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Evaluation of LH level at different time points during the follicular phase in ICSI cycle and correlation with pregnancy.

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ABSTRACT

Luteinizing hormone (LH) plays vital role for optimal follicular growth, maturation and is responsible for inducing ovulation, various protocols in ICSI cycles used to prevent the premature surge of luteinizing hormone. Currently, flexible GnRH antagonist protocols appears to be mostly used protocols. individualization of controlled ovarian hyper stimulation (COS) needs better timing of antagonist administration, assessing variation in LH levels during COS helps in determining appropriate initiation of inhibition .This prospective comparative observational analysis was made on one hundred twenty females with infertility who were undergoing ICSI flexible antagonist protocol at the "High Institute for Infertility Diagnosis and Assisted Reproductive Technologies/Al-Nahrain University" from October 2020 to April 2022, primary infertility was the with higher percentage (78.3%) than the secondary type. However; the main cause of infertility among the participant was male type (36.7%) fallowed by the combined one (20.8%). A highly significant difference was present in LH levels at all time points, with level decreasing from 4.99 ± 1.90 at baseline to 4.82± 2.15 at antagonist starting day to 1.63± 0.87 at trigger day and p value was > 0.001, no statistical difference in LH levels regarding the three time points between patients who get pregnant and non-pregnant women with pregnant patients having higher baseline lower antagonist starting day, and higher trigger LH levels 5.16 ± 1.87, 4.30 ± 1.72, 4.30 ± 1.72 respectively. Difference in LH level between cycle day 2 and the day of antagonist starting according to starting day of antagonist reveals that the basal LH values declined significantly (p= <0.001) until cycle day 7 of stimulation after which the basal LH start to increase and also with a high significant difference (negative values mean that the level decrease in comparison to the base line level).The comparable LH level in the current study reveals that earlier initiation of antagonist may serve no purpose and it is advisable to include the measurement of LH level to the criteria of antagonist initiation for better individualization and better outcome and to avoid un necessary earlier start of antagonist.

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KEYWORD

Flexible GnRH antagonist protocol, Antagonist initiation, Luteinizing hormone (LH), Pregnancy Outcome

1. Introduction

Luteinizing hormone (LH) is essential for normal follicular development and oocyte maturation, especially in the late follicular phase. Luteinizing hormone assists to generate small quantities of progesterone, stimulating positive feedback of estrogen, which is essential for the development of follicles and their maturation. Several studies have exposed the great significance of LH levels throughout COS in follicular growth and clinical consequences **(Hattori et al., 2018 [1]).** Previous research had indicated that oscillations in LH levels throughout the follicular period may have an important influence on the morphology and function of the oocyte and an additional effect on its meiotic status and its capability to be fertilized and may be harmful to the receptivity of the endometrium and hereafter to pregnancy rates **(Depalo et al., 2018 [2], Hattori et al., 2018 [1])**. Wide-ranging clinical trials have revealed that LH concentration in serum of ≥1.2 IU/L is essential to provide acceptable LH support to (FSH)-provoked follicular growth **(Hemsey et al., 2001 [3], Hopkisson et al., 2005 [4])**. A substantial proportion of patients exhibit an inadequate endogenous LH concentration throughout ovarian stimulation. Still, those patients cannot be noted earlier to ovarian stimulation using basal features alone. On behalf of these patients, with either fixed or flexible GnRH antagonist protocols, the initiation of an antagonist might decrease LH activity more and lead to adverse reproductive outcomes **(Haas et al., 2016 [5]).** Thus, these patients might not require antagonist administration **(Liu et al.,2019 [6]) .**

Findings that address the relationship between LH levels and IVF consequences have tested LH levels on different days through ovarian stimulation and had used various outcome methods (**Kolibianakis et al., 2006 [7]).** Yet, only a minor number of reports have been performed, and there

are no clear recommendations regarding the optimal serum LH concentration or time of supplementation; hence, these are areas worthy of more analysis **(Lv et al., 2021 [8])**.

2. Subject material and method

One hundred twenty infertile couples who were subjected to ICSI flexible antagonist protocol were enrolled in this study. 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. There were women with both primary and secondary forms of infertility. While some couples suffered infertility due to male factors or a combination of factors, and couples with unexplained infertility also contributed in this study, several of the women who participated complained of PCOS or tubal factors. Patients aged 18-40 years old. Early follicular phase FSH < 10 mIU/ml, and Estradiol < 60 pg/ml, and LH 10< IU/l. Body mass index 18-30 kg/ m2. All the one hundred twenty women enrolled in the current study were undergone pelvic ultrasound assessment to exclude ovarian cysts or any pathology, with assessing antral follicle count as well as a hormonal assay to assess basal plasma hormone level (FSH, LH, TSH, progesterone, prolactin, and estradiol) at day two of the menstrual cycle, the seminal fluid analysis was assessed according to WHO 2010. Controlled ovarian hyper-stimulation with 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. The flexible GnRH antagonist regimen was used to downregulate the pituitary when the group's predetermined criteria was reached. A daily

subcutaneous injection of 0.25 mg of cetrorelix acetate (Cetrotide®, Merck Serono, Geneva, Switzerland) was administered in accordance with the multiple-dose regimen. At the time of antagonist commencement, the number of follicles with leading follicles measuring between 12 and 14 mm was counted, and venous blood sampling at the day of GnRH-antagonist initiation and on the day of HCG trigger was done for assessment of serum LH. Triggering of final oocyte maturation was performed with recombinant Hcg with two ampoules Ovitrelle® 250μg subcutaneous injection (Ovitrelle®, Merck Serono) as soon as at least three leading follicles reach 17-18, followed by ovum pick-up 35-36 hours later. Oocyte pick-up is 35-36 hours following the ovulation trigger. Embryo transfer on day three following oocyte pickup. Cyclogest® 400 mg twice; Cox Pharmaceuticals, Barnstaple, UK (Vaginal progesterone) has been used to support the luteal phase since the day of oocyte retrieval. This treatment has been continued for 14 days as daily does (until β-HCG was performed; if it was positive, progesterone administration continued until 12 weeks, when the placenta begins to function). Data were entered and analyzed by using the 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). The difference between means of numerical variables was tested by the One-way ANOVA test. Considering the P-value equal to or less than 0.05 as a significant

3. Results

The mean age, BMI, AMH, and duration of infertility of patients enrolled in this study are shown in **(Table 1)**, with values of 31.04, 26.38, 2.59, and 7.91, respectively. While percentages of types of infertility are illustrated in **(Table 2)**, where primary infertility was the with higher percentage (78.3%) than the secondary type of infertility. However, the main cause of infertility among the participants was a male type (36.7%), fallowed by the combined one (20.8%), as it shown in the same table**.** Whole data comparison of LH levels at different time points of the follicular phase shown in **(Table 4)**, a highly significant difference was present in LH levels at all time points, with level decreasing from 4.99 ± 1.90 at baseline to 4.82± 2.15 at antagonist starting day to 1.63± 0.87 at trigger day and p-value was > 0.001. **(Table 5)** suggests that the difference in LH levels was statistically not different with respect to the three-time points between pregnant and non-pregnant women, with pregnant patients having higher baseline lower antagonist starting day, and higher trigger LH levels 5.16 ± 1.87, 4.30 ± 1.72, 4.30 ± 1.72 respectively. **(Figure 1)** shows a Difference in LH level between cycle day two and the day of antagonist starting according to the starting day of the antagonist, and as it is obvious from the figure that the basal LH values declined significantly (p= <0.001) until cycle day 7 of stimulation after which the basal LH start to increase and also with a high significant difference (negative values mean that the level decrease in comparison to the baseline level). These values of the mean difference and 95% Confidence Interval for Mean are illustrated in **(Table 6).**

Table (1): Basal Demographic characteristics of participant infertile women

Variables	Minimum	Maximum	Mean	Std. Deviation
Age (years)	18	40	31.04	5.47
BMI (kg/m^2)	20.2	34.2	26.38	3.04
AMH (ng/ml)	0.81	6.80	2.59	1.02
Duration of inf. (Year)	1	18	7.91	4.23

BMI: Body mass index, AMH: Anti-Mullerian Hormone

***(Table 2)*:** Infertility features of participant infertile women

Variables		Frequency	Percent
Type of infertility	Primary	94	78.3%
	Secondary	26	21.7%
Cause of infertility	Male	44	36.7%
	Combined	25	20.8%
	unexplained	8	6.7%
	PCOS	18	15.0%
	Ovulatory	23	19.2%
	Tubal	1	0.8%
	Endometriosis	1	0.8%

(Table 3): Main baseline features of participant infertile women

Variables	Minimum	Maximum	Mean	Std. Deviation
LH	1.51	8.40	4.99	1.90
FSH	1.23	11.80	5.93	1.88
E2	9.00	64.02	41.87	10.45
Prolactin	0.50	33.05	14.99	6.37
TSH	0.35	4.80	2.05	0.83
progesterone	0.130	0.98	0.51	0.20
Endometrial Thickness .mm	2.0	4.0	3.17	0.65

FSH: Follicle stimulating hormone; LH: Luteinizing hormone; TSH: Thyroid stimulating hormone; E2: Estradiol. p-value ≤0.05 (significant)

(Table 4): Whole data comparison of LH levels at different time points of the follicular Phase

Variables	Cycle day 2	Day of antagonist	Day of trigger	*P*-value (Repeated measure ANOVA test)
	(Mean ± Standard Deviation)			
LH (mIU/ml)	4.99 ± 1.90	4.82± 2.15	1.63± 0.87	< 0.001*

(Table 5): Whole data comparison of LH levels at different time points of the follicular Phase between pregnant and non-pregnant women

LH (mIU/ml)	Pregnant	Non-pregnant	P-value (Independent sample t-test)
	(Mean ± Standard Deviation)		
Basal LH	5.16 ± 1.87	4.95 ± 1.91	0.614
Day of the antagonist starts LH	4.30 ± 1.72	4.97± 2.25	0.106
Trigger day LH	1.87± 0.78	1.56 ± 0.89	0.104

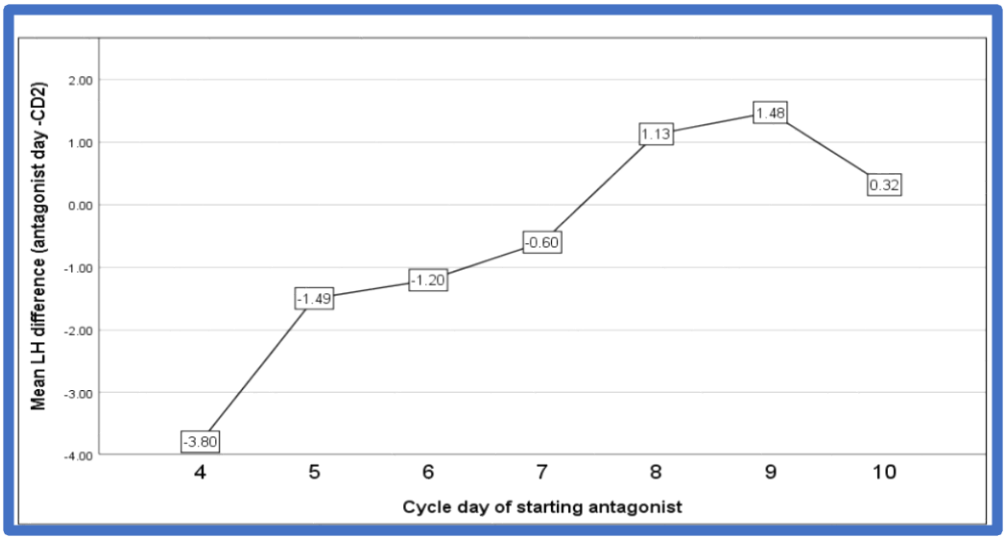
LH: Luteinizing hormone; *: p-value 0≤.05 (significant)

(Table 6): Difference in LH level between cycle day two and day of antagonist according to starting day of antagonist

Cycle day of starting antagonist	No.	Mean difference in LH	Standard Deviation	95% Confidence Interval for Mean		*P*-value
				Lower Bound	Upper Bound	
4	1	-3.80	-------	---------	-------	<0.001
5	13	-1.49	0.71	-1.92	-1.06	
6	25	-1.20	1.52	-1.83	-0.57	

7	34	-0.59	1.96	-1.28	0.08	(One-way ANOVA test)
8	32	1.12	1.81	0.47	1.78	
9	10	1.47	1.27	0.56	2.38	
10	5	0.32	3.38	-3.88	4.52	
Total	120	-0.17	2.05	-0.54	0.19	

*: p value 0≤.05 (significant)



(Figure 1): Difference in LH level between cycle day two and day of starting antagonist according to starting day of antagonist

4. Discussion:

The mean age, BMI, and AMH of the patients enrolled in this study were within the criteria chosen to include patients in this study to assure statistical matching and to avoid any variation in pregnancy outcome that can be attributed to this variation. Increased infertility duration and the cause of infertility have been shown to have an adverse effect on pregnancy outcomes following IVF **(Bhattacharya et al., 2013 [9])**. Whereas primary infertility was the with higher percentage (78.3%) than the secondary type in this study. However, the main cause of infertility among the participants was a male type

(36.7%), fallowed by the combined one (20.8%), as it shown in the same table; these results agreed with many studies **(Elhussein et al., 2019 [10])** concluded also, a high ratio of primary infertility. Yet, other revisions have conveyed that the infertility causes may be related to male factors (40%), female factors (40%), or the combination (20%) of both **(Moyo et al., 2013 [11]).** Several factors, for example, environmental, infectious, dietary, and genetic factors, could have a part in the causativeness of infertility, as hypothesized by **(Elhussein et al., 2019 [10])**. In the contrary,

other study stated that the female cause of infertility accounted for the uppermost proportion of infertility **(Moridi et al., 2019 [12])**. This inconsistency may be due to dissimilar study designs, features of the contributors, and sample size. Furthermore, most reports revealed that the incidence of male and female causes and other undetermined causes were 20-40%, 30- 35%, 35%, and 5-15%, respectively **(Masoumi et al., 2015 [13])** .

The mean baseline characteristics are shown in **(Table 3)**, where it was 4.99 for LH level and 1.88, 10.45, 6.37, 0.83, and 0.20 for FSH, E2, Prolactin, TSH, and progesterone, respectively, while the mean endometrial thickness was 0.65mm

baseline hormonal levels were within normal values in order to minimize the differences in cycle outcome that could arise as a result of differences in their levels. A highly significant difference was present in LH levels at all time points, with the level decreasing from 4.99 ± 1.90 at baseline to 4.82± 2.15 at antagonist starting day, then 1.63± 0.87 at trigger day, and the p-value was > 0.001, studies have revealed that serum LH levels must be between 1.2 and 5.0 IU/L for optimum follicular growth **(Liu et al., 2019 [6])**. Other reports established that when LH levels throughout COS are less than 1/3 of the LH levels on the day of trigger, the pregnancy rate declines, which

specifies that LH plays a vital role in oocyte growth **(Depalo et al., 2018 [2])**.

Many physiological articles had taken hormonal dynamics during the menstrual cycle, especially variation of LH level, in consideration. Marco et al. stated that the mean amplitude of LH pulse in the early follicular phase (6.5 ± 0.4 mlU/ml) reduced considerably by the mid follicular phase (MFP; 5.1 ± 0.8 mlU/ml; $P < 0.05$) and increased once again by the late follicular phase (7.2 ± 1.2 mlU/ml). **(Marco et al., 1989 [14])**. More recently, Murat et al. in 2022, reviewed many studies that evaluated serum LH levels and concluded that there were various definitions for the beginning of the LH surge have been settled and a rise in LH levels varying from 1.8- to 6-fold beyond the baseline LH value was exposed in seven revisions and a rise of at least two or three standard deviations beyond the mean of the previous LH measurements was theorized in two studies. An LH level above the 30% of the amplitude "peak-baseline LH level" of the LH surge was stated as the onset day by one report, though some other significant inter-personal variances in the time interval between the ovulation and the onset of the LH surge was seen, altering from 22 to 56 h, six revisions supposed the mean duration in hours between the onset of the LH surge and ovulation was 33.91 **(Murat et al., 2022 [15])**. LH levels was statistically not different with regarding the three-time points concerning pregnant and non-pregnant women, with pregnant patients having a higher baseline, lower antagonist starting day, and higher trigger LH levels 5.16 ± 1.87, 4.30 ± 1.72, 4.30 ± 1.72 respectively .

Comparable baseline serum hormonal levels between pregnant and non-pregnant women groups were expected since older patients and those with endocrine disorders were excluded from this study in an attempt to unify hormonal levels in study groups at the start of ovarian stimulation. However, one study stated

that the role of baseline serum LH in predicting the success of IVF in terms of infertility outcomes remains controversial **(Wang et al., 2017 [16])**. Some recent studies agree with the results of this study; Liu et al in 2019 suggested that in some patients with sustained low endogenous LH levels (LH < 4 IU/L), it may not be sufficient to completely support the growth of the follicles and may not require antagonist intake, and believed that LH values throughout COS essential to be considered and used as a marker for the onset of antagonist administration for a better ART outcome **(Liu et al., 2019 [6])**. Also, this was illustrated by Chen et al., as with low values of LH, starting antagonist could further lower the LH values and unfavorably affect reproductive outcomes, whereas higher values of LH in the follicular phase goes with adverse outcomes **(Chen et al., 2016 [17])**. Liu et al. in 2022 propose that the LH-depended antagonist protocol is not lower to the conventional flexible protocol **(Liu et al., 2022 [18])**; also, Luo et al., 2021, stated no important variance amongst different LH groups concerning the implantation and pregnancy rates. In approval with these studies, Benmachiche et al. stated that in contrast to higher LH levels, lower LH levels lessening the low birth rates in women who underwent COS using GnRH antagonists owing to the substantial adverse effect of low LH values following fresh embryo transfers, in other recent study it was stated that lower values of serum LH on the day of starting GnRH agonist trigger are coupled with decreased rates of live birth and increased early miscarriage **(Benmachiche et al., 2019 [19])**. However, Lainas et al., 2005 support the initiation of the antagonist earlier than day 6 of stimulation in a substantial ratio of patients, and in those patients, a higher pregnancy rate was observed. It has been reported that low serum concentration of LH had negative impacts on reproductive outcomes and lead to low serum

estradiol levels, impaired follicle growth, lower pregnancy rate, and increased miscarriage rate **(Lahoud et al., 2006 [20]; O'Dea et al., 2008 [21])**. This was in agreement with Benmachiche's study that suggested a serum LH value of 1.60 IU/L on the day of GnRH-a trigger regarded as the most suitable threshold to predict reproductive outcomes; hence, patients with LH > 1.60 IU/L exposed significantly superior reproductive outcomes than patients with LH < 1.60 IU/L **(Benmachiche et al., 2019 [19])**.

Previous studies indicated that profound LH suppression is detrimental for patients undergoing either GnRH agonist- or GnRH antagonist-treated cycles **(Shi et al., 2018 [22]; Chen et al., 2016 [17])**. Urman et al. in 2018 hypothesized that routine measurement of serum LH levels seems unjustified throughout stimulation cycles, neither to verify pituitary down-regulation in the long GnRH agonist protocol nor to define an endogenous LH surge through GnRH antagonist cycles, as plasma LH values rapidly drop after GnRH antagonist initiation **(Urman et al., 2018 [23])** . **(Figure 1)** and **(Table 6)** show a difference in LH level between cycle day two and the day of antagonist starting according to the starting day of antagonist, and as it is obvious from the figure that the basal LH values declined significantly (p= <0.001) until cycle day 7 of stimulation after which the basal LH start to increase and also with a high significant difference. These findings have a physiological explanation proposed by many studies. The frequency of LH secretion increases from the early follicular phase to the mid-follicular phase and late follicular phase, and the LH pulse amplitude decreases in the mid-follicular phase, suggesting increased negative feedback of estrogen on the hypothalamic-pituitary axis and/or a reduction in GnRH secretion at this stage, according to previous literature **(Marco et al., 1989 [14]).** Additionally, estrogen has a significant

impact on pituitary gonadotrophs. Up until mid-cycle, when the gonadotropin surge takes place, rising levels of circulating estrogen during the follicular phase make the pituitary more sensitive to GnRH. Other variables regulating and altering gonadotrophic production include Major FSH secretion inhibitors, include inhibitin and follistatin, whereas activin increases GnRH activity **(Besecke et al., 1996 [24])**. The gonadotropin surge-attenuating factor (GnSAF), which was proposed to be a non-steroidal ovarian factor, was named after the fact that numerous other researchers had reported a reduction in the pituitary sensitivity to the mid-cycle gonadotropin surge in women enduring controlled ovarian hyperstimulation. This impairment was thought to be caused by a biologic factor that was thought to be released by the ovary and that inhibited the pre-ovulatory **(Hendriks et al., 2011 [25])**. When serum oestradiol or inhibin concentrations did not significantly increase while receiving FSH treatment, a significant attenuation in LH response to GnRH was observed. This attenuation proved that the attenuation was caused by GnSAF rather than any other steroidal or non-steroidal ovarian substances **(Messinis et al., 1998 [26])**.

The evidence indicates that small follicles in the ovary releasing hormone the hormone, and with a rise in the number of follicles sized 12-15mm, gonadotropin surge-attenuating factor (GnSAF) rises as well. Vega et al. stated in 2015 that high levels of (GnSAF) inhibit LH surge and that (GnSAF) is specific for LH **(Vega et al., 2015 [27])**. This suggests that the likelihood of the LH surge being significantly diminished or inhibited as a result of excessive GnSAF synthesis increases with the intensity of ovarian stimulation **(Turkgeldi and Ata, 2021 [28])**. The combination of these two changes in cycles with only FSH prevents the LH surge in about 80% of them **(Messinis et al., 2021 [29])**. In

simulated cycles, there is a fast spike in serum oestradiol levels that surpass the threshold of the positive feedback before entire follicle maturation occurs, as well as a major increase in the bioactivity of gonadotrophin surge-attenuating factor (GnSAF).

Nevertheless, this research demonstrates that LH levels begin to rise beyond the baseline around cycle day 8. This could be explained by 1998 research by Messinis et al., which found that raising oestradiol levels above physiological levels during FSH cycles might reduce the attenuating effects of GnSAF and increase pituitary responsiveness to GnRH during the mid-follicular phase. However, despite the continued rise in oestradiol concentrations, there was no further improvement in the pituitary's response to GnRH during the late follicular phase. This suggests that GnSAF, whose production increased throughout the treatment period, limited the capacity of oestradiol to sensitize the pituitary to GnRH in the FSH cycles **(Messinis et al.,1998 [29]; Alzaidi, Z et al.,2021[30])**.

5- Conclusion

The comparable LH level in the current study reveals that earlier initiation of the antagonist may serve no purpose since both pregnant and non-pregnant having a mean LH level that was more than 4 IU/L at the antagonist starting day. Yet, no adverse outcome from the delaying inhibition was observed provide it was away from the surge, since this study hypothesized the role of GNSAF in attenuating LH activity, this would potentiate the opinion of this study to include the measurement of LH level to the criteria of antagonist initiation for better individualization and better outcome. Considerable number of PCOS patients participated in this study, yet, no adverse correlation with hormonal levels on antagonist day and no cycle cancelation due to premature LH surge or OHSS.

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Author Contribution Sahar Muhssin performed the study, and Lubna Amer Al-Anbari supervised the work .

Conflict of Interest

The authors declare no conflict of interest .

Ethical Clearance

The study was approved by the Ethical Approval Committee.

Financial Disclosure

There is no financial disclosure.

Ethical Clearance

The study was approved by the Ethical Approval Committee.

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