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Original Article

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Is there a Need for Luteinizing Hormone (LH) Estimation in Patients Undergoing Ovarian Stimulation with Gonadotropin-Releasing Hormone (GnRH) Antagonists and Recombinant Follicle-Stimulating Hormone (rFSH)?

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ABSTRACT

Aim: To find out effect of serum luteinizing hormone (LH) levels on Day 1 (Day 2 of cycle) and Day 5 of stimulation and Day of trigger hCG in controlled ovulation stimulation with antagonist protocol of IVF-ICSI cycle on cycle outcome.

Materials and Methods: This retrospective study was conducted in a University Hospital setting. One hundred and 62 patients underwent 165 cycles of controlled ovarian stimulation (COS) with recombinant Follicle stimulating hormone (rFSH) and Gonadotropin releasing hormone (GnRH) antagonist protocol in one year were included. Serum LH levels estimated on Day 1, Day 5 and the day of trigger hCG injections were divided into three groups based on the percentile and outcome measured.

Results: The average number of follicles >18 mm in size were significant in the patients with LH levels between 25th to 75th percentile group on Day 1, Day 5 and Day of trigger hCG. The fertilization rate was significant (p= 0.04) in the patients with LH levels < 25thpercentile on the day of trigger hCG. Oocyte recovery rate, oocyte maturation rate and average number of best quality embryos (Grade 1) were not affected significantly in all three groups.

Conclusion: In GnRH antagonist and rFSH protocols, low serum LH concentrations on the day of trigger hCG has better fertilization rate. LH levels between 25th and 75th percentile have an influence on the average number of > 18 mm size follicles. However, the LH level on Day 1, Day 5 and Day of hCG does not affect the cycle outcome in COS with antagonist protocol of IVF cycle. Hence, LH estimation is not mandatory in ART cycles with GnRH antagonist protocol.

Keywords: Luteinizing hormone, recombinant Follicle stimulating hormone (rFSH), Gonadotropin releasing hormone (GnRH) antagonist

INTRODUCTION

The gonadotropins regulating steroidogenesis and folliculogenesis are Luteinizing Hormone (LH) and Follicular Stimulating Hormone (FSH) and GnRH plays a key role in secreting these hormones. The role of LH in both oocyte maturation and ovulation is very important and is well established. However, the actual mechanism for these is still not understood [1]. A rise in LH levels by the mid-follicular phase of the menstrual cycle coincides with the acquiring of LH receptors by the granulosa cells [2] and a premature LH surge may cause early luteinisation and follicular atresia in cases of controlled ovarian hyperstimulation [3]. High LH levels results in a premature secretory transformation of the endometrium causing an asynchrony between the embryos and the endometrium to which they are transferred and lead to decreased pregnancy rates [4-6].

GnRH antagonists results in rapid inhibition of LH release by competitively binding to the pituitary GnRH receptors, leading to profound, immediate, and dose dependent LH suppression. Hence, various antagonist protocols have been developed to prevent the premature LH surge and improve outcomes in IVF cycles.

Given this background, in the present study, we set out to determine the effect of serum LH levels on Day 1, Day 5 and Day of trigger hCG on the cycle outcome in a rFSH - GnRH antagonist IVF/ICSI protocol.

MATERIALS AND METHODS

We retrospectively analyzed the outcomes in 162 women who underwent 165 cycles, aged between 23 and 48 years, during the period January to December, 2011 at the Division of Reproductive Medicine, Department of Obstetrics and Gynecology, Kasturba Medical College, Manipal, Manipal University, Karnataka, India.

The Body Mass Index (BMI) ranged between 18 and 29 kg/m2. Patients had regular menstrual cycles, no existence of polycystic ovaries, no presence of endometriosis or uterine abnormalities in the ultrasound, no previous adnexal surgery, and normal basal hormonal levels during the cycle before stimulation (cycle day 2: FSH <10 IU/L; LH<10 IU/L; E2 <60 pg/mL).

Women considered as “poor responders”, those with polycystic ovarian syndrome and those detected with polycystic ovaries at ultrasound scan were excluded. The choice of retrospective study was made because it is difficult to randomize the patients before the start of the FSH stimulation and after the measurement of the LH concentration on day of trigger hCG of the cycle. The recombinant FSH (rFSH) (Gonal-F: Serono S.A., Madrid, Spain; Recagon: R Organon, Ireland) was started at a dose of 150-225 IU subcutaneously per day on day 2 of the cycle (which is considered as Day 1 of stimulation) according to number of the antral follicles, baseline FSH and age of patient. The ovarian response was monitored by ultrasound, serum LH and Estradiol (E2) on day 5 of the stimulation. The need for further doses of rFSH was determined according to follicular growth, as assessed by ultrasound and serum E2 measurements. The criteria used to start GnRH antagonists were: (1) Estradiol > 400 pg/ml, (2) 6 follicles of size >11 mm and (3)

Groups	LH value in percentile on Day 1				LH value in percentile on Day 5				LH value in percentile on Day of trigger hCG			
Charecteristics	< 25th n=41	25th to 75th n=83	>75th n=41	P Value	< 25th n=41	25th to 75th n=84	>75th n=40	P Value	< 25th n=41	25th to 75th n=84	>75th n=40	P Value
Age	34.26 ± 4.56	32.30 ± 4.20	33.60 ± 4.27	0.506	34.27 ± 4.56	32.33 ± 4.18	33.57 ± 4.32	0.689	34.27 ± 4.56	32.33 ± 4.18	33.57 ± 4.32	0.689
P* on the day of HCG trigger	0.91 ± 0.46	1.01 ± 0.60	1.03 ± 0.70	0.679	0.91 ± 0.46	1.01 ± 0.59	1.04 ± 0.71	0.591	0.91 ± 0.46	1.01 ± 0.59	1.04 ± 0.71	0.591
E2** on the day of HCG trigger	3065.82± 2657.69	2861.90± 1552.22	2615.52± 1858.34	0.492	3065.82± 2657.69	2884.73± 1556.97	2561.41± 1849.01	0.474	3065.82± 2657.69	2884.73± 1556.97	2561.41± 1849.01	0.474

[Table/Fig-1]: Patient characteristics based on LH levels on Day 1, Day 5 and Day of trigger hCG, *P: Progesterone, ** E2: Estradiol

Groups	LH value in percentile on Day 1				LH value in percentile on Day 5				LH value in percentile on Day of trigger hCG			
Outcomes	< 25th n=41	25th to 75th n=83	>75th n=41	P Value	< 25th n=41	25th to 75th n=84	>75thn=40	P Value	< 25th n=41	25th to 75th n=84	>75th n=40	P Value
Follicles >18mm	4.92 ± 2.35	7.18 ± 3.88	6.82 ± 3.94	0.020	4.92 ± 2.35	7.20 ± 3.86	6.77 ± 3.98	0.057	4.92 ± 2.35	7.20 ± 3.86	6.77 ± 3.98	0.005
Oocytes retrieved	6.14 ± 4.14	7.43 ± 4.15	6.65 ± 4.52	0.509	6.14 ± 4.14	7.42 ± 4.13	6.65 ± 4.57	0.606	6.14 ± 4.14	7.42 ± 4.13	6.65 ± 4.57	0.170
Oocyte maturation rate	0.79 ± 0.19	0.86 ± 0.15	0.81 ± 0.19	0.282	0.80 ± 0.16	0.83 ± 0.20	0.84 ± 0.14	0.168	0.84 ± 0.14	0.82 ± 0.19	0.82 ± 0.19	0.392
Oocytes fertilised	4.43 ± 3.12	5.08 ± 3.02	4.17 ± 3.26	0.105	4.43 ± 3.12	5.09 ± 3.00	4.12 ± 3.29	0.200	4.43 ± 3.12	5.09 ± 3.00	4.12 ± 3.29	0.131
Fertilization rate	67.40 (33.33-100)	72.35 (27.77-100)	70.60 (18.18-100)	0.375	68.69 (33.33-100)	70.70 (20-100)	72.71 (18.18-100)	0.188	73.38 (33.33-100)	70.06 (20-100)	69.25 (18.18-100)	0.040
No. of Grade 1 embryos	1.29 ± 1.50	2.16 ± 1.21	2.48 ± 2.64	0.557	1.29 ± 1.50	2.17 ± 1.21	2.47 ± 2.67	0.700	1.29 ± 1.50	2.17 ± 1.21	2.47 ± 2.67	0.173
Embryos transferred	2.87 ± 1.07	2.97 ± 0.97	2.41 ± 1.04	0.364	2.87 ± 1.07	2.98 ± 0.97	2.37 ± 1.02	0.086	2.87 ± 1.07	2.98 ± 0.97	2.37 ± 1.02	0.068
Implantation rate	11.17 (0-100)	14.55(0 -133.33)	16.86 (0-100)	0.666	11.17 (0-75)	14.68 (0-133.33)	16.66 (0-100)	0.320	13.82 (0-75)	11.50 (0-100)	20.62 (0-133.33)	0.127
Pregnancy: n(%)	12/41 (28.57)	28/83 (31.86)	13/41 (33.33)	0.880	13/41 (30.23)	25/84 (28.88)	15/40 (38.09)	0.688	15/41 (34.88)	25/84 (29.21)	13/40 (32.55)	0.744

[Table/Fig-2]: IVF/ICSI Cycle Outcomes based on LH levels on Day 1, Day 5 and day of trigger hCG

1 follicle of size > 14 mm. Patients with low LH levels were given Inj. hMG 75 IU in addition to rFSH.

After satisfying the criteria to start the antagonist, patients received GnRh antagonist i.e; Inj Cetrorelix 0.25 mg (Ares-Serono, Geneva, Switzerland) or Inj Ganirelix (N.V. Organon, BH Oss, The Netherlands) subcutaneously, daily till the day of hCG trigger. When the leading follicle was >18 mm on follicular imaging, patients received Inj hCG (250 µg SC; Ovitrelle, Serono S.A.) 13000 IU S/C (2 ampoules of 250 µg) and oocyte retrieval was performed 35 hours later by trans-vaginal ultrasound– guided follicle aspiration.

After oocyte retrieval, oocytes were allowed to mature in vivo in culture media, and IVF/ICSI was performed and successful fertilization was documented after 16-18 hours. A maximum of four embryos were transferred after 2 or 3 days in culture, and the surplus transferable embryos were cryopreserved. The luteal phase was supported by daily Injections of Gestone (Nordic, Reading UK) vaginal administration of 200 mg micronized P beginning 2 days before Embryo Transfer (ET) and continuing until the day of performing serum β-hCG levels on 14th day of ET. Patients were divided, based on LH levels on Day 1 of rFSH stimulation (i.e Day 2 of cycle), Day 5 of rFSH injection and day of hCG trigger, into following groups: Group 1: LH < 25th percentile, Group 2: LH = 25th to 75th percentile and Group 3: LH > 75th percentile.

The primary outcome measure of interest in this study was pregnancy rate. Secondary outcome measures included the follicles of >18 mm, oocytes retrieved, oocyte maturation rate, oocytes fertilized, fertilization rate, number of high quality embryos, embryos transferred and the implantation rate.

Statistical analysis was done using SPSS 16. Results were expressed as mean ± SD. Differences between the three groups of patients were tested statistically by use of the Chi square test. P-value of 0.05 was considered statistically significant.

Of the 162 women who underwent 165 cycles, the patient characteristics [Table/Fig-1] were comparable in all the three LH groups with regard to age, BMI, basal FSH and E2 levels and the cause of infertility.

The average number of follicles >18 mm in size were significantly more in the patients with LH levels between 25th to 75th percentile group on Day 1, Day 5 and Day of trigger hCG. The fertilization rate was significantly better (p value= 0.04) in the patients with LH levels < 25th percentile on the day of trigger hCG. But the oocyte recovery rate, oocyte maturation rate and average number of best quality embryos, implantation and pregnancy were not affected significantly in all three LH level groups on Day 1, Day 5 and Day of trigger hCG [Table/Fig-2].

DISCUSSION

In our retrospective study of 165 GnRH-antagonist IVF cycles performed over a one year period, we found that the varying LH levels did not have an impact on the final cycle outcome. We found that the number of follicles reaching more than 18 mm were significantly more when the LH value is between 25th to 75th percentile. In a study by Bosch et al., [7] the author did not find any difference in the number of dominant follicles in groups having premature luteinization and the other group with no premature luteinization and their study LH values in both the groups were comparable. But in our study progesterone factor was not studied. Merviel et al., [8] found that follicles >15mm were more in the group with LH <0.5 IU/L. They also found that oocyte retrieval rate and the better embryos were seen in the same group, contrary to our study where we did not find any difference between different groups of LH. However, they also did not find any difference in the implantation and pregnancy rate which is similar to our study.

In a similar study by Bosch et al., [9] found there was no difference

in the number of oocytes retrieved, fertilization rate, number of embryos transferred, implantation rate and pregnancy rate in various LH groups, but they had commented on the number of mature follicles.

Nicole et al., [10] in their study state that a transient elevation of LH in a patient undergoing IVF with a GnRH antagonist protocol does not impair clinical pregnancy rate per ET and therefore, should not be a reason to cancel a cycle. In our study also, we did not have any cycle cancellations based on the LH values.

Hence, it demonstrates that the serum LH levels detected during an IVF-ET cycle with rFSH and a GnRH antagonist are not indicative of IVF–ET cycle outcome in normogonadotropic women in terms of oocyte retrieval rate, oocyte maturation rate, fertilization rate, number of Grade 1 embryos, implantation rate and clinical pregnancy rate. This mainly accounts to the protocol of starting GnRH antagonist before the recruitment of LH receptors. Therefore, it's not mandatory to estimate serum LH levels during the IVF-ET cycles with antagonist protocol in patients having normal hormonal profile.

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