Comparative study of serum E2, P and E2/P ratio on ovulation induction day as a predictor of success of ICSI outcome

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Abstract: Background: Since infertility represents a major physiological and psychological problem to a growing proportion of the population, governments worldwide are investing heavily in assisted reproductive technology (ART), which has led to significant improvements in our understanding of male/female reproductive systems, gamete preservation and gamete manipulation**.** ART now accounts for 7% of all births in some developed countries. Worldwide, ~1 million ART treatments are performed each year and over 8 million ART babies have been born worldwide. **Objective:** To evaluate the effect of high serum estrogen (E2), progesterone (P4) levels and E2/P ratio on the day of human chorionic gonadotropin (hCG) administration on outcome of ICSI cycles. **Patients and Methods:** This study was conducted from June 2017 till May 2018 on 150 patients attending for IVF/ICSI programs in private IVF centers. It was a prospective observational study that included 150 patients accessed for eligibility, after examination and exclusion of patients with uterine and ovarian abnormalities only 120 patients underwent IVF as a treatment of infertility. **Results:** The results of our study analysis as regard demographic data of females; mean age, parity and BMI was (28.12, 0.5 and 24.3) respectively, and mean duration of infertility was (6±3.9) years.70% show primary infertility versus 30% had a secondary fertility, and 34% of females did previous ART. Mean of basal hormonal profile in day 2 among the studied group; FSH, LH, estrogen, PRL and TSH was (5.5±2), (4.8±2.5), (47.1±17.4), (20.2±11.2) and (2.3±0.93) respectively. **Conclusion:** We concluded that an increase in serum progesterone levels on the day of hCG administration in GnRH agonist protocol was detrimental to IVF pregnancy outcome more than P/E2 ratio by reducing clinical pregnancy. In the case of estradiol, our results showed no association was found between estradiol levels on the day of hCG administration and pregnancy achievement.
[Hanaa Mohammed Shalabi, Yehia Abdelsalam Wafa, Mona Al-Sayed El-Kafrawy, Ayaelrahman Salaheldin Mahmoud. **Comparative Study of Serum E2, P And E2/P Ratio on Ovulation Induction Day As A Predictor of Success of ICSI Outcome.** *Nat Sci* 2019;17(11):1-13]. ISSN 1545-0740 (print); ISSN 2375-7167 (online). http://www.sciencepub.net/nature. 1. doi:10.7537/marsnsj171119.01.

Keywords: Estradiol, antral follicle concentration, chronic endometritis

1. Introduction

Infertility is becoming a health challenge worldwide. A couple is considered to be infertile if conception has not occurred after 12 months of sexual activity without use contraception.

It has been documented that infertility rate in developing countries is 4%-17% [(1)]. This is catered by assisted methods of reproduction; In vitro fertilization and Intracytoplasmic Sperm Injection (ICSI).

Intracytoplasmic sperm injection (ICSI) is an advanced technique employed in assisted reproductive clinics for treatment of infertile couples. The reproductive endocrinologists try their best to identify factors that enhance success rate after ICSI.

The success of inviter fertilization (IVF-ICSI) depends on controlled ovarian hyperstimulation resulting in multi-follicular response. The follicles contain granulosa cells which secrete hormone estradiol (E2). Serum estradiol (E2) plays an important role in oocyte/follicular maturation and preparation of the uterus for implantation [(2)].

The primary aim of controlled ovarian hyperstimulation (COH) in ICSI is to produce a large cohort of mature oocytes for ICSI. Elevated secretion of ovarian steroid hormones is inevitably associated with COH. Serum estradiol (E2) levels can be increased more than 10-fold over those found during spontaneous cycles [(3)].

Changes in endometrium are regulated by ovarian steroid hormones, the increased ovarian steroid hormone secretion from COH may compromise endometrial receptivity for embryo implantation [(4)].

The effect of such supraphysiologic E2 levels on the outcome of IVF-ET has been the subject of intense debate with conflicting evidence. Some investigators have shown that supraphysiologic E2 levels have a detrimental influence on endometrial receptivity and IVF outcome [(5)].

However, others did not find high E2 levels to be a detrimental to IVF outcome [(6)]. Adverse effect of a supraphysiologic E2 levels may include alterations in both endometrial receptivity and oocyte/embryo

quality. The role of elevated serum progesterone (P) in the late follicular phase of COH is still controversial. Elevated serum P has been reported with wide clinical spectrum of ovarian response or IVF and clinical outcomes [7].

One of the major reasons for controversy has been the diverse definition of premature luteinization. Most studies considered the occurrence of premature luteinization when serum P exceeded a certain level, in a range from 0.8 to 2 ng/ml [8].

Premature elevation of serum P on or before the day of hCG administration, referred to as premature luteinization (PL), is presumably the result of an early preovulatory LH elevation and occurs in 5%-30% of controlled ovarian hyperstimulation (COH) with GnRH agonist suppression cycles [9].

Aim of the Work

The purpose of this study is to evaluate the effect of high serum estrogen (E2), progesterone (P4) levels and E2/P ratio on the day of human chorionic gonadotropin (hCG) administration on outcome of ICSI cycles.

2. Patients and Methods

This study was conducted from June 2017 till May 2018 on 150 patients attending for IVF/ICSI programs in private IVF centers.

It was a prospective observational study that included 150 patients accessed for eligibility, after examination and exclusion of patients with uterine and ovarian abnormalities only 120 patients underwent IVF as a treatment of infertility.

Inclusion criteria:

Age from 18 to 40 years. BMI less than 18-35Kg/m^2. Regular cycles (25-35 days). Basal FSH (day 2) serum level < 10 IU/mL. Basal E2 (day 2) serum level < 80 P/mL. Both ovaries present with no morphological abnormalities detected by ultrasound. Fresh IVF-ET cycle. Duration of infertility more than 1 year.

Exclusion criteria:

Abnormal uterine anatomy.

Patient evaluation:

Full history:

Personal history: age and marital life. Menstrual history: Age of menarche, Rhythm and LMP. Past history: DM, HT and operations, previous trials for IVF. Family history: Consanguinity, familial diseases as DM, HT and congenital anomalies.

Examination:

General examination and estimation of BMI. Abdominal examination. Local examination including: PV, bimanual examination and speculum examination.

Vaginal U/S. assesses the pelvic organs specially:

The uterus (any abnormalities in shape or any myomas). Ovaries; ovarian size and antral follicle count to assess ovarian reserve.

Lab investigations:

Basal FSH, LH, E2, thyroid function test and PRL.

Method:

One Hundred Twenty women undergoing intracytoplasmic sperm injection (ICSI). Down regulation by long luteal-phase protocol will be followed on all patients. Once down regulation is confirmed after measuring level of serum E2 < 50 P/ml., induction will start using gonadotropins, doses and protocol of induction depends on: age, ovarian reserve and previous medical treatment.

The ovarian response will be assessed by ultrasound and serum E2 at day 9. When at least 3 follicles reach 18 mm. in two diameters, blood sample will be obtained on the day of hCG administration (10,000-20,000 IU) for estrogen and progesterone level detection.

Ultrasound-guided transvaginal oocyte retrieval will performed 34-36 hours after hCG administration under general anasthesia. Oocytes will be evaluated by embryologist according to the following: Clarity of the cytoplasm or absence of cytoplasmic granules. Thickness of the zona pellucida. Presence or absence of polar body. Morphological assessment of polar body. Size of perivitelline space.

After insemination by intracytoplasmic sperm injection, assessment of percentage of fertilization and cleavage of the oocyte is monitored.

Embryos quality will be assessed; 24, 48 and those with 6-10 cells with no fragmentation and equal blastomere size (grade 1) or allowing for up to 20% fragmentation (grade 2) were qualified as a good-quality embryos will be transferred 72 hours after follicular aspiration (Day 3).

All patients undergoing will receive progesterone orally (Duphastone 10 mg) 2 tablets 3 times daily and progesterone vaginal pessaries (Cyclogest 400 mg) twice per day from the day of ovum pick up for luteal support.

Pregnancies will documented by measuring serum hCG 14 days after embryo transfer.

Number and quality of retrieved oocytes, number of embryo transferred, clinical pregnancy and miscarriage rates will be evaluated as a main outcomes.

Assessment:

The outcome was assessed through the following parameters: The general data, laboratory data, type of infertility. The dose of gonadotrophins used for ovarian stimulation. The number of follicles, number of oocytes retrieved, endometrial thickness and Number of fertilized oocytes. Progesterone and

estradiol on day of hCG administration. Occurrence of pregnancy, the result is not regarded as being positive except for B- hCG values exceeding 50IU/liter and after U/S detection of gestational sac 2-4 weeks thereafter.

Statistical methods:

Data were collected and coded to facilitate data manipulation and double entered into Microsoft Access and data analysis was performed using SPSS software version 18 in windows 7 (IBM, Chicago, IL, USA).

Simple descriptive analysis in the form of numbers and percentages for qualitative data, and arithmetic means as central tendency measurement, standard deviations as measure of dispersion for quantitative parametric data, and inferential statistic test:

For quantitative parametric data: In-depended **student t-Test** used to compare measures of two independent groups of quantitative data.

For quantitative non parametric data: Mann-whitney test in comparing two **independent** groups.

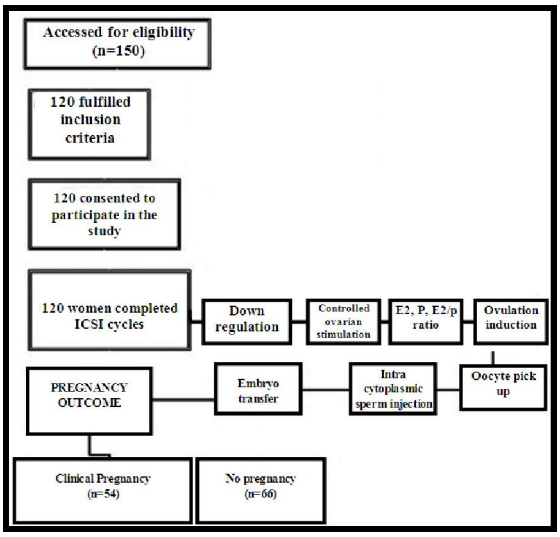

Fig. (1): Flow chart.

For qualitative data: Chi square test to compare two of more than two qualitative groups. **Bivariate Pearson correlation test** to test association between variables**. Sensitivity and specificity test with ROC curve "**Receiver Operating Characteristic" were used to find out the best cut off and validity of certain variable. Sensitivity = true +ve /true +ve, false –ve = Ability of the test to detect +ve cases. Specificity = true -ve/true-ve, false +ve = Ability of the test to exclude negative cases. PPV (positive predictive value) = true+/true+ve +false +ve = % of true +ve cases to all positive. NPV = true-/true-ve + false –ve = % Of the true –ve to all negative cases. The **P-value ≤ 0.05** was considered the cut-off value for significance, **P-value** >0.05 insignificant. **P-value** <0.05 significance. **P-value** <0.01 highly significant.

3. Results

Table (1): Description of demographic data of females in the studied group.

Variables (n=120)	**Patients**	
	Mean ±SD	
Age (years)	28.12±5.9	
Parity	0.5±0.8	
BMI (kg/m^2)	24.3±5.6	
Duration of infertility	**Mean ±SD**	
(years)	6±3.9	
Duration of each type of infertility (years)	**Mean ±SD**	
Primary	5.9±4.4	
Secondary	6.2±2.8	
Types of infertility	**No.**	**%**
Primary	84	70%
Secondary	36	30%
Previous ART		
No	79	66%
Yes	41	34%

Table (2): Description of basal hormonal profile in day 2 among the studied group.

Variables	**Range**	**Mean±SD**
Base line assessment		
FSH (IU/mL)	1.8-9.6	5.5±2
LH (IU/mL)	0.9-12.6	4.8±2.5
E2 (P/mL)	16-88	47.1±17.4
PRL (ng/mL)	0.50-61	20.2±11.2
TSH (uIU/mL)	1.01-4.74	2.3±0.93

Table (3): Description of husband demographic data among the studied group.

Variables (n=120)	**Husband**	
	Mean ± SD	
Age (years)	35.2±6.6	
Special habits (smoking)	**No.**	**%**
No	72	60%
Yes	48	40%
Previous ART		
No	72	60%
Yes	48	40%
Seminal analysis		
Normal	45	37.5%
OTA*	54	45%
AZO*	21	17.5%
Previous operation		
No	84	70%
Yes	36	30%

*OTA: oligozospermia, teratozospermia, asthenospermia; AZO: Azospermia

Table (4): Different causes of infertility among the studied group.

Positive finding	**Number** (n=120)	**%**
Tubal Factor		
Hydrosalpinx	30	25%
Salpingectomy and drainage	13	43.3%
Disconnection salpingectomy	17	56.7%
Ovarian Factor		
Anovulation	18	15%
Other investigations		
Abnormal HSG	54	45%
Do Laparoscopy	38	31.6%
Do Tuboplasty & adhysolysis	28	23.4%
Surgical History		
Positive surgical history	54	45%
Appendectomy	10	8%
Cholecystectomy	9	7.5%
Ovarian cystectomy	5	4.2%
Cesarean section	30	25%
Obstetric complication	24	20%
Unexplained	7	6%
Mixed causes	19	16%

Table (5): Incidence of pregnancy among studied group.

Pregnancy	**Number** (n=120)	**%**
No	66	55%
Yes	54	45%

Table (6): Comparison between the studied groups (pregnant and non pregnant) as regard demographic data.

Variables	**No pregnancy** (n=66)	**Pregnancy** (n=54)	**p-value**	**Sig.**
Demographic data				
Age (years)	28.1±7.1	28.1±4.5	0.9	NS
BMI (kg/m^2)	24.8±5.5	23.7±5.6	0.2	NS
Types of infertility				
1ry	42 (63.6%)	41 (76%)	0.2	NS
2ry	24 (36.4%)	13 (24%)		
Duration of infertility				
Years	4.2±1	4.2±2	1	NS

Table (7): Comparison between the studied groups (pregnant and non pregnant) as regard basal hormonal profile.

Variables	**No pregnancy (n=66)**	**Pregnancy (n=54)**	**p-value**	**Sig.**
Base line assessment				
FSH (IU/mL)	5.9±2.1	**4.9**±1.8	**0.001**	**HS**
LH (IU/mL)	5.6±2.8	**3.9**±1.7	**<0.001**	**HS**
E2 (pg/mL)	42.4±17.7	**51.9**±15.8	**0.001**	**HS**
PRL (ng/mL)	17.9±5.9	**22.5**±14.4	**0.01**	**S**
TSH (uIU/mL)	2.16±0.85	2.4±1	0.1	NS
Before trigger time				
E2-day9(pg/mL)	2219.1±1450.6	2284.8±1611.9	0.8	NS
E2 (pg/mL)	4965.8±4530	4270.7±2550.4	0.2	NS
P4 (ng/ml)	1.9±0.83	**1.38**±0.62	**<0.001**	**HS**
P/E2 ratio	0.593±0.41	**0.459**±0.39	**0.01**	**S**
Others				
Dose of gonadotropin (IU)	435±129.9	**393**±100.8	**0.02**	**S**
Endometrial thickness (mm.)	11±4	**10**±5	**1**	NS

Table (8): Sensitivity, specificity, positive and negative predictive values of E2, P4, P/E2 ratio and AFC as regard pregnancy rate.

Variable	Sensitivity	Specificity	+ve Predictive value	-ve Predictive value	Accuracy
E2 before trigger (pg/mL)	72%	36%	57.9%	51.2%	50.9%
P4(ng/mL)	68%	52%	68.7%	75.2%	70.5%
P/E2 ratio	76%	56%	67.5%	67%	63.1%
AFC/overy	80%	38%	61.2%	60.9%	53.9%

Table (9): Cutoff points of E2, P4, P/E2 ratio and AFC as regard pregnancy rate.

Variable	Cutoff point
E2 before trigger (pg/mL)	2557.5
P4(ng/mL)	1.7
P/E2 ratio	0.458
AFC/overy	5.5

Table (10): Comparison between the studied groups as regard AFC/ovary, number of withdrawn and fertilized oocytes and oocyte quality.

Variables		Non pregnant (n=66)	Pregnant (n=54)	p-value	Sig.
		Mean ±SE	Mean ±SE		
AFC/ovary		7.6±3.4	7.9±2.6	0.5	NS
Number of withdrawn oocytes		8.2±0.7	11.4±0.8	**0.003**	**HS**
Number of fertilized oocyte		5.5±0.5	7.3±0.6	**0.02**	**S**
Integrity of cytoplasm	Normal	4±0.6	7.3±0.6	**<0.001**	**HS**
	Abnormal	3.4±0.3	3.7±0.4	0.6	NS
Thickness of ZP	Normal	4±0.5	10±1.3	**<0.001**	**HS**
	Abnormal	3.3±0.5	2.3±0.4	0.1	NS
Polar body	Present	5.9±0.6	8±0.6	**0.01**	**S**
	Absent	1.6±0.1	1.9±0.5	0.5	NS
Morphology of PB	Normal	4.8±0.5	7±0.5	**0.004**	**S**
	Abnormal	2.3±0.3	3.1±0.5	0.1	NS
Size perivitalline space	Regular	5.3±0.6	8.7±0.7	**<0.001**	**HS**
	Irregular	1.9±0.3	1.3±0.4	0.2	NS

4. Discussion

Progesterone (P) and estradiol (E_2) are required for successful conception, both to prepare the endometrium for blastocyst implantation and pregnancy. During IVF cycles, the endometrium and embryo are exposed to supra-physiological concentrations of estradiol and progesterone during ovarian stimulation, which could influence pregnancy outcomes [(10)]. E_2 initiates hypertrophy and hyperplasia of endometrial epithelia, but its role in the luteal phase remains poorly understood. How E_2 influences endometrial synchronization and blastocyst implantation is also not well described [(11)]. Progesterone transforms the E_2-prepared endometrium into a secretory tissue and creates a hospitable environment for embryo attachment. The effects of elevated progesterone and oestradiol on the day of hCG administration on pregnancy outcomes is a controversial topic. However, the researches on these effects are scarce. Previous studies on the relationship between sex hormones and pregnancy outcomes are limited to elevated progesterone or oestradiol concentrations separately, not in combination. Some studies have mentioned that elevated progesterone concentrations often following elevated oestradiol concentrations [(12)].

The purpose of this study was to evaluate the relation between estradiol (E2) & progesterone (P4) levels on the day of human chorionic gonadotrophin (hCG) administration and pregnancy rate in ICSI cycle.

This study was conducted from June 2017 till May 2018 on 120 patients attending for IVF/ICSI programmes.

This study demonstrated the demographic data of females in the study as regard age, parity and BMI

(28.12±5.9) (0.5±0.8) and (24.3±5.6) respectively, and the mean duration of infertility was (6±3.9) years.

As regard type of infertility, 70% of study group showed primary infertility versus 30% had a secondary infertility, and 34% of females did previous ART as shown in Table (3).

However, a study of ***Yan et al.*** [13] showed that a total of 10864 patients underwent day 3 ET in ICSI cycles, the mean age and BMI of these patients were (31.3±5.1) and (22±3) respectively, and mean duration of infertility was (3±4) years.

Also, in previous mentioned study, 49.2% of patients had primary infertility versus 50.8% had a secondary infertility, 15.7% of them did previous ART.

Basal FSH concentration measured prior to the treatment cycle is widely used in many IVF programmes. A level exceeding 25mIU/ml (12mIU/ml using current assay) has been correlated with a very low chance of pregnancy [14].

In the present study, the mean of basal hormonal profile in day 2 among the studied group as regard FSH, LH, estrogen, PRL and TSH was (5.5±2), (4.8±2.5), (47.1±17.4), (20.2±11.2) and (2.3±0.93) respectively as shown in Table (4).

And the value of measuring serum LH in routine fertility and endocrinological conditions remain to be confirmed by further studies [15].

These agree with ***Toftager et al.*** [16] study showed that the median basal FSH, basal LH and day 3 estradiol were (7.4±3.4), (6.3±4.7) and (0.19±0.44) respectively.

Also increased day 3 estradiol has been associated with both diminished ovarian reserve and enhanced ovarian reserve (PCO), interestingly, cancellation is increased with either low (<20 pg/ml) or high (>80 pg/ml) estradiol levels [17], but these levels did not predict pregnancy rate in those not cancelled but the combined FSH and estradiol in screening for diminished ovarian reserve appears to be more sensitive than either test alone [18].

Male factor is assumed to be responsible in about 50% of the infertile couples. Not only defects in hormone production, testicular structure, ejaculation and/or the spermatozoa themselves can adversely affect the chances of conception, but also genetic defects can affect the fertility [19].

According to husband demographic data results showed that the mean age of the husbands was (35.2± 6.6) years old, 40% of husbands were smokers, 40% of them did previous ART, 30% had a past history of operation (varicocele, hydrocele, inguinal hernia) and for seminal analysis 45% of samples show OTA (oligozospermia, teratozospermia, asthenospermia) and 17.5% show AZO (Azospermia) while 37.5% had normal seminal analysis as shown in Table (5).

This goes in agreement with ***Lund and Larsen*** [20] who said that the most common identifiable cause of male subfertility is a varicocele, which can be associated with infertility. The term "subclinical varicocele" refers to a lesion too small to be detected by physical examination. Over the past decade, however, several studies have established an association between the presence of varicocele and abnormal semen parameters in infertile patients.

Also, lifestyle and environmental factors, including smoking, can affect gamete, leading to subfertility/infertility [21].

As regard different causes of infertility among the studied group, results showed that the most common cause was abnormal HSG and patients with past history of pelvic operations, patients with hydrosalpinx and unexplained (45%, 45%, 25% and 6%) as shown in table (6) respectively.

These agree with results in ***Yan et al.*** [13] study found that the most common cause of infertility was tubal pathology, male factor, advanced age, PCOS and endometriosis (44.5%, 27.5%, 11.1%, 6.25% and 3.5) respectively.

These findings disagree with ***Lai et al.*** [22] study, in which the most common cause of infertility was mixed causes, male factor, tubal factor, ovarian dysfunction, unexplained and endometriosis (59.7%, 16.5%, 7.2%, 7.2%, 5.8% and 3.6%) respectively. This difference could be due to larger patient sample 583, different exclusion criteria of patient group as follow: infertility due to endocrine abnormalities, such as hyperprolactinemia, embryo transferred numbers > 4 and previous COH with documented poor response.

Regarding the incidence of pregnancy in this study, 54 women (45%) became pregnant and 66 women (55%) were not pregnant as shown in table (7).

This incidence goes in agreement with the results of the study ***(ESHRE, 2016)*** which found that the implantation and pregnancy rates for fresh ET was 45.3% (95% CI, 42.7–47.9), odds ratio (OR) 1.31 (95% CI, 1.13–1.51).

Also, study of ***Yan et al.*** [13] found that clinical pregnancy rates (CPRs) in fresh day 3 ET cycles was 48% versus 52% failure rate.

This is on contrary to recent retrospective cohort study ***Keefe et al.*** [23] who found that in fresh IVF cycles, clinical pregnancy rate was 40.8% versus 59.2% failure rate. This difference could be due to larger sample of study 923 patients, different study design and different inclusion criteria.

In this study, the difference was statistically non-significant between the studied groups as regard age and BMI. Mean of both were higher in non-pregnant group than pregnant group; the mean age in pregnant group was (28.1±4.5 yrs.) compared to (28.1 ±7.1

yrs.) in non pregnant group, the mean BMI in pregnant group was (23.7±5.6 Kg/m^2) compared to (24.8±5.5Kg/m^2) in non-pregnant group as shown in Table (8).

This goes in agreement with the major findings in the study of ***Barbara et al.*** [24] on ART patient population of over 45 000 embryo transfers included that failure to achieve a clinical intrauterine gestation increased significantly with advancing age and increasing BMI. Also it goes in agreement with other studies showing a progressive decline in pregnancy rates with rising obesity [25].

Previous pregnancy had a significantly positive impact on the chance of success with IVF with the effect being stronger for pregnancies resulting in a live birth. This positive association with previous live birth was even stronger if it had followed IVF pregnancy [26].

While duration of infertility has been shown to be associated with the chance of spontaneous pregnancy, its impact on the chance of success with IVF treatment has been less clear, were able to show in their analysis of factors affecting outcomes in IVF that there was a significant decrease in age adjusted live-birth rates with increasing duration of infertility [27].

In this study, results also showed that the pregnancy rate in primary infertility was 76% & in secondary infertility was 24% and according to duration of infertility who became pregnant was (4.2±2 yrs.) compared to (4.2±1 yrs.) in non pregnant. But no statistically significant difference between both groups as regard type and duration of infertility as shown in Table (8).

As regard basal hormonal profile, the results showed that there was statistically highly significant difference as regard FSH, LH and E2.

FSH in pregnant group was (4.9±1.8) compared to (5.9±2.1) in non-pregnant group as shown in Table (9).

This agrees with ***Broekmans et al.*** [28] study in which a systematic review of tests predicting IVF outcome had shown that the measurement of basal FSH in regularly cycling women is accurate in the prediction of non-pregnancy only at very high threshold levels.

However, this is on contrary to a meta- analysis of ***Bancsi et al.*** [29] which showed that the performance of basal FSH concentration for predicting poor response was moderate and the performance for predicting no pregnancy was poor.

Also, ***van Montfrans et al.*** [30] study demonstrated that screening for elevated FSH concentrations was of no additional value in the prediction of fecundity in a general subfertility population with ovulatory menstrual cycles.

In the present study, LH in pregnant group was (3.9±1.7) compared to (5.6±2.8) in non-pregnant group as shown in Table (9).

Some publications have found no effect and have questioned the value of measuring LH [15,31].

On contrary to the results of this current study, studies have shown that low LH concentrations were associated with negative treatment outcomes [32,33].

Day-3 E2 in pregnant group was (51.9±15.8) & in non pregnant group (42.4±17.7), the difference was statistically highly significant as shown in Table (9).

This goes in agreement with ***Westergaard et al.*** [34] study which reported that mid-follicular phase levels of circulating E2 and LH is of significance for the outcome of assisted reproductive treatment (ART) after long GnRH agonist protocol and FSH stimulation.

Also, ***Ranieri et al.*** [18] reported that increased day 3 estradiol has been associated with both diminished ovarian reserve and enhanced ovarian reserve (PCO). This makes interpretation of this test problematic without further information. The combined FSH and estradiol in screening for diminished ovarian reserve appears to be more sensitive than either test alone.

E2 at the day of hCG administration in pregnant group was (4270.7±2550.4) & in non-pregnant group was (4965.8±4530). There is no statistically significant difference between both groups in relation to the estradiol concentrations on the day of hCG administration as shown in Table (9).

In this study, cutoff point of E2 was (2557.5) with sensitivity (72%), specificity (36%), positive predictive value (57.9%) and negative predictive value (51.2%) as shown in Table (10, 11).

So, the results showed no association between estradiol levels and pregnancy achievement, This is in agreement with ***Yu et al.*** [35] and ***Kyrou et al.*** [36] who used 25th and 75th percentiles to divide the patients into three groups according to oestradiol concentrations on the day of hCG administration (<1142, 1142–2446,>2446 pg/ml). Their results showed that in patients with oestradiol concentrations higher than the 75th percentile (oestradiol concentration >2446 pg/ml), the pregnancy rates remained the same as compared with the medium and lower percentile group, although the embryo quality was better than the two other groups.

Also, the systematic review by ***Kosmas et al.*** [5] had shown that E_2 levels do not affect treatment outcome in GnRH agonist down-regulated IVF/ICSI cycles.

And other results showed that there was still no consensus concerning any adverse role of elevated peri-implantation E2 levels on IVF outcome. However, that there was a threshold peak E2 level

above which pregnancy and implantation rates were decreased, but this threshold was likely to be 5,000 pg/mL from the results and other publications [37].

Also, five out of nine studies, including 1875 patients (55.9%), did not support the presence of an association between estradiol on the day of hCG administration and pregnancy achievement [11].

However, this disagrees with ***Makkar et al.*** [38] who showed that a high serum E2 level had a negative effect on endometrium may account for the lower implantation and pregnancy rates. This difference due to different investigations as uterine flushings and endometrial biopsies were collected after hCG injection in stimulated cycles, also the study showed cutoff value of E2 at 2000 pmol/L.

The results of the study by ***Chen et al.*** [39] support that increasing E_2 levels on the day of hCG administration is associated with improved pregnancy rates when embryo transfer is performed on Day 5.

Regarding progesterone concentrations, the data showed that an increase in progesterone on the day of hCG administration impairs pregnancy rate. As results in the current study showed that progesterone in pregnant group was (1.38±0.62) & in non pregnant group was (1.9±0.83) that pregnant cases had a lower level of progesterone compared to non pregnant group with highly significant difference as shown in Table (9), and as regard P4, the cutoff point was (1.7) with sensitivity (68%), specificity (52%), positive predictive value (68.7%) and negative predictive value (75.2%) Tables (10, 11).

This is in agreement with a study by ***Cui et al.*** [40] who reported that increase in progesterone on the day of hCG administration impairs pregnancy, implantation and live birth rates.

Also, 1045 GnRH agonist cycles by ***Kilic Dag et al.*** [41], in which 251 infertile patients undergoing IVF/embryo transfer with the uniform GnRH agonist down-regulation and stimulation were prospectively studied. All the cycles were grouped according to serum progesterone concentration on the day of hCG administration. The pregnancy rate was significantly lower (25.9 versus 48.75%; $P < 0.001$) in the elevated progesterone group.

The mechanism by which increases in serum progesterone may impact on pregnancy rates is unclear, with data suggesting that elevated progesterone levels may impair endometrial receptivity rather than oocyte quality [42].

Also another study showed that although serum P elevation on the day of hCG was inversely associated with the probability of pregnancy, the numbers of total oocytes and mature oocytes retrieved were higher in the elevated P group. Moreover, it did not appear to have a negative effect on oocyte performance in terms of fertilization, cleavage rates, and ongoing PRs in FET- cycles regardless of different ovarian responses [43].

Papanikolaou et al. [44] analyzed 628 infertile patients. Progesterone increase on the day of hCG administration impaired pregnancy outcome in day-3 single-embryo transfers, while it had no effect on day-5 single blastocyst transfer. It was thought that the extreme progesterone concentration affected the embryo-endometrium cross-dialogue.

However, this disagree ***Venetis et al.*** [45] in his study, in which a meta-analysis suggested that the increase in circulating progesterone levels did not correlate with cycle outcome in terms of pregnancy rate, this is due to correlation between progesterone and term pregnancy, while in this current study the correlation between progesterone and pregnancy rate.

Also, ***Melo et al.*** [46] reported that progesterone elevation had no influence on fertilization and embryo quality, but this study used a higher number of patients 240 patients and lower cutoff point of progesterone 1.2 ng/ml.

In women undergoing IVF-ET, a positive correlation has been found between late follicular serum P and E2 levels.

P/E2 ratio was calculated by P (in nanograms per milliliter) ×1000/E2 (in picograms per milliliter).

From the study results, P/E2 ratio was with low mean in pregnant group compared to non pregnant group as (0.459±0.39) compared to (0.593±0.41) respectively and the difference was statistically significant as shown in table (9).

In this study, we first conducted a ROC analysis to search the most efficient cutoff value for P/E2 ratio to pregnancy rate in patients with IVF-ET cycles.

The cutoff point of P/E2 ratio was (0.458) with sensitivity (76%), specificity (56%), positive predictive value (67.5%) and negative predictive value (67%) table (10, 11).

This goes in agreement with ***Elgindy*** [47] study performed on a total of 240 women undergoing long agonist protocol with at least four grade I day 3 embryos, women were randomized in a 1:1 ratio to undergo day 3 or day 5 embryo transfer. Using ROC, cutoffs for P and P/E2 ratio were 1.5 ng/mL and 0.55, respectively. Patients with $P \leq 1.5$ ng/mL and $P/E2 \leq 0.55$ undergoing cleavage-stage ET had higher clinical pregnancy rate (CPR). Using multiple regression, P/E2 ratio was the only independent predictor for pregnancy. The P and P/E2 cutoffs were not correlated with CPR in blastocyst transfers.

This disagrees with the cutoff point determined by ***Rafael Levi et al.*** [48] who studied 248 patients who had undergone ART for infertility treatment between 2001 and 2002. The patients were separated into two groups according to P/E2 ratios on hCG administration day. Group A consisted of the patients

whose P/E2 ratio was 1 (n = 116) and Group B consisted of the patients with PL of which P/E2 ratio was > 1 (n =132). Although the difference between the fertilization rates in Group A and Group B was not statistically significant (P > 0.05), the clinical pregnancy rates seemed to be affected adversely in the Group B patients with premature luteinization (41.4%*versus* 28%, respectively; P < 0.05).

Younis et al. [49] definition of P/E2 >1 on the day of hCG administration as PL needs more reliable assessment.

In this current study, results showed no statistically significance of measuring E2/P ratio.

As regard dosage of gonadotropins used for ovulation induction, mean was (393±100.8) in pregnant groups versus (435±129.9) in nonpregnant group as shown in table (9).

This is in agree with retrospective study analyzed more than more than 650,000 assisted reproductive technology cycles, ***Barbara et al.*** [50] analyzed total of 658,519 fresh autologous cycles of in vitro fertilization (IVF) reported to the Society for Assisted Reproductive Technology from 2004 to 2012, the results of this study showed live birth rate significantly decreased with increasing FSH dose, regardless of the number of oocytes retrieved. The statistically significant decrease in live birth rate with increasing FSH dose remained in patients with good prognosis, and regardless of female age, except for women aged ≥35 years with 1–5 oocytes retrieved.

Endometrial thickness in pregnant group was (10±5) & in non pregnant group was (11±4), there was no statistically significant difference between both groups as shown in Table (9).

Some authors did not show a significant correlation between endometrial thickness and pregnancy rates in IVF patients [51].

While ***Mazdak et al.*** [52] demonstrated endometrial thickness is significantly higher in pregnant women compared to non-pregnant. As this study used a large number of IVF cycles included in 14 studies. The meta-analysis with a random effects model was performed using comprehensive meta-analysis software. They calculated the standardized mean difference, odds ratio (OR), and 95% CIs.

This study also reported that AFC cutoff value was 5.5 table (11) with sensitivity (80%), specificity (38%), positive predictive value (61.2%) and negative predictive value (60.9%). Sensitivity and specificity test for AFC will illustrate probability of being true positive is (53.9%) more than being false positive when repeat test 100 times as shown in Table (10).

And AFC in the pregnant group & in non-pregnant group was (7.9±2.6) & (7.6±3.4) respectively, but the difference was statistically non significant Table (12).

Hansen et al. [53] showed that AFC did not change after pituitary down-regulation.

In accordance with ***Huang et al.*** [54] they demonstrated that AFC determined on day 6 or 7 after gonadotrophin stimulation was predictive of the ovarian response. Similarly, the combination of AFC on day 3 and day 7 had high positive and negative predictive values respectively of ovarian response during IVF treatment [55].

Low number of ovarian antral follicles (<10 total follicles with a diameter between 2 and 10 mm in both ovaries) indicates reduced ovarian reserve and diminished chance for pregnancy after ART [56].

Also in this study, retrieved oocyte in pregnant group was (12.6±6.1) compared to (7.9±5.4) in non pregnant group, fertilized oocyte in pregnant group was (8.5±4.7) compared to in (5.5±4.1) non pregnant group, there was high statistically significant difference between both groups as shown in Table (12).

This agrees with ***Kably et al.*** [57] who demonstrated that pregnancy rates increased when more oocytes were retrieved. This is due to the fact that increase total number of oocyte retrieved leads to increase number of embryos developed which gives more chances in selecting the best embryos to be transferred.

However, this disagrees with ***Gleicher et al.*** [58] study who found no association between total numbers of follicles with high pregnancy outcome. Also, ***Hendriks et al.*** [59] in their meta-analysis found that the total oocyte number is clearly poor for predicting pregnancy. They believed that this test merely represents the quantitative aspect of ovarian reserve and the occurrence of pregnancy in IVF is largely dependent on oocyte quality.

And as regard other factors determining the oocyte maturity, results of this study showed that there was statistical significance difference between pregnancy outcome group as regard number of oocytes with normal integrity of cytoplasm, normal zona pellucida (ZP) thickness, number of oocytes with polar body (PB), normal PB morphology, and regular perivitalline space (PVS) size with high mean among pregnant group (11.4±0.8) (7.3±0.6) (7.3±0.6) (10±1.3) (8±0.6) (7±0.5) and (8.7±0.7) respectively.

It is generally recognized that a normal human metaphase II (MII) oocyte should have a round, clear ZP, a small PVS containing a single, not fragmented first polar body (IPB) and a pale moderately granular cytoplasm with no inclusions [60].

This goes in agreement with some authors who have reported a correlation between oocyte morphology and embryo developmental potential, regarding the cumulative effect of multiple morphological features, ***Xia*** [61] showed that oocyte

grading based on IPB morphology, size of PVS, and cytoplasmic inclusions was correlated with its developmental potential after ICSI.

And this is in contrary with some authors who had suggested that all oocytes could be fertilized by ICSI independently from their morphological appearance [62, 63]. These studies included larger sample of couples and with normal semen characteristics.

Furthermore, no impact on embryo quality had been associated with oocyte morphology. Similar clinical pregnancy and implantation rates were also obtained after transferring embryos derived from abnormal oocytes compared with those obtained with embryos derived from normal appearing oocytes ***(La Sala et al., 2009).*** These findings observed irrespective of patient's age and included large patient's sample.

Conclusion

According to our study we found that an increase in serum progesterone levels on the day of hCG administration in GnRH agonist protocol was detrimental to IVF pregnancy outcome more than P/E2 ratio by reducing clinical pregnancy. In the case of estradiol, our results showed no association was found between estradiol levels on the day of hCG administration and pregnancy achievement.

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