Relationship between Oestradiol Level and Number and Quality of retrieved Oocyte in Cases of Assisted Reproductive Technology with Gonadotropin Releasing Hormone Agonist

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Abstract: This study conducted on 1000 cases at Al-Azhar Islamic center for population and researches, from 2016-2018. IVF indications included, male factor, tubal factor, endometrosis stage I & II, PCO, unexplained infertility. All female had criteria: - Basal FSH ≤ 10 IU - Age: 20-35. - Long of agonist protocol - First ICSI cycle. Over a period of 2y, 1000 female were recruited with a range of follow up from 4 wks to 12 wks. All 1000 studied female underwent to hormonal profile before ICSI cycle, but percentage of females underwent to different invasive investigations (HSG, hysteroscopy and laparoscopy) Only laparoscopy had a significant impact on the pregnancy rate. As expected there were significant positive correlations between estradiol levels at the time of oocyte pickup and outcomes of ICSI cycle, implicating that the numbers of oocytes retrieved, embryos obtained, and embryos transferred were increased as the estradiol levels increased, there was a significant moderate positive correlation between B-HCG resulted and estradiol levels. There was significant association between the level of estradiol and quality of oocytes retrieved and quality of embryos obtained. Female getting older, having higher BMI, having older & husbands were significantly with lower estradiol with no significant correlations between estradiol level and duration of infertility, age at menarche, FSH, LH, serum prolactin, TSH and baseline estradiol. There was a significant difference in the mean of value of estradiol level among those with negative ovarian factor, PCO, ovarian insufficiency. Although female with PCO had a mean estradiol level higher than those with negative ovarian factor. So, female without unexplained cause of infertility had a significant correlation level of estradiol than those with unexplained cause of infertility. It was found through the study that the cutoff value for estradiol level at time of pick up was chosen around 3430-3460pg. So, generally if the level of estradiol after our procedure becomes more than 3430, it is better to continu8e the procedure as this female is more likely to get pregnant. In addition, the test had a predictive accuracy of 74.4%.

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1. Introduction

Two principal parameters used clinically in the follow up of follicle development in controlled ovarian hyperstimulation (COH) in ICSI cycles are oestradiol level and follicle size. In general, as oestradiol secretion rises in the follicular phase, so does follicle size ***(Var et al., 2010).***

The prediction of a successful outcome during in-vitro fertilisation (IVF) critically depends on optimising ovarian-stimulation protocols that are aimed to provide good quality oocytes, the final goal is achieving and maintaining pregnancy in which there are many factors affecting the outcome ***(Anifandis et al., 2005).***

The assessment of oocyte/embryo quality in human IVF is increasing attention to embryologists. The qualities of oocytes and embryos play important roles in pregnancy rates (PR) and the factors affecting the qualities are not well known ***(Sharara and McClamrock, 1999).***

Estradiol plays pivotal roles both in the regulation of follicle/oocyte maturation and in the endometrial receptivity. However, its role beyond that stage remains a controversial issue in reproductive medicine. Some authors supported that high E2 levels were not detrimental to the IVF outcome while others demonstrated that high serum E2 levels on the day of human chorionic gonadotrophin (hCG) injection are detrimental to uterine receptivity without affecting embryo quality ***(Ozdegirmenci et al., 2011).***

Currently, no high-quality evidence exists to support or deny an association between serum levels of E2 on the day of hCG administration and pregnancy achievement in IVF cycles. In this study, we aimed to investigate the influence of the ratios of serum E2 to either the number of follicles > 14 mm on the day of hCG administration or the number of oocytes retrieved

during oocyte pick up (OPU) session on the oocyte quality, maturation, quality of viable embryos, fertilisation rate, implantation, clinical and ongoing pregnancy rates during ICSI cycles to find out the optimal predictive E2 level for the prognosis ***(Kolibianakis et al., 2009).***

Thereby, on hCG and OPU days, the role of E2 per mature follicle and oocyte retrieved would be investigated rather than total levels. Therefore, it is important to evaluate whether there is an association between total serum E2 levels on the day of embryo transfer and implantation, clinical and ongoing pregnancy rates. Our target was to identify the optimum concentration range of oestradiol conferring optimal oocyte and embryo quality with high pregnancy rates ***(Joo et al., 2010).***

Retrieved oocytes will be denuded by 80 IU/mL hyaluronidase (Vitrolife, Sweden) enzyme, and the morphology of oocytes at the time of ICSI will be evaluated under an inverted microscope with Hoffman modulation at 400 × magnification (Olympus IX71, Olympus Co., Japan). Morphology assessment was performed based on the previously suggested morphological features ***(Balaban et al., 1998)***.

Basically, abnormal features were grouped as extracytoplasmic, including fragmented first polar body, abnormal first polar body, large perivitelline space, abnormal zona pellucida and abnormal oocyte shape; and cytoplasmic, including vacuoles, granularity, refractile body and brown oocytes. Morphologically evaluated oocytes will be scored from best to worst as Score 7; MII oocyte with no abnormal feature, Score 6; MII oocytes with one abnormal feature, Score 5; MII oocytes with more than one abnormality, Score 4; MI oocyte with no abnormal feature, Score 3; MI oocytes with one abnormal feature, Score 2; MI oocytes with more than one abnormality and finally Score 1 for GV with any abnormality ***(Rienzi et al., 2008)***.

Good-quality embryos were defined equal-sized, symmetric blastomeres with ≤10% fragmentation under inspection in an inverted microscope. Fertilisation rate was defined as the percentage of fertilised embryos (2PN) in all the oocytes. The implantation rate was the proportion of embryo transferred resulting in an intrauterine gestational sac. Pregnancies were confirmed 2 weeks after ET, when the serum hCG was elevated. and intrauterine gestational sac was documented by transvaginal ultrasound 1 week after hCG elevation. A clinical pregnancy is defined by the presence of one or more gestation sacs ***(Turgut et al., 2011)***.

Estradiol (E2 or 17 β-estradiol, also oestradiol) is a sex hormone. Estradiol is abbreviated E2 as it has two hydroxyl groups in its molecular structure. Estrone has one (E1) and estriol has three (E3). Estradiol is about 10 times as potent as estrone and about 80 times as potent as estriol in its estrogenic effect. Except during the early follicular phase of the menstrual cycle, its serum levels are somewhat higher than that of estrone during the reproductive years of the human female. Thus it is the predominant estrogen during reproductive years both in terms of absolute serum levels as well as in terms of estrogenic activity.

During menopause, estrone is the predominant circulating estrogen and during pregnancy estriol is the predominant circulating estrogen in terms of serum levels. Estradiol is also present in males, being produced as an active metabolic product of testosterone. The serum levels of estradiol in males (14 - 55 pg/mL) are roughly comparable to those of postmenopausal women (< 35 pg/mL). Estradiol in vivo is interconvertible with estrone; estradiol to estrone conversion being favored. Estradiol has not only a critical impact on reproductive and sexual functioning, but also affects other organs, including the bones.

Aim of the Work

The aim of this study is to identify the optimal concentration range of oestradiol level conferring optimal oocyte and embryo quality with high pregnancy rates.

2. Patients & Methods

The current study was conducted on 1000 cases as a prospective study at Al-Zhar University Center for Population Studies and Researches.

Data was accepted by University Ethical Committee. IVF indications included tubal factor, male factor, endometriosis (stages 1 and 2), polycystic ovarian syndrome and unexplained infertility.

Each patient was subjected to the following:

(1) Full history taking: including:

1- Name
2- Age
3- Parity
4- Menstrual history
5- Obstetric history
6- Medical illness (hypertension, DM, thyroid disease).
7- Family history of medical diseases and malignancy.

(2) Clinical examination: including:

1- General examination: vital data, BMI, chest and heart examination, thyroid examination, palpable LNs and lower limbs examination.
2- Abdominal examination: for masses, ascites.
3- Pelvic examination: for uterine size, adnexial masses.

(3) Investigations: including:

1- Laboratory investigations: menstrual cycle day 2 serum FSH, LH, and serum prolactin, TSH.

2- Transvaginal ultrasound for uterine size, endometrial thickness and any pelvic pathology.

Inclusion criteria:

1- A basal day 2 FSH level less than ≤10 mIU/l.
2- Age between 20-35 years.
3- Long protocol with GnRH-a and HPFSH.
4- First cycle of ICSI treatment.

Exclusion criteria:

1- Poor responders (women who achieved an E2 level < 500 pg/ml on the day of hCG administration and women in whom < 5 oocytes were retrieved).
2- Patients with known pelvic pathology like severe endometriosis, uterine fibriod.
3- Severe male factor.
4- Frozen-thawed cycles and other stimulation protocols.

Methods

All the patients were subjected to:

1. Informed consent.
2. Detailed history taking about age, parity, previous uterine surgery.
3. Physical examination
4. All patients applied long agonist stimulation protocol.
5. Transvaginal Ultrasound study for: detection of any uterine anomalies, normal uterine measurements, no residual ovarian cysts.
6. Follicular growth was monitored by serial transvaginal ultrasounds.
7. Serial serum E2 levels for monitoring of follicular growth & guarding against ovarian hyper stimulation.
8. Oocyte pick-up was performed 36 hours after the administration of HCG.

Stimulation protocols:

All patients was started 0.035 mg oestradiol/ 0.15 mg desogestrel oral contraceptive pill. Subcutaneous 0.5mg/ dl Leuprolid acetate (lucrin daily flacon, 1 mg Abort, Cedex, Istanbul) was used for the inhibition and it was started from midluteal phase of previous menstruel cycle up to 14 days.

The ovarian stimulation was initiated when serum oestradiol levels were lower than ≤50pg/ml and follicules smaller than 10 mm. HPFSH. was used for ovarian stimulation based 150 IU initiation dose on the third day of vaginal bleeding. After the initiation of gonadotrophin stimulation, the dosage of lucrin was reduced to 0.25 mg/dl and continued until the day of oocyte retrieval.

The starting regimen was fixed for the first 3 days, with the dose of HpFSH subsequently adjusted according to individual ovarian response. One injection of HCG (10,000 IU.) was administrated when an adequate follicular response was recorded with ultrasound. Criteria for hCG administration was at least three ovarian follicles with a mean follicle diameter > 17 mm.

No specific E2 level was given in this protocol as a binding criteria for HCG administration, but the serum E2 level was to be within an acceptable range for the number of follicles according to the investigator's experience. After hCG administration, standard oocyte pick-up.

Retrieved oocytes was denuded by 80 IU/mL hyaluronidase (Vitrolife, Sweden) enzyme, and the morphology of oocytes at the time of ICSI were evaluated under an inverted microscope with Hoffman modulation at 400 × magnification (Olympus IX71, Olympus Co., Japan). Morphology assessment was performed based on the previously suggested morphological features.

Basically, abnormal features were grouped as extracytoplasmic, including fragmented first polar body, abnormal first polar body, large perivitelline space, abnormal zona pellucida and abnormal oocyte shape; and cytoplasmic, including vacuoles, granularity, refractile body and brown oocytes. Morphologically evaluated oocytes were scored from best to worst as Score 7; MII oocyte with no abnormal feature, Score 6; MII oocytes with one abnormal feature, Score 5; MII oocytes with more than one abnormality, Score 4; MI oocyte with no abnormal feature, Score 3; MI oocytes with one abnormal feature, Score 2; MI oocytes with more than one abnormality and finally Score 1 for GV with any abnormality.

Good-quality embryos were defined equal-sized, symmetric blastomeres with ≤10% fragmentation under inspection in an inverted microscope. Fertilisation rate was defined as the percentage of fertilised embryos (2PN) in all the oocytes. The implantation rate was the proportion of embryo transferred resulting in an intrauterine gestational sac. Pregnancies were confirmed 2 weeks after ET, when the serum hCG was elevated. and intrauterine gestational sac was documented by transvaginal ultrasound 1 week after hCG elevation. A clinical pregnancy is defined by the presence of one or more gestation sacs.

ICSI and ET procedures were performed. Embryos were evaluated according to Gl-5 grading system. All transfers were completed on Day 3, and no more than 3 Grade 1 embryos were transferred.

Ethics committee:

After approval of the research and ethics committee of El-Azhar university hospital, Cairo, Egypt in accordance with local research government requirement, the trial was registrated with Federal Clearing House for randomized trial, clinical trials government.

Blood sampling:

For measuring E2 serum level 5ml. blood sample centrifuged to extract serum, strips of VIDAS estradiol II from BIOMERIEUX SA, were used.

VIDAS estradiol II is an automated quantitative test for the quantitative measurement of total 17 B-estradiol in human serum or plasma, using the ELFA technique (Enzyme linked fluorescent assay).

Principle:

The assay principle combines a competition method with a final fluorescent detection (ELFA).

The Solid Phase Receptacle (SPR) serves as the solid phase as well as the pipetting device for the assay.

Reagents for the assay are ready-to-use and pre-dispensed in the sealed reagent strips. All of the assay steps are performed are performed automatically by the instruments. The reaction medium is cycled in and out of the SPR several times.

The sample is transferred into the well containing the conjugate, which is an alkaline phosphatase-labled estradiol derivative. The estradiol present in the serum and the estradiol derivative in the conjugate compete for the anti-estradiol specific antiboby sites coated to the inner surface of the SPR.

Unbound components are eliminated during the washing steps.

During the final detection step, the substrate (4-Methyl-umbelliferyl phosphate) is cycled in and out of the SPR. The conjugate enzyme catalyzes the hydrolysis of this substrate into a fluorescent product (4-Methyl-umbelliferone), the fluorescence of which is measured at 450 nm.

The intensity of the fluorescence is inversely proportional to the concentration of antigen present in the sample.

At the end of the assay, results are automatically calculated by the instrument in relation to the calibration curve stored in memory.

The SPR: the interior of the SPR is coated during production with polyclonal anti-estradiol immunoglobulins (rabbit). Each SPR is identified by the E2II code.

Results and interpretation:

Results are analyzed automatically by the computer. Fluorescence is measured twice in the reagent strips. The first reading is a background reading of the substrate cuvette before the substrate is placed in contact with the SPR. The second reading is taken after incubating the substrate with the enzyme remaining on the interior of the SPR. The (RFV) relative fluorescence value is calculated by subtracting the background reading from the final result.

The VIDAS Estradiol II assay is calibrated against the ID-GCMS (Isotope dilution-gas chromatography mass spectrometry technique.

3- Results

Over a period of 2 years, 1000 females were recruited with a range of follow up from 4 weeks to 12 weeks, the median age of these females was 29 years with a range of ages from 20-41 years, the mean BMI was 30.356±5.701, 52% of these females were obese, with a median age at menarche of 14 years, 78% of them had regular cycles, the median duration of infertility was 5.277±2.849, 61.5% of females had a primary infertility while 38.5% had secondary infertility, table (1).

Table (1): Demographic characteristics of study females

Characteristics	**No. %**
Age at ICSI	
Median	29 years
Mean ±SD	29.15±4.496
Min-max	20-41years
Age at menarche	
Median	14years
Mean ±SD	13.8025±1.4079
Min-max	11-17 years
Husband's age	
Median	35years
Mean ±SD	35.825±6.107
Min-max	24-58 years
BMI (mean±SD)	30.356±5.701
Underweight <18.5%	5 0.5%
Normal weight18.5-24.9%	195 19.5%
Overweight 25-29.9%	289 28%
obese≥30%	520 52%
Regularity of cycle	
Regular	780 78%

Characteristics	**No.**	**%**
irregular	220	22%
Duration of infertility	5.277±2.849	
Type of infertility Primary secondary	 615 385	 61.5% 38.5%
Previous surgery	270	27%
Chronic disease	110	11%
Previous abortions	260	26%
Previous pregnancies	170	17%

Results expressed as median, mean±SD, number and percentages

Table (2): Laboratory investigations before ICSI cycle

Lab.	Mean ±SD
FSH Min-max	5.865±1.838 0.50-13.30
LH Min-max	4.256±2.233 0.25-12.50
Estradiol Min-max	49.446±23.095 10.0-210.0
Serum prolactin Min-max	16.422±6.406 0.50-40.0
TSH Min-max	2.6602±1.236 0.05-6.90

Data expressed as mean±S, range.

The mean FSH among studied females was 5.865±1.838 IU/ml, the mean LH was 4.256±2.233 IU/ml, while the mean estradiol level was 49.446±23.095, serum prolactin was 16.422±6.406 and the mean TSH was 2.6602±1.236, hormonal profile of 1000 studied females was demonstrated in table (2).

Table (3): Invasive investigation done to detect the cause of infertility and its relation to pregnancy results

Investigation	Pregnancy result				
	positive 545 54.5%	negative 455 45.5%	*P<0.05*	Wald	Exp (B)
Hystrosalpingography (N=500 50%)	305 56.1%	195 42.9%	0.066	3.378	1.694
Hysteroscopy (N=235 23.5%)	100 10%	135 13.5%	0.554	3.778	0.491
Laparoscopy (N=215 21.5%)	115 11.5%	100 10%	0.050*	0.350	1.252

Data expressed as number, percentages, logestic regression with Wald test to detect significance and likelihood ratio

Percentages of females who underwent different invasive investigations to detect the cause of infertility were illustrated in table (3), only laparoscopy had a significant impact on the pregnancy rate, with 11.5% of pregnant females underwent laparoscopy versus 10% of non pregnant females with P value 0.05.

Table (4): Correlations between the level of Estradiol at time of oocyte pickup and different protocol results

	Estradiol level at time of pick up	
	r	*P<0.05*
Number of oocyte retrieved	0.577	0.0001***
Number of embryo obtained	0.522	0.0001***
Number of embryo transferred	0.465	0.0001***
Mean B-HCG ±SD (528.524±116.860)	0.431	0.0001***

r=Pearson correlation coefficient, *** means highly significant

As expected there were significant positive correlations between estradiol levels at the time of oocyte pickup and outcomes of ICSI cycle, implicating that the numbers of oocytes retrieved, embryos obtained, and embryos transferred were increased as the estradiol levels increased, there was a significant moderate positive correlation between B-HCG resulted and estradiol levels, table (4).

Table (5): Relation between Estradiol level at pickup and pregnancy results

		Estradiol level	*P value<0.05*
Clinical pregnancy	Negative= 455	2737.857±1513.724	0.0001
	Positive= 545	4302.22±1815.422	
Implantation	Negative=475	2769.958±1575.136	0.0001
	Positive=525	4332.771±1777.264	
Ongoing pregnancy	Negative=465	2759.462±1529.274	0.0001
	Positive=310	4262.774±2071.208	
	Unknown=225	4381.444±1400.415	

Tests of significance include independant samples Mann-Whitney U test, Kruskal-Wallis test (the data were considered as nonparametric)

There were significant differences in the estradiol levels between pregnant females after ICSI cycle with success rate of pregnancy of 54.5% and non pregnant females with P value of 0.0001, table (5).

There was a significant association between the level of estradiol and the qualities of oocytes retrieved (P<0.0001), and qualities of embryos obtained (P<0.0001).

Table (6): Relation between Estradiol levels at time of pickup and quality of oocytes and quality of embryos obtained

		Estradiol level	
		Mean ±SD	P<0.05
Quality of oocyte	Degenerated=50	593.100±322.711	0.0001
	Good=950	3748.189±176.877	
Quality of embryo	G1=730	3867.877±1819.604	0.0001
	G2=180	3579.611±1523.132	
	G3=30	2783.333±670.790	
	G4=10	940.000±141.421	

Tests of significances; independant t-test, one-way Anova test

Table (7): Correlation between Estradiol level and demographic data of study females

	Estradiol level	
	r	p<0.05
Age	-0.249	0.0001***
Duration of infertility	-0.120	0.091
BMI (30.357±5.701)	-0.190	0.007**
Age at menarche	-0.060	0.401
Husband age	-0.269	0.0001***
FSH	-0.073	0.302
LH	0.003	0.967
Serum prolactin	0.012	0.867
TSH	-0.073	0.601
Estradiol	0.108	0.127

Data expressed as mean±SD, r=Pearson correlation coefficient,**moderately significant

Getting older, having higher BMI, having older husbands were correlated significantly with lower estradiol levels (P<0.0001, 0.007, 0.0001 respectively), with no significant correlations between estradiol levels and duration of infertility, age at menarche, FSH, LH, serum prolactin, TSH, and baseline estradiol.

Table (8): Relation between pregnancy results and possible causes of infertility

Cause		Pregnancy result		*P value<0.05*
		Positive	Negative	
Male factor	Positive Negative	200 345	105 350	0.01*
Tubal factor	Positive Negative	135 410	95 360	0.013*
Ovarian factor	Positive Negative	145 400	60 395	0.001**
Gonadal factor	Positive Negative	15 530	15 440	0.533
Uterine factor	Positive Negative	25 520	45 410	0.143
Unexplained	Positive Negative	80 465	165 290	0.0001***

Data was calculated using Chi square test, *significant, **moderately significant, ***highly significant.

Patients with negative male factor, tubal factor, ovarian factor, negative unexplained factors had significantly higher success rates of pregnancy with (P <0.01, 0.013, 0.001, 0.0001respectively).

Table (9): Relation of pregnancy rate to E2/oocyte ratio

	pregnancy		P-value
	Positive (54.5%)	negative	
E2/oocyte ratio	460.556±247.08	386.656±233.95	0.006 sig.

Data were analyzed using independant sample Mann-Whitney U test, P<0.05

This table demonstrated that pregnant females had a significantly higher E2/oocyte ratio than non pregnant females with P<0.006.

This meant that the predictive accuracy of E2/oocyte ratio was 61.3% in determining the success rate of pregnancy, and the cutoff value of this ratio was ≈ 385.42, subsequently the success of pregnancy was higher when this ratio was ≥ 385.42.

Based on this cutoff value, patients were classified into two groups ≥385 and <385, when we compared between both groups, we found significant difference in the mean number of oocytes retrieved, in the mean E2/o ratio, and E2 at time of oocyte pick up but not in the number of embryos obtained nor transferred, also no significant difference in the mean B-HCG, table (10).

Table (10): differences in the protocol outcomes according to E2/o ratio

Pregnancy outcomes	**E2/o ≥385**	**E2/o<385**	**P<0.05**
E2/o ratio	584.856±254.19	275.202±85.027	0.0001
E2 at time of pick up	4372.01±1936.52	2839.51±1415.59	0.0001
Number of oocytes	10.21±3.97	8.31±4.19	0.0001
Number of embryos obtained	2.89±1.5	2.86±1.4	0.721
Number of embryos transferred	2.34±0.824	2.32±0.924	0.967
B-HCG 2wks after ET	660.529±1261.835	402.588±1062.171	0.002

Data expressed as mean ±SD, independant sample Mann Whitney-U test

Table (11): difference in the pregnancy results according to the ratio

Observed	Predicted pregnancy			B ±SE	Wald	P<0.05	Exp (B) LR
	Negative ratio<385	Positive ratio>385	% correct				
Pregnancy negative	280	175	61.5%	-0.784± 0.290	7.323	0.007	0.456
Positive	230	315	57.8%				
Overall %			59.5%				

Data calculated using binary logestic regression test, LR= likelihood ratio

Interestingly we found a significant difference in the pregnancy result among females with E2/o ratio ≥385 versus those females with ratio <385 (LR=0.456, P<0.007)

Table (12): Relation between Estradiol level and possible factors of infertility

factor	Estradiol level at time of oocyte pick up Mean ±SD	P-value
Male factor Positive negative	 3724.193±1684.730 3530.341±1927.120	0.287 nonsignificant
Tubal factor Negative Bilateral tubal block Unilateral tubal block	 3588.123±1766.176 3457.242±2136.322 3955.923±2196.644	0.791 nonsignificant
Ovarian factor Negative PCO Ovarian insufficiency	 3496.616±1845.490 4181.432±1784.636 1853.000±1203.234	0.015 significant
Gonadal factor Negative Hypogonadism unilateral oophrectomy	 3644.098±1834.529 1653.200±1880.501 2866.000±021	0.075 nonsignificant
Uterine factor Negative Positive	 3616.871±1882.264 3239.214±1420.661	0.714 nonsignificant
Unexplained Yes No	 3232.530±1684.730 3706.576±1927.120	0.044 significant

Data expressed as mean ±SD, Mann-Whitney U test, and Kruskal-Wallis test for significance

There was a significant difference in the mean value of estradiol level among those with negative ovarian factor, PCO, and ovarian insufficiency P<0.015, although females with PCO had a mean estradiol level higher than those with negative ovarian factor, however, the latter group had other factors that might be incompatible with pregnancy. Females without unexplained cause of infertility had a significantly higher level estradiol than those with explained cause of infertility P<0.044.

4. Discussion

Although close observation of serum estradiol (E2) levels remains a mainstay of assessing clinical response to controlled ovarian stimulation, the prognostic value of any change in E2 levels after administration of gonadotrophin agonist remains unclear ***(Kondapalli et al., 2012)***.

The prediction of a successful outcome during in-vitro fertilisation (IVF) critically depends on optimizing ovarian-stimulation protocols that are aimed to provide good-quality oocytes. The final goal is achieving and maintaining pregnancy in which there are many factors affecting the outcome. The role of oestradiol (E2) in IVF cycles is well known up to the fertilisation stage ***(Anifandis et al., 2005)***.

E2 serum level plays pivotal roles both in the regulation of follicle/oocyte maturation and in the preparation of the uterus for implantation. However, its role beyond that stage remains a controversial issue in reproductive medicine. Some authors supported that high E2 levels were not detrimental to the IVF outcome ***(Ozlem Ozdegirmenci, et al, 2011)***.

It is not surprising that the researchers have focused on the relationships between serum E2 and pregnancy rates in IVF cycles. Several reports were concerning about this issue ***(Joo BS et al., 2010, Orvieto R et al., 2007)***.

However, the results of these studies were not consistent to each other. In our study, we investigated the role of E2 on oocyte quality, maturation, fertilization rate, quality of viable embryos, implantation, clinical and ongoing pregnancy rates.

Oocyte maturation and endometrial receptivity are the two major factors that seem to be related with a successful outcome in ART many reports suggest that oocyte immaturity accounts for a loss of efficiency in ART mainly due to the poor quality of the embryos and their inability to develop normally.

Tesarik and Mendoza (1995) concluded that E2 influenced in-vitro oocyte cytoplasmic maturation by acting at the oocyte surface, resulting to better fertilisation and cleavage rates after IVF. Our results

came in agreement with this study where there were significant positive correlations between E2 levels and numbers of oocytes retrieved.

Although a consensus regarding the impact of E2 on the quality of oocytes and embryos and subsequently pregnancy rate was not reached in the previous study, however, our results revealed a significant positive impact of E2 on quality of oocytes and embryos and subsequently pregnancy rates.

In accordance with Pena JE et al., who stated that elevated E2 levels were associated with larger of oocytes and embryos, our results revealed significant positive correlations between E2 and oocyte, and embryo numbers ($r=0.577$, $P<0.0001$, $r=0.522$, $P<0.0001$ respectively).

Three previous reports demonstrated a reduced pregnancy rate with increasing E2/oocyte ratio.

Loumaye's group studied patients undergoing COH for IVF using the long GnRH-agonist suppressive protocol, with different GnRH-agonist types and modes of administration. While pregnancy rate was found to be the highest whenever E2/oocyte ratio ranged between 70 and 140 pg/ml, it was not different from pregnancy rates in patients with E2/oocyte ratio exceeding 210 pg/ml. Yang's group studied the influence of various E2/oocyte ratios on reproductive outcome in women undergoing IVF using the flare-up GnRH-agonist protocol. They found that IVF cycles with an elevated E2/oocyte ratio correlated with lower pregnancy and implantation rates.

Orvieto et al. (2006) concluded that while neither E2/follicle nor E2/oocytes ratio has a role in the prediction of IVF outcome of normal-to-high responder patients undergoing the long suppressive GnRHagonist protocol, E2/oocyte ratio correlates with pregnancy rates in low responders and in patients undergoing the GnRH-antagonist.

Our study results were not comparable with the previous ones where we found a significant positive association between E2/oocyte ratio and pregnancy rates possibly due to unexpectedly very high levels of E2 compared to the number of oocytes retrieved.

Upon sub grouping these females based on E2/oocyte ratio into two categories, we detected a cutoff value of 385.42 pg/ml for categorization which was associated with success of pregnancy, our results agreed with Ozlem Ozdegirmenci et al., 2011, who reported that clinical and ongoing pregnancy rates were highest in patients with E2/follicle ratio ≥ 540 pg/ml.

Loumaya et al. analysed the ratio of E2 level to number of oocytes retrieved in patients treated with GnRH agonist and FSH. They concluded that it is a strong index for success rate of pregnancy; our results were more or less comparable to Loumaya et al.

In our opinion, it would be better to use the level of E2 on the day that the oocytes were retrieved. So we defined E2/o ratio as E2 per retrieved oocytes on the day of oocyte pickup not on the day of hCG administration as previous studies.

Mitwally et al. (2006) have investigated the association of E2 level during COH and peak E2 pregnancy outcome in 270 patients and reported the positive correlation between estradiol during COH and pregnancy outcome, but not between E2 on hCG administration day and pregnancy outcome. In their study, they used GnRH agonist and gonadotrophin. Our results partly agreed with these results with E2 measured at time of oocyte pick up.

Contrary to a recent study by ***Wu et al. (2012)*** on infertile women undergoing a short or long protocol GnRH agonist/ recombinant FSH or HMG treatment also demonstrated that high serum estradiol concentration on hCG day did not affect the IVF pregnancy outcome. E2 may disrupt the implantation process through endometrial damage which is responsible for the negative effect of E2 on IVF–ICSI outcome, there are some controversies in different studies; this is due to different stimulation protocols in comparison to our study.

Females on long agonist protocol had higher rates of pregnancy, implantation, higher levels of LH, and E2 secretion, subsequently higher number of oocytes retrieved, with the possibility of higher embryos obtained and transferred.

A study conducted by ***Rabinson*** et al***. (2008)*** also favored the agonist protocol in patients with normal body mass index (BMI) and showed no difference in the efficacy between the agonist and antagonist protocols in patients with high BMI (>40); Therefore, BMI may be an important factor in deciding the starting dose of gonadotropin and the treatment protocol, High dose gonadotropin was required in patients with a high BMI to produce good ovarian stimulation, in the present study, we proved a significant negative correlation between E2 level at time of pickup and BMI and indirectly with success of pregnancy.

Manal et al. (2018) demonstrates a significant adverse effects of the age on the number of mature oocytes (MII), in addition the number of good quality embryo decrease as the age increase while no effect of age on the other IVF parameters, our results coincided to greet extent with this study.

Furthermore, in our study, there were significant negative correlations between E2 level at time of oocyte pickup with age, and BMI, but not with FSH and duration of infertility a little bit different than these results of ***Fatemeh Foroozanfard et al. (2016)*** who reported no association.

Obesity has become a major health problem across the world. In women, it is known to cause anovulation, sub-fecundity, increased risk of fetal anomalies and miscarriage rates. However, in women going for assisted reproduction the effects of obesity on egg quality, embryo quality, clinical pregnancy, live birth rates are controversial. Reduced fecundity in obese women is probably related to multiple factors including aberrations in endocrine and metabolic functions that in turn can affect follicular growth, implantation and development of a clinical pregnancy ***(Fedorcsák et al., 2004)*.**

Several investigators have found a decrease in the number of retrieved oocytes in overweight and obese compared with normal weight women, most likely due to decreased response to control ovarian hyperstimulation with increased BMI, whereas others have found no difference.

Overall, the GnRH agonist long protocol showed better fulfillment in the purpose of controlled ovarian stimulation, which is to attain a greater number of mature follicles.

5. Conclusion and Recommendations

1- Thorough investigation of the cause of infertility as 24.5% of females had unexplained cause.

2- Laparoscopy, hysteroscopy, hystrosalpingography, should be performed whenever possible because females underwent laparoscopy had a significantly higher success rate of pregnancy.

3- The success rate of pregnancy among females in our study treated with long agonist protocol was 54.5%, subsequently it was recommended based on this result which was comparable to many studies recommending long agonist protocol as a better option for IVF compared to the antagonist and other stimulation protocols.

4- It is recommended to perform E2/O ratio for all females under long agonist protocol and to continue this protocol if this ratio > 385 with accuracy of 61.3%.

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