Evaluating Endometrial Thickness at Antagonist Starting Day as an Adjuvant Criterion to Decide Gnrh Initiation in Flexible Antagonist Protocols

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Abstract

Background Currently, GnRH antagonist protocols may be most prevalent protocols designed to prevent the luteinizing hormone surge in ICSI cycles, the flexible start protocols with more individualized timing of initiating the inhibition were mostly used, however; different criteria were proposed to determine the optimal time of GnRH antagonist administration, mostly leading follicular size was depended as criterion to start the inhibition. However; Leading follicular size, serum estradiol level, serum LH level was used in this study to initiate antagonist administration, and will correlate the endometrial thickness and pattern to the other optimal start criteria for antagonist administration and this is attributed for being noninvasive and easy method of assessment via transvaginal ultrasound, Objectives: Evaluate the optimal endometrial thickness at time of initiating GnRH antagonist in different groups of patients treated with ICSI and to correlate endometrial thickness at antagonist starting day and endometrial pattern with pregnancy rates. Materials and Methods: This prospective comparative observational study was made on one hundred twenty women who were experiencing ICSI flexible antagonist protocol at the High Institute for Infertility Diagnosis and Assisted Reproductive Technologies/Al-Nahrain University from October 2020 to April 2022, Cycles included were fresh transfer cycles and frozen cycles excluded. Those patients were randomized in to three groups according to the criterion they fulfilled to initiate the antagonist. Group A: 40 patients where the antagonist was started when the leading follicle size was (12-14). Group B: 40 patients where the antagonist was started when the serum E2 was exceeding 500pg/ml and group C: 40 patients where the antagonist was started when the serum LH level > 5 IU/l. vaginal ultrasound was used to assess the endometrial thickness and pattern in the three different groups during controlled ovarian hyper stimulation. Data were analyzed by (SPSS) version 26.0 using $\chi 2$, t-test, ANOVA, Fisher exact tests. "ROC curve" was also used to find the best cutoff value for continuous variables predicting pregnancy. Results: There was significant difference in endometrial thickness at antagonist day among study groups with (p= 0.016) and lowest endometrial thickness value being in the A group. There was no significant difference among the three study groups with regard to the endometrial thickness (mm) on the day of ovulation trigger (p= 0.060) with higher thickness was in the B group compared to other two groups. A highly significant difference (p= 0.001) regarding the presence of triple layer pattern at antagonist starting day was seen, 27(50%) of patients in the A group have no triple layer pattern, while higher percentage of patients 29(43.9%) have triple layer pattern in group B compared to other two groups. The difference was not significant in basal endometrial thickness between pregnant and non-pregnant groups, however, there was significant difference at antagonist day (p= 0.003) and highly significant difference was on trigger day (P= <0.001). Triple layer pattern was significantly higher in pregnant group. The best cutoff value of endometrial thickness at day of antagonist in predicting pregnancy was >6.45 Conclusion: The endometrial thickness at day of antagonist correlates significantly with pregnancy rates and it can be taken as criteria to initiate GnRH antagonist with a sensitivity level of 78 % and a specificity level 57%. Triple layer pattern was higher in pregnant group, but however, without statistical difference.

Keywords: Flexible GnRH antagonist protocol, criteria of antagonist initiation, endometrial thickness, triple line endometrial pattern.

1. Introduction

The GnRH antagonist protocol has been broadly used in ICSI cycles and have several advantages when related with the GnRH agonist long protocol, involving, short duration of treatment, lower dose of gonadotropin, with no disproportionate pituitary suppression and flare-up side effects, and a decrease the occurrence of severe ovarian hyper stimulation syndrome (OHSS) (Lambalk et al, 2017).Despite the comparable live birth rate between both protocols, a lower pregnancy rate with the GnRH-ant protocol was reported (Xu et al., 2017).

as the main reason for This difference in pregnancy rate may be related to the hostile effects of GnRH-ant on endometrial receptivity as postulated by many studies (Xu et al, 2017, Xu et al., 2018). Furthermore, studies also revealed the dose-related detrimental effects of GnRH-ant on endometrial receptivity (Zhang et al., 2018). (Dieamant etal., 2019) stated that low pregnancy rates in antagonist cycles may be due to the detrimental effect of antagonist on endometrial receptivity rather than embryo quality.

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A study by (Huang etal, 2018) suggested the adverse influence of GnRH inhibition on the endometrium may be investigated by several histological, gene expression analysis, and endometrial transcriptome study.

Since, ultrasound (US) is important in medical fields, and this is attributed for being noninvasive, fast, and painless method. Endometrial thickness, endometrial pattern, endometrial volume and Doppler study of uterine arteries are the main parameters of endometrial receptivity that revealed by ultrasound (Murtinger and Schuff, 2019). Endometrial thickness and pattern have been widely accepted as prognostic indicators for endometrial receptivity (Laurel and Ilan, 2019).

Many studies show that an endometrial thickness of >7 mm and triple line development is a prognostic factor for pregnancy. Thin endometrium commonly measuring <7 mm, are assumed to be less competent to support implantation and pregnancy and more than a few reports have identified small pregnancy rates in existence of a thin endometrium (Mahajan N & Sharma S, 2016). Furthermore, the endometrial thickness is reliant on a number of influences comprising phase of menstrual cycle, reproductive age, concentration of ovarian hormone estrogen and progesterone, and endometrial hormone receptor density, the thickened endometrium provides a spot for attachment, and it is the source of nourishment for an implanting embryo for the period of its first few weeks until development of placenta but, thin endometrium which fails to respond to hormones leads to early miscarriages and implantation failure due to lack of blood supply (Baradwan S et al.,2018).“Endometrial pattern can be defined as the comparative echogenicity of the endometrium and the neighboring myometrium as imagined on a longitudinal scan of ultrasound. “Triple line” morphology reached throughout the proliferative phase of the menstrual cycle (Figure1). However, the central echogenic line signifies the uterine cavity; the outer lines resemble to the endometrial basal layer, or the interface concerning the endometrium and myometrium. The hypo-echogenic regions concerning the two outer lines and central line may denote the functional endometrial layer (Hamdi et al., 2018). “This image is attributed to the glandular disposition, reduced secretion and scarce stromal edema” (Luis et al., 2012).

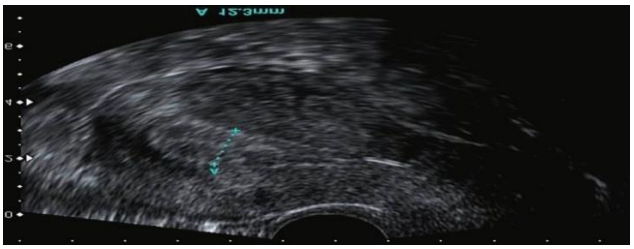


Figure1: “triple line” morphology, (Pillai et al., 2018), (Ultrasound in Reproductive Healthcare Practice).

The triple -layer ultrasound pattern reveals normal follicular/proliferative subtleties. In the pre-hCG phase, its presence was reported to predict a better outcome, but not constantly. clinical pregnancy rates were Significantly higher in patients with triple-layer pattern on the day of hCG injection among IVF cohorts (Hershko-Klement and Tepper, 2016).

It has been found that the structure of triple line is correlated with a receptive endometrium and to be more predictive marker of implantation than other parameters (AboAlyazid et al., 2018). The lining of endometrium; however, develops into this classic pattern that is identifiable through transvaginal ultrasonography in reaction to the estradiol from ovaries as was stated by (Zhang et al., 2018). Furthermore, the triple echo disappears after ovulation and the endometrium appears more homogenous and echo-bright, as it continues to thicken due to secretory glandular proliferation was suggested by (Pillai et al., 2018).

An endometrial pattern of low grade, characterized by an entirely homogeneous, hyper-echogenic pattern without a central echogenic line, was frequently associated with non-conception cycles, although “its presence did not altogether preclude the chance of implantation observed a higher prevalence of poor endometrial grade in women aged >40 years than in younger ones, as well as in patients with uterine pathology in contrast to women with healthy uteri, these cases may represent a group of women with a reduced potential for endometrial proliferation” (Behnoud et al.,2019).

2. Materials and Methods

One hundred twenty infertile women enrolled in this prospective comparative study, who were subjected to ICSI flexible antagonist protocol in the “Infertility Center of High Institute for Infertility Diagnosis and Assisted Reproductive Technologies Al-Nahrain University”, during the period from November 2020 until April 2022. The morphological valuation of the oocytes aspirated, and their embryos resulted was done in the ICSI laboratory of the Institute. Cycles included were fresh transfer cycles and excluded if they were transferred to freeze all or if they were designed to be frozen cycles from the beginning. Those patients were randomized in to three groups according to the criterion they fulfilled to initiate the antagonist:

Group A: 40 patients where the antagonist was started when the leading follicle size was (12-14).

Group B: 40 patients where the antagonist was started when the serum E2 was 500-600 pg/ml.

Group C: 40 patients where the antagonist was started when the serum LH level > 4 IU/l. Primary and also, secondary types of infertility were included. Some of the women enrolled were complaining of PCOS, tubal factor while other couples had male cause of infertility or combined reasons, also couples with unexplained cause of infertility participated in this study, aged 18-40 years old. Early follicular phase FSH < 10 mIU/ml, and Estradiol < 60 pg/ml and LH 10< IU/l while body mass index 18-30 kg/m2, only grade 1 (G1) embryo was transferred. All

those patients subjected to vaginal ultrasound assessment to exclude ovarian cyst or any pathology, with assessing antral follicle count, follow ovarian response to stimulation and assessing endometrial thickness and pattern. As well basal assessment of serum LH, FSH, E2, day of GnRH-antagonist initiation and day of HCG trigger assessment of serum E2 was done. Trans vaginal ultrasonic fallow up done starting from the fourth day of stimulation daily to assess the follicular size, number, endometrial thickness and pattern till the day of staring the antagonist inhibition and then it was done accordingly to assess the ovarian response to the stimulation that is done till the day of HCG trigger with assessment of endometrial thickness and pattern. For male partners, the seminal fluid analysis was assessed according to WHO 2010. Controlled ovarian hyper-stimulation using recombinant follicular stimulating hormone (r-FSH 75 IU FSH activity/ampule) regular fixed daily subcutaneous injection with doses depended on the BMI, age of women, previous response to ovulation induction and AFC. Pituitary inhibition was performed with a flexible protocol of GnRH antagonist when the criterion that was set according to their group was met. Briefly, 0.25 mg cetrorelix acetate (Cetrotide®, Merck Serono, Geneva, Switzerland) daily subcutaneous injection according to the multiple-dose regime was given. based on the patient's characteristics, follicular development, and provided that LH levels not exceeding 10 IU/l, HMG (Menogone) 75IU (Ferring, GmbH/ Germany) was administered when the follicles growth was slow or asynchronized and the hormone measurements performed from the day of GnRH-antagonist supplementation. final oocyte maturation and triggering was performed with recombinant Hcg with two ampoules Ovitrelle® 250µg subcutaneous injection (Ovitrelle®, Merck Serono) as soon as at minimum three leading follicles reach the size 17-18, followed by ovum pick-up 35-36 hours later. The morphological assessment of the oocytes aspirated, and their resulting embryos was done in the ICSI laboratory of the "High Institute for Infertility Diagnosis and Assisted Reproductive Technologies", where incubation of the cumulus oocytes complexes was done for 2 hours after retrieval. after which, the meiotic grade of retrieved oocytes was assessed after the denudation of their cumulus and corona layers by by means of hyaluronidase enzyme and also, mechanical pipetting. the presence of an extruded first polar body (IPB) in the perivitelline space (PVS) and the absence of a germinal vesicle (GV) oocyte are used as indicators of nuclear maturity (MII oocyte). The procedure of intracytoplasmic sperm injection (ICSI) was carried out. Fertilization and then pronuclear estimation were done16–18 hours after sperm injection. fertilization was characterized by the presence of two pronuclear and two polar bodies. However, scoring of cleavage-stage embryos was established depending on the Istanbul consensus workshop "ASRM and ESHRE Special Interest Group of Embryology in 2011", and were classified into grade I, II, and III. Embryo transfer was done at day 3 where grade one embryos (fragmentation < 10% and an even blastomere). The luteal phase support from the day of retrieval of oocytes with vaginal progesterone (Cyclogest® 400 mg twice: Cox Pharmaceuticals, Barnstaple, UK). Serum ß-HCG analyze was done on day 14 after the day of embryo transfer informative of biochemical pregnancy.

Data were entered and analyzed by using Statistical Package for Social Sciences (SPSS) software program version 26. All categorical variables were presented by frequency and percentages while numerical continuous variables were represented by mean (a measure of central tendency) and standard deviation (a measure of dispersion). Association between categorical variables was assessed by the Chi-Square test or Fisher's Exact Test (if > 20% of expected cell counts are less than 5) accordingly. The difference between means of numerical variables was tested by the One-way ANOVA test and independent samples t-test according to the number of involved groups. Considering P-value equal to or less than 0.05 as a significant. "ROC curve" also utilized to find the best cutoff value for continuous variables expecting pregnancy.

3. Results

There was no significant difference in mean age, AFC and AMH (ng/ml) of the study groups as was demonstrated in table (1), while there was significant difference in body mass index (BMI) among the leading follicle size group, serum E2 group and the serum LH group with p value equal to (0.022) with lowest value in the serum E2 group (25.30± 2.93). Causes of infertility also shown in table (2), with no significant difference in between the three studied groups. (p=0.076).

Basal hormonal levels of the study groups were illustrated in table (3). There was significant difference regarding LH level (p=0.049) while there was no significant difference regarding FSH level (p=0.260) and E2 (p=0.306). Endometrial thickness at day tow was illustrated in the same table with no significant difference (p=0.658).

Table 1: Cycle Day 2 Demographic features of infertile patients among the three groups

Variables		Group A	Group B	Group C	P-value (One-way ANOVA test)
Quantitative variables	Units	(Mean ± Standard Deviation)			
Age	Years	31.8±5.39	31.4±5.20	29.93± 5.78	0.275
BMI	(kg/m2)	26.88±2.85	25.30±2.93	26.95±3.11	0.022*
AFC (count)		14.05 ± 7.53	13.90± 3.68	15.23 ± 6.11	0.558
AMH (ng/ml)	(ng/ml)	2.53±1.04	2.95±3.34	2.82± 1.23	0.675
BMI: Body mass index, AMH: Anti-Mullerian Hormone, AFC: Antral Follicle Count*: p value ≤ 0.05 (significant)					

Table (2): comparison of infertility features among the groups of study				
features	Group A	Group B	Group C	P-value
Causes of infertility				
Male factor (MF)	11(25%)	22(50%)	11(25%)	076 (Fisher's Exact Test 0.)
Ovulatory disorders	11(47.8%)	6(26.1%)	6(26.1%)	
Ovulatory disorders + MF	4(36.4%)	3(27.3%)	4(36.4%)	
Tubal factor	0(0%)	1(100%)	0(0%)	
Tubal factor + MF	0(0%)	1(100%)	0(0%)	
Unexplained	4(50%)	2(25%)	2(25%)	
Endometriosis	1(100%)	0(0%)	0(0%)	
PCOS	6(33.3%)	4(22.2%)	8(44.4%)	
PCOS+ Male factor	3(25%)	1(8.3%)	8(66.7%)	
PCOS+ tubal	0(0%)	0(0%)	1(100%)	

Table (3): Comparison of basal hormonal levels among the study groups at cycle day 2.				
Hormone	Group A	Group B	Group C	P-value (One-way ANOVA test)
	(Mean ± Standard Deviation)			
FSH (mIU/ml)	6.20 ± 1.56	5.50 ± 2.15	6.05 ± 1.87	0.260
LH (mIU/ml)	4.92 ± 1.79	4.52 ± 1.80	5.55 ± 2.00	0.049*
Endometrial thickness (mm)	3.12 ± 0.64	3.14 ± 0.66	3.25 ± 0.66	0.658
E2 (pg/ ml)	41.78 ± 11.38	40.11 ± 8.92	43.72 ± 10.83	0.306
FSH: Follicle stimulating hormone; LH: Luteinizing hormone; E2: Estradiol. *: p value ≤ 0.05 (significant)				

Endometrial thickness, E2 levels, total number of follicles at antagonist time and total gonadotropin dose with total antagonist dose shown in table (4), significant difference in endometrial thickness at antagonist day among study groups was shown with (p= 0.016) and lowest endometrial thickness value being in the leading follicle size group, also the difference was significant regarding the E2 levels with higher levels of E2 in serum LH group (P= 0.017), while total number of follicles at antagonist time, total gonadotropin dose with total antagonist dose were comparable among the different groups. There was no significant difference among the three study groups with regard to the endometrial thickness (mm) on the day of ovulation trigger (p= 0.060) with higher thickness was in the serum E2 group compared to other two groups, the mean serum concentration of E2, was comparable between the three time points (p= 0.085) as it shown in table (5), other stimulation characteristics among the study groups also have no statistical significance, table (6).

Table (4): Comparison of Endometrial thickness, LH and E2 levels at antagonist day among the groups of study				
features	Group A	Group B	Group C	P-value (One-way ANOVA test)
	(Mean ± Standard Deviation)			
Endometrial thickness (mm) at day of antagonist	5.99 ± 1.48	6.81 ± 1.06	6.54 ± 1.28	0.016*
E2 (pg/ ml)	626.19±533.27	557.32± 42.60	830.84±534.42	0.017*
Total number of follicles at antagonist time	14.28 ± 7.98	14.70 ± 5.59	14.95 ± 6.08	0.900
Total GT dose (IU)	1558.38±505.56 (760-2850)	1564.30±411.11 (900-2400)	1673.73±717.11 (900-3450)	0.585
Total antagonist dose	1.00±0.35(0.50-1.75)	0.90± 0.21 (0.50-1.25)	0.91±0.25(0.50-1.75)	0.190
LH: Luteinizing hormone; E2: Estradiol *: p value ≤ 0.05 (significant)				

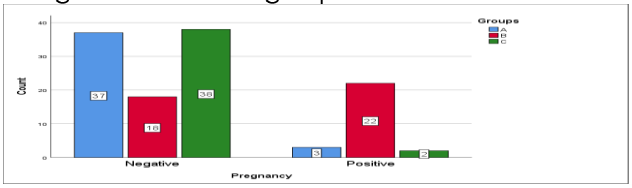
Table (5): Comparison of Endometrial thickness, and E2 levels at trigger day among the study groups.				
features at trigger day	Group A	Group B	Group C	P-value (One-way ANOVA test)
	(Mean ± Standard Deviation)			
Tigger day Endometrial thickness (mm)	9.85 ±2.79	11.09 ±2.03	10.90 ±2.58	0.060
Trigger E2	1233.17± 796.67	1314.46 ±463.31	1574.75±817.11	0.085
E2: Estradiol. *: p value ≤ 0.05 (significant)				

Table (60): Comparison of stimulation characteristics between the study groups				
features	Group A	Group B	Group C	P-value (One-way ANOVA test)
	(Mean ± Standard Deviation)			
Injected Oocytes	10.25 ± 6.86	10.75 ± 4.71	10.80 ± 5.29	0.892
Metaphase II (MII)	7.23 ± 5.90	7.38 ± 3.58	7.28 ± 4.50	0.990
fertilized oocytes	6.13 ± 4.64	6.85 ± 3.01	6.35 ± 4.13	0.708
Grade 1 embryos	3.35±2.37	4.18±1.86	3.68±2.59	0.274
Number of embryos transferred	2.38 ± 1.19	2.95 ± 0.93	2.48± 1.26	0.058
*: p value ≤ 0.05 (significant)				

A highly significant difference (p= 0.001) regarding the presence of triple layer pattern was seen in table (7), 27(50%) of patients in the leading follicle size group have no triple layer pattern. to 11(20.4%) and 16(29.6%), while higher percentage of patients 29(43.9%) have triple layer pattern in serum E2 group compared to other two groups, table (7).

Table (7) Comparison of Presence of Triple layer pattern and cycle day of Triple layer pattern appearance among the study groups				
Triple layer pattern /cycle day	A	B	C	P-value
	No. (%)			
No	27(50%)	11(20.4%)	16(29.6%)	0.001* (Chi-square test)
Yes	13(19.7%)	29(43.9%)	24(36.4%)	
*: p value ≤ 0.05 (significant)				

The pregnancy rate among patients enrolled in the current study is shown in Figure (1). A highly significant difference (P= <0.001) in pregnancy rate among the three different groups seen with highest percentage being in the serum E2 group.



(Chi-square test) (P= <0.001)

Figure (2): pregnancy rate among study groups

Table (8) shows no statistical difference in endometrial thickness concerning pregnant ant non-pregnant groups at cycle day two, however, there was significant difference at antagonist starting day (p= 0.003) and highly significant difference was on trigger day (P= <0.001). Triple layer pattern was higher in pregnant group but without statistical difference, table (9).

Table (8) Comparison of Endometrial thickness on three time points between pregnant and non-pregnant groups.			
Endometrial thickness (mm)	Pregnant	Non- pregnant	P-value (Independent sample t-test)
	(Mean ± Standard Deviation)		
At cycle day 2	3.14 ± 0.73	3.18 ± 0.63	0.828
At day of antagonist starting	7.01 ± 0.98	6.28 ± 1.36	0.003*
At trigger day	12.22 ± 1.96	10.15 ± 2.49	<0.001*
*: p value ≤ 0.05 (significant)			

Table (9) Comparison of Presence of Triple layer pattern at antagonist starting day between pregnant and non-pregnant groups.			
Triple layer pattern on antagonist starting day	Pregnant	Non-pregnant	P-value
	No. (%)		
No	8(29.6%)	46(49.5%)	0.068 (Chi-square test)
Yes	19(70.4%)	47(50.5%)	
*: p value ≤ 0.05 (significant)			

The best cutoff value of endometrial thickness at day of antagonist started predicting pregnancy was >6.45 with a sensitivity level of 78% and a specificity level 57% and this result was significant with a P-value= 0.006, table (10), figures (3).

Table (10): Characteristics of ROC curve and best cutoff value of Endometrial thickness at day of antagonist starting for predicting pregnancy	
Characteristics	Endometrial thickness (mm)
Cutoff	>6.45
Area under curve (AUC)	0.673
95 % Confidence interval (CI)	0.570-0.776
P-value	0.006*
Sensitivity	78%
Specificity	57%
*: p value ≤ 0.05 (significant)	

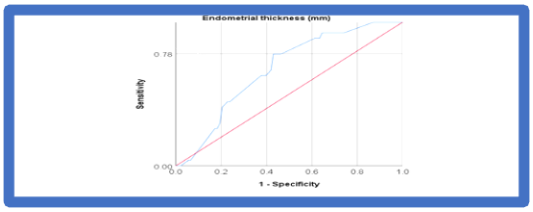


Figure (3): Characteristics of the ROC curves, sensitivity, and cutoff value of Endometrial thickness at day of antagonist starting for predetecting pregnancy

4. Discussion

Different criteria were proposed to determine time of starting the inhibition in GnRH antagonist cycles like cycle day, leading follicular size, serum estradiol level, however; the antagonist is administered only after certain endocrine and/or sonographic criteria indicating a risk for LH rise is present and these criteria have differed between studies (Kolibianakis *et al*, in 2011).

Age of patients, embryo quality and endometrial receptivity are the most essential influences for the success of IVF. Even so, endometrial thickness (EMT) was recognized as an indicator for endometrial receptivity (Yu et al., 2014), for that reason this study will correlate the endometrial thickness to the other optimal start criteria for antagonist administration and this is attributed for being noninvasive and easy method of assessment via transvaginal ultrasound. There was no statistical difference in demographic features as (age, AFC, and causes of infertility) among the study groups, so it is agreed with Dashti *et al.* study in 2019 when found that no statistical differences in baseline characteristics regarding to the age, type and duration of infertility (Dashti et al., 2019). there was significant difference in body mass index (BMI) among the study groups with *p* value equal to (0.022) with lowest value in the serum E2 group, this may be related to different sample size, socioeconomic statutes and habits of patients enrolled, Moreover, PCOS patients where somewhat lower in group B. The role of BMI in ICSI cycles studied by Al-Obaidi et al., as they hypothesized that there are negative effects of age and BMI on the oocytes number and quality (Al-Obaidi et al., 2018). However, another study by Banker et al (2017) reported that women's weight and height have marginal or no effect on oocyte quality or endometrial thickness and hence on the overall probability of achieving pregnancy following IVF treatment (Banker *et al.*, 2017). There was significant difference regarding basal LH level (p=0.049) while there was no significant difference regarding basal FSH level, E2 and Endometrial thickness, however; it was not of value since these values were within normal. This study agreed by Qiu etal study 2019, when found that no statistically significant differences in baseline clinical parameters regarding endometrial thickness and AFC. (Qiu *et al.*, 2019). significant difference in endometrial thickness at antagonist starting day among study groups with (p= 0.016) and lowest endometrial thickness value being in the leading follicle size group, also the difference was significant regarding the E2 levels with higher levels of E2 in serum LH group (P= 0.017), Few data are available about the endometrial thickness at antagonist day since most studies evaluate the endometrial thickness at trigger day, however; Weerakkody et al., in 2022 stated that the endometrium in the late proliferative phase (day 6-14) develops a trilaminar appearance: outer echogenic basal layer, middle hypoechoic functional layer, and an inner echogenic stripe at the central interface with a measurement of 5-7 mm (Weerakkody et al., 2022). The endometrium is an essential factor for the success rates of artificial reproduction techniques, and it is correlated to estrogen stimulation (mostly E_2) throughout the follicular phase, which is demonstrated by the hyperplastic development of endometrial glands and stroma (Zhang et al., 2021). In the first group the antagonist started when the LFS was (12-14) there was lower endometrial thickness than the other two groups, possible explanation is that earlier start of antagonist has a detrimental effect on the endometrium. Some previous studies may agree with this as it was reported that during COS low serum LH levels may affect the function of corpus luteum or endometrium receptivity (Liu et al., 2019). while total number of follicles at antagonist starting time, total gonadotropin dose with total antagonist dose were comparable among the different groups. It has been reported that optimal follicular development and embryos with good morphological quality are positively related with pregnancy after IVF and endometrial characters, such as endometrial thickness, have been assessed as prognostic factors (Wang 2017). There was no significant difference among the three study groups with regard to the endometrial thickness (mm) on the day of ovulation trigger (p= 0.060) with higher thickness was in the serum E2 group compared to other two groups. the mean serum concentration of E2, was comparable also, May be related to the fact that the magnitude of uterine growth stimulation is largely dependent upon the duration of bioavailable E_2, as stated by (Grotius., et al., 2007). There is an association between endometrial thickness on hCG day and clinical consequence in normal responder patients after GnRH antagonist inhibition. The lower pregnancy rate was in women with endometrial thickness measures less than 7 mm in comparison with patients with endometrial thickness more than 7 mm. (Wu Yet al., 2014). other stimulation characteristics among the study groups also have no statistical significance, other stimulation characteristics among the study groups also have no statistical significance, this may be attributed to the comparable patient's characteristics and nearly comparable basal and trigger day hormonal profile among the three study groups. A highly significant difference (p= 0.001) regarding the presence of triple layer pattern with higher percentage of patients 29(43.9%) have triple layer pattern in serum E2 group compared to other two groups. and that may be explained by the acceptable hormonal profile specially E2 in that time limit in the same group, pattern and thickness may independently affect pregnant results. a three-line pattern with a moderate thickness of endometrial seemed to be correlated with a good clinical consequence (Zhang et al., 2021). One study found that significantly lower implantation and also, pregnancy rates were seen in women having no triple-line pattern of endometrium (Detti et al., 2011), furthermore, a thinner thickness of endometrium with a three-line pattern is related with increased clinical pregnancy rate as compared to a thinner endometrium having no triple line pattern. (Alfer et al, 2021). A highly significant difference (P= **<**0.001) in pregnancy rate among the three different groups seen with highest percentage being in the serum E2 group. No statistical difference in endometrial thickness concerning pregnant ant non-pregnant groups at cycle day two, however,

there was significant difference at antagonist day (p= 0.003) and highly significant difference was on trigger day (P= <0.001). Triple layer pattern was higher in pregnant group but without statistical difference, it is anticipated since endometrial thickness largely correlated with reproductive outcome as it was agreed by many studies, optimal endometrial receptivity and synchronization between embryonic and endometrial development serve important roles in successful pregnancies resulting from FET cycles (An et al., 2020), endometrial features, for example endometrial thickness, have been valued as prognostic factors by (Wang et al., 2017). some studies have found decreased pregnancy and live birth rates with a thin endometrium, but other studies have not found the same association as stated by (Liu et al., 2018). It was stated that "triple line" endometrium is the marker of ultrasound that most precisely reveals endometrial receptivity, while the 'non-three-line' pattern is commonly associated with non-conception cycles (Luis et al., 2012). Other study by Abunayla et al., 1n 2019 stated that the mean endometrial thickness, endometrial layering, myometrial contractions, and zone three myometrial blood flow did not affect pregnancy outcome significantly (Abunayla et al., 2019) Zhao et al., stated that and endometrial thickness and endometrial pattern had no predictive value on the outcome of in vitro fertilization-embryo transfer (Zhao et al., 2014) and that when patients have moderate thickness of endometrium the endometrial pattern can be considered. The best cutoff value of endometrial thickness at day of antagonist was >6.45 with a sensitivity level of 78% and a specificity level 57% and this result was significant with a P-value= 0.006. Very few statistics available concerning the endometrial thickness on antagonist day, however; although many factors other than serum E2 level effect the endometrial thickness but still, it can be taken as criteria to initiate GnRH antagonist with a sensitivity level of 78% and a specificity level 57%.

Conclusion: The endometrial thickness at day of antagonist correlates significantly with pregnancy rates and it can be taken as criteria to initiate GnRH antagonist with a sensitivity level of 78 % and a specificity level 57%. Triple layer pattern was higher in pregnant group but; However, without statistical difference.

5. Acknowledgment

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Author Contribution

Muhammad SM, performed the study, examined and reviewed results, and manuscript writing with the help and supervision of LA Al-Anbari, and S Muayad

Conflict of Interest

The author declares no conflict of interest.

Ethical Clearance

The study was approved by the Ethical Approval Committee.

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