Section: Reproductive Medicine and Surgery

Original Research Article

ASSOCIATION OF LEVELS OF LUTEINIZING HORMONE DURING CONTROLLED OVARIAN STIMULATION ON OOCYTE RETRIEVAL, FERTILIZATION & EMBRYO QUALITY

Ohri Sugandh[1], Basandani Sapna[2], Gahlot Alka[3]

[1]MCH Resident, Department of Reproductive Medicine and Surgery, National Institute of Medical Sciences and Research Centre, Jaipur, India.
[2]Professor, Department of Reproductive Medicine and Surgery, National Institute of Medical Sciences and Research Centre, Jaipur, India.
[3]Professor and Head, Department of Reproductive Medicine and Surgery, National Institute of Medical Sciences and Research Centre, Jaipur, India.

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Corresponding Author:
Dr. Sugandh Ohri,
Email: ohrisugandh@gmail.com

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Abstract

Background: Adequate ovarian stimulation using purified or recombinant gonadotrophins is a crucial factor for the success rate of in vitro fertilization and embryo transfer (IVF-ET). The maturation of oocytes and follicular development depend on luteinizing hormone (LH). Aim and Objectives: 1. To investigate the effects of Luteinizing hormone (LH) concentrations during controlled ovarian stimulation on oocyte retrieval, fertilization, and embryo development in patients undergoing in vitro fertilization (IVF).2. To find the association of level of Luteinizing hormone during Controlled Ovarian Stimulation on oocyte retrieval, fertilization and embryo quality. **Materials and Methods:** Study Design: prospective cohort study. Study place: Department of Reproductive Medicine and Surgery, National Institute of Medical Sciences and Research Centre, Jaipur, India. Study Duration: from July 2024 to December 2024. Study population: The study was conducted on all the patients who underwent fixed antagonist. Study sample: 38. **Result:** The findings revealed that, serum LH levels gradually declined during controlled ovarian stimulation, with the effect being more noticeable in the late follicular phase. The decreasing level of serum LH level on the day of ovulation trigger was more in Group B than Group A and this was statistically significant. Considering oocyte retrieval, it was found to have significant differences among the two groups, the number of fertilized oocytes and cleavage rate were comparable among the two groups. (76.69±15.21 and 87.69±14.42 versus ±13.7 and 88.95±15.86 respectively). **Conclusion:** Exogenous LH during late follicular phase may be considered as a strategy in women with decrease in levels of LH during ovarian stimulation to improve oocyte quality.

INTRODUCTION

Adequate ovarian stimulation using purified or recombinant gonadotrophins is a crucial factor for the success rate of in vitro fertilization and embryo transfer (IVF-ET).[1] The maturation of oocytes and follicular development depend on luteinizing hormone (LH).[2] The GnRH antagonists lead to a rapid inhibition of LH release from competitive binding to pituitary GnRH receptors.

Studies have suggested that profound suppression of LH during the midfollicular phase of ovarian stimulation might have detrimental effects on the outcome of IVF treatment.[3] A strong correlation has been noted by other authors between low serum levels of LH and both inefficient oocyte retrieval and poor reproductive results. This study aims to investigate the effects of LH concentrations on oocyte retrieval, fertilization, and embryo development in patients undergoing in vitro fertilization/ intracytoplasmic sperm injection during Controlled Ovarian Stimulation.

Aim and Objectives

1. To investigate the effects of Luteinizing hormone (LH) concentrations during controlled ovarian stimulation on oocyte retrieval, fertilization, and embryo development in patients undergoing in vitro fertilization (IVF).
2. To find the association of level of Luteinizing hormone during Controlled Ovarian Stimulation on oocyte retrieval, fertilization and embryo quality.

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MATERIALS AND METHODS

Study Design: prospective cohort study.
Study place: Department of Reproductive Medicine and Surgery, National Institute of Medical Sciences and Research Centre, Jaipur, India.
Study Duration: from July 2024 to December 2024.
Study population: The study was conducted on all the patients who underwent fixed antagonist.
Study sample: 38
Inclusion criteria:
The study included all the patients who underwent fixed antagonist protocol Age between 18 and 39 years, body mass index 18–29 kg/m2, regular menstrual cycles, normal uterus and ovaries at transvaginal sonography, DAY 2- FSH- <10 IU/L, LH- 1.37 to 9 IU/L and E2<50pg/ml.
Exclusion Criteria

1. The study excluded patients with other endocrinological, metabolic, or autoimmune diseases.
2. Polycystic ovarian disease and poor ovarian reserve.

Study size and Power Calculation
At 95% Confidence interval and proportion of ovarian stimulation at 10% margin of error, the sample size came out to be 38.
Approval for the Study
Written approval from Institutional Ethics committee was obtained beforehand. Written approval of Department of Reproductive Medicine and Surgery and other related department was obtained. After obtaining informed verbal consent from all patients visiting our institute during study period according to exclusion and inclusion criteria in Department of Reproductive Medicine and Surgery of tertiary care centre such cases were included in the study. The study was approved by the Office of the Institutional Ethics Committee at National Institute of Medical Sciences and Research Centre (IEC/P-769/2024)
Sampling Technique: Convenient sampling technique used for data collection. All patients attending Department of Reproductive Medicine and Surgery during study period from July 2024 to December 2024
Methods of Data Collection and Questionnaire: Predesigned and pretested questionnaire was used to record the necessary information. Questionnaires included general information, such as age, Medical history- Infertility history, past history, general examination, systemic examination, menstrual history, BMI, AFC, AMH, type and duration of infertility, or IVF treatment indications
Study procedure:
Treatment protocol:

1. **Ovarian stimulation:**

All patients undergoing controlled ovarian stimulation with antagonist protocol meeting inclusion and exclusion criteria were taken. On day 2 of the menstrual cycle, a transvaginal scan (TVS), serum follicle-stimulating hormone (FSH), luteinizing hormone (LH), estradiol, and progesterone were done, endometrial thickness less than 4 mm, and no ovarian cyst, the ovarian stimulation was started with a daily gonadotrophin (HMG/recombinant FSH) injection in the abdominal wall. The starting dose was based on the patient's characteristics and history. Addition of GnRH antagonist (Cetrorelix) was started from day 6 of ovarian stimulation. On day 6, LH level were taken and both Human Menopausal- HMG /Recombinant Follicle Stimulating hormone- FSH and antagonist were continued until (and including) the day of ovulation trigger (Group B -19). In the group A, Recombinant LH was added from Day 6 onwards (Group A- 19). On the day when at least three follicles ≥18 mm were observed by ultrasound, ovulation was triggered using injections of GnRH Agonist or Human chorionic gonadotrophin. Intracytoplasmic sperm injection (ICSI) was performed 35-36 h after trigger.

1. **Oocyte Preparation and ICSI Procedure:**

From the aspirated follicular fluid the cumulus-corona-oocyte complexes were identified in sterile plastic dishes rinsed, transferred to IVF-50 medium and incubated at 37°C in an atmosphere of 5% CO2 in the air until ICSI. Immediately before ICSI, the cumulus and corona cells were removed by brief exposure to HEPES-buffered medium. To enhance enzymatic removal of the cumulus and corona cells, the oocytes was aspirated in and out of a hand-drawn Pasteur pipette. Denudation was performed in a four-well culture dish. Denuded oocytes were washed twice and incubated in IVF-50 medium. Oocytes were then examined under an inverted microscope at ×200 magnification to assess the integrity and maturation stage. Only morphologically normal mature oocytes with a visible first polar body were microinjected. ICSI was performed in microdroplets under oil using plastic culture dishes under a microscope at ×400 magnification.

2. **Embryo Culture**

After ICSI, embryos were placed in 4-well Petri dishes and cocultured at 37°C in a 5% CO2 incubator. Pronucleus formation was verified 16–20 h post ICSI, and the progression of embryo growth was recorded daily. Pronucleus formation and/or the timely cleavage of nucleated embryos were used as fertilization measures for oocytes subjected to ICSI.

3. **Embryo Grading**

Embryo scoring according to Depa-Martynow et al,[14] as depicted in [Table 1].

Table 1: Embryo grading

Grade	Description
A	Embryo with 8 blastomeres and 20% cytoplasmic fragmentation
B	Embryo with 8 blastomeres and over 20% fragmentation
C	4-6 cell embryo with maximum 20% fragmentation

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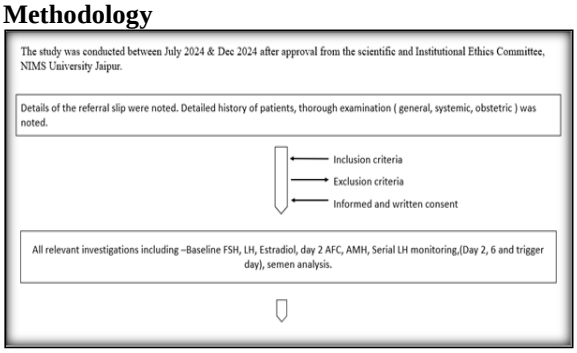
D	4-6 cell embryo with over 20% fragmentation
Necrosed	Embryo with visible signs of necrosis

Variable: The primary outcome was to find the association of the levels of Luteinizing hormone during controlled ovarian stimulation on oocyte retrieval, fertilization and embryo quality.

Statistical Analysis: Descriptive statistical analysis, including mean and standard deviation for continuous variables and count and percentage for categorical variables were determined. Normality of the data is tested using Shiparo Wilk test. Independent t test is used to compare continuous values between Group A and Group B and fischer's exact test is used to compare categorical variables between two groups. All the reported p-values <0.05 are considered to indicate statistical significance. All data entries and statistical analyses are performed by using SPSS® Version 23.0 software.

Methodology



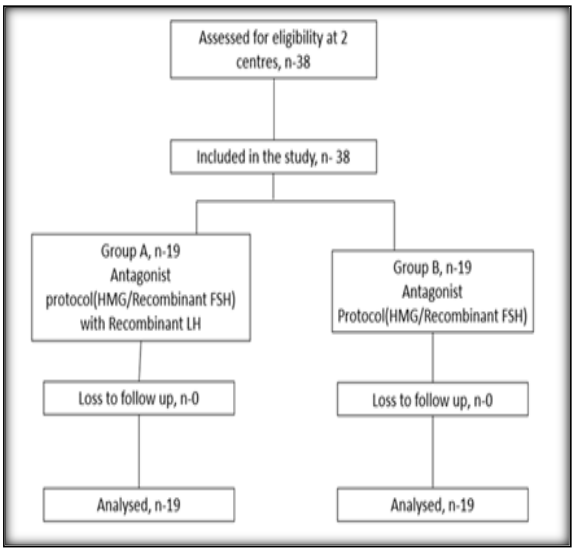


Figure 1: Methodology Flow diagram

RESULTS

Data are presented as mean ± standard deviation. Categorical data presented as number and percentage. BMI, body mass index; FSH, follicle stimulating hormone; AFC, antral follicle count; AMH, anti-Mullerian hormone; ICSI, intracytoplasmic sperm injection; E2, Estradiol.

Table 2: Demographic details.

		Group		P-value
		A(n-19)	B(n-19)	
Age (in Years)		28.05±2.72	28.93±2.31	0.325
Duration of infertility (Years)		4.63±1.71	5.63±1.92	0.098
BMI		24.07±1.79	23.23±2.09	0.188
Type of infertility				
Primary %		84.21	63.10	
Secondary %		15.78	36.8	
Ovulatory %		10.52	15.78	
Tubal factor %		26.31	42.10	
Male factor %		36.84	10.52	
Unexplained %		31.57	36.84	
No of previous ICSI cycles	No	15(78.95)	14(73.68)	1
	Yes	4(21.05)	5(26.32)	
No of previous pregnancies	No	17(89.47)	17(89.47)	1
	Yes	2(10.53)	2(10.53)	
No of previous miscarriages	No	17(89.47)	15(78.95)	0.66
	Yes	2(10.53)	4(21.05)	
No of previous live births	No	17(89.47)	19(100)	0.486
	Yes	2(10.53)	0(0)	
Basal FSH (IU/L)		4.95±1.55	3.59±1.3	0.006
AMH		2.45±0.86	2.26±0.77	0.47
AFC (ng/dl)		9.53±2.09	9.47±2.39	0.943
DAY 2 E2		25±8.45	16.89±6.81	0.002

Table 3: LH levels from day 3 to day 12 of ovarian stimulation in the whole cohort.

	Group		P-value
	A	B	
DAY 2LH	4.31±2.17	3.98±1.35	0.583
DAY6 LH	3.65±1.87	3.25±1.39	0.453
Trigger day LH	3.26±1.64	1.84±1.37	0.006

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Data are presented as mean ± standard deviation. LH, Luteinizing hormone.

The overall LH levels decreased from Day 2, during stimulation (Day 6) and before ovulation trigger in both the groups as depicted in [Table 3]. In Group the Mean Serum LH level on Day 2 was 4.31±2.17 IU/L, on Day 6 was 3.65±1.87 IU/L and on the day of ovulation trigger was 3.26±1.64 IU/L. In Group B we observed a similar trend: in detail, mean serum LH levels decreased from 3.98±1.35 IU/L on Day 2 to 3.25±1.39 IU/L and reached 1.84±1.37 IU/L on the day of ovulation trigger. The decreasing level of serum LH level on the day of ovulation trigger was higher in Group B than Group A with the difference being statistically significant as depicted in [Table 3] (p-value- 0.006).

Table 4: Oocyte retrieval and Embryo grading in the whole cohort

	Group		P-value
	A	B	
Total No of oocyte	8.21±3.43	6.95±1.68	0.158
No of M2 oocyte	6.37±2.89	5.05±1.68	0.095
Number % of oocyte fertilized (%)	76.69±15.21	72.73±13.7	0.405
Cleavage stage (%)	87.69±14.42	88.95±15.86	0.8
Grade A (%)	75.63±24.31	76.38±15.25	0.91
Grade D (%)	18.11±26.68	5.69±11.79	0.072
Necrosed (%)	6.51±10.48	18.33±17.84	0.018

Data are presented as mean ± standard deviation. M2, Mature oocyte

Data are presented as a percentage, considering the number of embryo grade A, D or Necrosed according to Deepa Martynow et al.[14] In Group A 75.63 % ±24.31 grade A embryos, 18.11 % ±26.68 grade B embryos and 6.51 % ±10.48 necrosed embryos and in Group B 76.38 %±15.25 grade A embryos; 5.69 %±11.79 grade B embryos and 18.33 %±17.84 necrosed embryos.

DISCUSSION

Normal follicular growth requires the complementary action of both follicle stimulating hormone (FSH) and luteinizing (LH). By encouraging theca cell differentiation and proliferation for androgen secretion, LH can increase estrogen production in a synergistic manner.[4] LH contributes to the production of trace amounts of progesterone in the late follicular phase, fostering positive estrogen feedback that is essential for maturation and development.[5]

Numerous studies have emphasized the significance of LH levels during controlled ovarian stimulation for adequate follicular development and favourable clinical outcomes.[6] In fact, variations in LH levels during the follicular phase significantly influence the oocyte's morphological and functional changes, as well as its meiotic status and its ability to fertilize.[7]

The ovarian follicle needs a certain minimium level of LH for optimal steroidogenesis, considered as LH threshold. However excess amounts of LH also have been known to be linked with lower fertilization, lower implantation and poorer pregnancy rates. The higher levels of LH may subdue aromatase activity and suppress cell growth. The synergism between LH and FSH is essential for steroidogenesis and to develop the subsequent capacity of the follicle to ovulate and luteinize when exposed to the mid-cycle LH surge.

By around days 7–9 (follicle diameter about 10–12 mm), granulosa cells stimulated by the effect of FSH begin developing LH receptors in preparation for the final stages of follicle maturation. LH plays an increasingly important role after day 6 in regulating the final stages of oocyte maturation. According to the threshold theory of LH in ovarian function, the ovarian follicle requires a minimal amount of LH for steroidogenesis (< 1% of receptors attached by LH). The LH ceiling is dependent on timing of the menstrual cycle but for optimal follicle development, this concentration is typically 1.2 IU/l and 5 IU/l.[8] As a consequence, an increasing LH concentration would promote leading follicle progression (being below its ceiling) and degeneration of secondary ones (by overcoming their ceiling).

The GnRH antagonists lead to a rapid inhibition of LH release from competitive binding to pituitary GnRH receptors. Studies have suggested that profound suppression of LH during the midfollicular phase of ovarian stimulation might have detrimental effects on the outcome of IVF treatment.[3]

In a large cohort of GnRH antagonist cotreated with IVF/ICSI treatment cycles, Benmachiche and colleagues examined the relationship between the LH level on the day of trigger and reproductive outcome. They found that patients with LH>1.6 Miu/ml had significant better reproductive outcomes than those with LH<1.6 Miu/ml. Furthermore, low serum LH levels have been strongly linked by other authors to poor reproductive outcomes and poor oocyte retrieval.[9]

In a study done by Giovanni, the cumulus cells aspirated from granulosa cells had lower rate of apoptosis when recombinant LH was added in low responders, suggesting that addition of LH improves chromatin quality of cumulus cells involved in the control of oocyte maturation.[10]

Our data suggests that serum LH levels decrease progressively during controlled ovarian stimulation. Accordingly, the LH levels may decrease significantly during the late follicular phase after the suppressive effect of GnRH antagonist or due to negative feedback of the ovarian hormones.[11] Therefore follicles that have reached 12-13 mm

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diameter have externalized and amplified LH receptors, the smaller follicles that express low levels of LH receptor may not have completed the maturation process, hence reducing the number of mature oocytes retrieved. By using GnRH antagonist, with different size and maturity of the follicles, the largest would be using the circulating LH to complete maturation.

Recombinant LH has been incorporated in IVF protocols, as it doesn't affect pharmacodynamics of FSH during coadministration. Since LH has a precise therapeutic window, it requires a precise control over the activity of exogenous LH administered. Recombinant LH, when given subcutaneously has a terminal half-life of 24 hours, functionally and structurally analogous to endogenous LH characterized by high purity, precision of dosing and consistency. Supplementation of recombinant LH has shown to decrease cumulus cells apoptosis, possibly indicating improved oocyte quality in LH supplemented studies.[12]

In this study scenario, data confirms that low levels of serum LH negatively impacts the oocyte quality despite the number of oocytes retrieved though not statistically significant. This study also concluded that the declining levels of LH may also because of low embryo quality although we acknowledge that several other possible factors could explain low oocyte/embryo quality such as suboptimal initial gonadotrophin dose, suboptimal dose adjustment, suboptimal triggering agent and differences in culture media.[13] The limitation of the study was small sample size. The main strengths of the study were its prospective nature with good patient compliance and simple regimen.

Therefore, the study confirms that serum LH levels progressively decrease during controlled ovarian stimulation. Based on these findings, exogenous LH may be considered a strategy in women with a decrease in LH levels during ovarian stimulation to improve oocyte quality and reproductive outcome.[14]

CONCLUSION

Exogenous LH during late follicular phase may be considered as a strategy in women with decrease in levels of LH during ovarian stimulation to improve oocyte quality.

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