between steroids seemed to be the most important aspect of the oocyte maturation process. Using the steroid ratios in FF, some researchers hypothesized that the progesterone/estradiol ratio would be the most accurate measure of maturity. Progesterone/estradiol, progesterone/testosterone, and estradiol/testosterone ratios were higher in FF from follicles of mature oocytes, according to preovulatory follicular fluid steroid levels in stimulated and unstimulated cycles triggered with human chorionic). The metabolism of C21 (progestogens) to C19 (androgens) decreases with an increase in the progesterone/testosterone ratio. It may be possible to use these indices to predict oocyte maturity based on the levels of these steroids in FF, as evidenced by the positive relationship between estradiol and testosterone and the rise in the estradiol/testosterone ratio, which suggests that the fall in estradiol levels may be caused by a reduction in testosterone rather than a reduction in aromatase activity [35].

Endometrial receptivity [9], which is a function of embryo quality and implantation potential, is crucial for the success of IVF programs in achieving pregnancy. Up until the fertilization stage, estradiol has a beneficial effect on in vitro fertilization (IVF); however, its effects during the implantation and pregnancy stages are debatable [36].

Conclusion

It appears that the E2 content in fibrous fluid was fairly predictive of oocyte maturation, fertilization, embryo quality, chemical pregnancy, and clinical pregnancy. However, it was a separate predictor of the generation of oocytes of MH grade. Therefore, in an effort to enhance pregnancy outcomes for patients having ICSI, it is advised to conduct additional research to evaluate various markers in the follicular fluid that may be utilized to predict the quality of oocytes recovered and embryo grading. Additional research to link follicular fluid E2 for each eash follicle and the likelihood of conception for each chosen embryo.

Conflict of interest

None

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Table I: Characteristics of the whole study population: Numerical variables

Variable	Mean	SD	Maximum	25th Percentile	Median	75th Percentile
Age (yr)	31	5	38	28	32	35
Weight (kg)	66	5	76	63	66	70
Height (cm)	164	3	177	163	164	167
BMI (kg/m²)	24.4	1.4	26.3	23.7	24.8	25.6
Duration of infertility	5	3	12	3	4	6
Parity				P0	113	62.8%
				P1	50	27.8%
				P2	16	8.9%
				P3	1	0.6%
Cause of infertility				Unexplained	26	14.4%
				Tubal factor	81	45.0%
				Male factor	67	37.2%
				Tubal and male factors	6	3.3%

Table II: Characteristics of the whole study population: Categorical variables

Variable	Mean	SD	Minimum	Maximum	25th Percentile	Median	75th Percentile
Follicuar fluid E2 (ng/ml)	247	199	0	700	60	230	385
Serum E2 (pg/ml)	2361	1583	100	7589	940	2276	3486
Total number of oocytes	10	6	1	26	5	10	15
Total number of MII oocytes	6	4	1	20	3	6	9
Number of good-quality MII occytes	4	3	0	17	1	3	6
Number of poor-quality MII occytes	2	1	0	7	1	2	3
Percentage of good-quality MII occytes	53.0	28.3	0.0	100.0	36.7	59.2	75.0
Percentage of poor-quality MII occytes	47.0	28.3	0.0	100.0	25.0	40.8	63.3

Varialble		Count	%
MII oocyte maturation	Grade 2 or 3	53	29.4%
	Grade 1	127	70.6%
Fertilization	Negative	0	0%
	Positive	180	100%
Day of embryo transfer	D3	77	42.8%
	D5	103	57.2%
Number of transferred embryo	Single embryo	46	25.6%
	2 Embryo	69	38.3%
	3 Embryo	65	36.1%
Quality of transferred embryo	Poor quality	65	36.1%
	Good quality	115	63.9%
Frozen embryo transfer			
	Positive	11	6.1%
Chemical pregnancy	Negative	90	50.0%
	Positive	90	50.0%
Clinical pregnancy	Negative	114	63.3%
	Positive	66	36.7%

Table III: Relation between follicular fluid or serum E2 concentration and MII oocyte quality

	MII oocyte maturation Grade 2 or 3 (N=53)		Grade 1 (N=172)				
Variable	**Median**	**IQR**	**Median**	**IQR**	**U**	**Z**	**P-value†**
Follicuar fluid E2 (ng/ml)	45	20 - 130	281	165 - 440	1094.5	-7.128	<0.001
Serum E2 (pg/ml)	800	390 - 1655	2822	1800 - 3798	1219.5	-6.735	<0.001

Table IV: Relation between follicular fluid or serum E2 and embryo quality

Quality of transferred embryo							
	Poor quality (N=65)		**Good quality (N=115)**				
Variable	**Median**	**IQR**	**Median**	**IQR**	**U**	**Z**	**P-value†**
Follicuar fluid E2 (ng/ml)	55	25 - 170	290	180 - 420	1494.5	-6.681	<0.001
Serum E2 (pg/ml)	800	430 - 1908	2871	1800 - 3887	1393.5	-6.981	<0.001

Table V: Relation between follicular fluid or serum E2 concentration and chemical pregnancy

Chemical pregnancy							
	Negative (N=90)		**Positive (N=90)**				
Variable	**Median**	**IQR**	**Median**	**IQR**	**U**	**Z**	**P-value†**
Follicuar fluid E2 (ng/ml)	80	30 - 260	300	200 - 440	1971.5	-5.947	<0.001
Serum E2 (pg/ml)	1079	540 - 2859	2874	1898 - 3765	2179.5	-5.351	<0.001

Table VI: Relation between follicular fluid or serum E2 concentration and clinical pregnancy

Clinical pregnancy							
	Negative (N=114)		Positive (N=66)				
Variable	Median	IQR	Median	IQR	U	Z	P-value†
Follicuar fluid E2 (ng/ml)	111	36 - 320	315	220 - 476	1887.5	-5.565	<0.001
Serum E2 (pg/ml)	1561	630 - 2859	3241	2388 - 3988	1748.5	-5.977	<0.001

Table VII: Receiver-operating characteristic (ROC) curve analysis for predictive value of follicular fluid E2

Outcome				
ROC curve parameter	MII maturity	Embryo quality	Chemical pregnancy	Clinical pregnancy
AUC	0.837	0.800	0.757	0.749
SE	0.034	0.038	0.037	0.036
95% CI	.0775 to 0.888	0.734 to 0.856	0.687 to 0.817	0.679 to 0.811
z statistic	9.839	7.892	7.002	6.995
P-value (AUC_0=0.5)	<0.0001	<0.0001	<0.0001	<0.0001
Youden index J	0.58	.053	0.44	0.43
Associated criterion	>170	>130	>144	>160
Sensitivity	74.8	80.9	83.3	86.4
Specificity	83.0	72.3	61.1	57.0

Table VIII: Receiver-operating characteristic (ROC) curve analysis for predictive value of serum E2

Outcome				
ROC curve parameter	MII maturity	Embryo quality	Chemical pregnancy	Clinical pregnancy
AUC	0.819	0.814	0.731	0.768
SE	0.037	0.036	0.039	0.035
95% CI	0.755 to 0.872	0.749 to 0.868	0660 to 0.794	0699 to 0827
z statistic	8.635	8.732	5.988	7.752
P-value (AUC_0=0.5)	<0.0001	<0.0001	<0.0001	<0.0001
Youden index J	0.58	0.56	0.44	0.46
Associated criterion	>1768	>1099	>1059	>2134
Sensitivity	77.2	91.3	94.4	80.3
Specificity	81.1	64.6	50.0	65.8