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Influence of Late Follicular Luteinizing Hormone Concentrations on Ovarian Response and Fertilization Outcome in Patients Undergoing in Vitro Fertilization with a Short Protocol

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The objective of this study was to investigate the effect of luteinizing hormone levels on the day of human chorionic gonadotropin administration (late follicular phase) on ovarian response and pregnancy outcome in women receiving pituitary down-regulation with gonadotropin releasing hormone-agonist administration in a short protocol. Blood samples were collected from 74 women undergoing assisted reproduction and analyzed retrospectively. Based on luteinizing hormone levels on the day of human chorionic gonadotropin administration, patients were divided into two groups (<5 and ≧5 IU/L). The number of follicles ≧15 mm in size and fertilized oocytes showed a highly significant correlation with luteinizing hormone concentration on the day of human chorionic gonadotropin administration. The levels of luteinizing hormone in the late follicular phase had a significant impact on the ovarian response and the quality of retrieved oocytes.

Key words: GnRH agonist, luteinizing hormone, ovarian response, pituitary down-regulation, short protocol

INTRODUCTION

Normal follicular growth is controlled by pituitary secretion of FSH and LH. Both two hormones are essential for normal follicular estradiol biosynthesis and described by the two-cell two-gonadotropin model[1,2]. LH stimulates androgen production in the theca cells, which are aromatized into estradiol under the action of FSH in the granulosa cells. During the midfollicular phase, granulosa cells gain LH receptors in addition to FSH receptors, and both are of importance for pre-ovulatory estradiol production and the production of competent oocytes[3].

It is well-known that LH levels which are too high or too low levels during the follicular phase may exert adverse effects on normal follicular maturation and the possibility of achieving pregnancy[4,5]. The importance and the amount of LH necessary for optimal follicular stimulation have been topics of debate[6]. Among several protocols currently used for IVF programs, the long protocol is the most commonly used. However, the short protocol has been recognized as effective and convenient. The administration of GnRH agonist in the short protocol has a dual effect: 1) an initial release of endogenous gonadotropins which participate in follicular recruitment, and 2) a subsequent decline in gonadotropin secretion related to hypophyseal desensitization. The latter effect starts shortly after the flare-up effect of GnRH agonist, lasts as long as its administration is continued, and prevents an endogenous LH surge[7].

The goal of the present study was to further evaluate the ovarian response and pregnancy outcome in relation to LH levels on the day of hCG administration (late follicular phase) in patients receiving pituitary down-regulation with GnRH-agonist administration with a short protocol.

METHODS

Patients and hormonal treatment

We performed a retrospective review of the medical charts of 74 women undergoing IVF or ICSI at the Tri-

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Service General Hospital between January 2002 and June 2008. The indication for IVF or ICSI and embryo transfer included tubal factor, male factor, idiopathic infertility, endometriosis, mixed diagnosis, and ovulation defects. Controlled ovarian hyperstimulation (COH) was conducted using the short protocol, down-regulation with GnRH agonist (leuprolide acetate; Grunenthal GmbH, Stolberg, Germany), followed by daily stimulation with an individual dose of SC recombinant FSH. Transvaginal ultrasound was performed to monitor follicular growth and the dose of FSH administered was adjusted if necessary. HCG (Pregnyl, 10,000 IU; N.V. Organon, Oss, Holland) was administered IM as a single bolus to induce final follicular maturation on the day the diameter of the leading follicle was at least 18 mm and at least 3 follicles ＞10 mm were present. Oocyte retrieval was performed 35 hours later by transvaginal ultrasound-guided follicle aspiration. Embryos were transferred on days 3 or 5 after retrieval. Luteal phase support was initiated after oocyte retrieval by daily administration of progesterone. A clinical pregnancy was defined as an intrauterine gestational sac with a fetal heartbeat 3 weeks after a positive hCG test. The implantation rate was defined as the ratio of gestational sacs determined by ultrasound after 7 weeks in relation to the total number of embryos transferred.

Blood samples and hormone assays

Blood samples were taken on stimulation day 2 and on the day of hCG administration. The sera were immediately separated and analyzed. Serum LH was quantified by using a two-site sandwich immunoassay and direct chemiluminescence technology (Bayer, Tarrytown, NY, USA). Serum estradiol was quantified using a competitive immunoassay and direct chemiluminescence technology (Bayer). Interassay coefficients of variation (CVs) of the LH assay were 6.2%, 5.2%, and 6.2% at concentrations of 7.2, 36.5, and 96.5 IU/mL, respectively. Interassay CVs of the estradiol assay were 9.8%, 4.2%, and 8.7% at concentrations of 68, 234, and 771 pg/mL, respectively. The detection limit of LH and estradiol was 0.07 IU/L and 10 pg/mL, respectively.

Statistical methods

The SPSS program, version 13 for Windows (SPSS, Chicago, IL, USA) was used for statistical analyses. Most values are expressed as the mean ±SEM. Statistical differences were evaluated using analysis of χ^2 test or linear regression analysis, as appropriate. The significance level was defined as $P < 0.05$.

Table I Patient characteristics related to serum LH concentrations on day of hCG administration

	Concentration of LH (IU/L) on day of hCG administration	
	<5	≧5
No. of cycles	45	29
Age (years)[a]	35.8±0.7	35.9±1.0
Infertility diagnosis:		
No. with tubal factor (%)	11 (24.4%)	7 (24.1%)
No. with male factor (%)	21 (46.7%)	12 (41.4%)
No. with idiopathic infertility (%)	2 (4.4%)	1 (3.4%)
No. with endometriosis (%)	1 (2.2%)	1 (3.4%)
No. with mixed diagnosis (%)	8 (17.8%)	6 (20.7%)
No. with ovulation defect (%)	2 (4.4%)	2 (6.9%)
No. of previous IVF attempts		
None (%)	23 (51.1%)	19 (65.5%)
One (%)	12 (26.7%)	4 (13.8%)
Two or more (%)	10 (22.2%)	6 (20.7%)

[a] values are given as the mean ± SEM.

RESULTS

Based on LH measurements on the day of hCG administration, women were allocated to two groups: <5 IU/L and ≥5 IU/L. Forty-five patients (61%) had a mean day of hCG administration <5 IU/L and 29 patients (39%) had a mean day of hCG administration ≥5 IU/L.

The patient characteristics as a whole, and the patient characteristics divided into two groups according to the LH levels on the day of hCG administration, are shown in Table I. There were no differences among the groups regarding infertility diagnoses, number of previous IVF/ICSI attempts, and age.

The number of follicles≧15 mm in size were significantly increased in the LH<5 IU/L group (P<.05), as shown in Table II. The consumption of FSH, the duration of gonadotropin stimulation, and the level of estradiol on the hCG day did not differ between the two groups.

The number of fertilized oocytes was significantly increased in the LH < 5 IU/L group (P < .05), as shown in Table II. The number of retrieved oocytes was more in the group with LH < 5 IU/L than the group with LH≧5 IU/L (10±1.2 vs. 6.5±1.0); however, this difference did not reach statistical significance. The rate of fertilization, number of pre-embryos transferred, number of clinical pregnancies, and the implantation rate of embryos did not differ between the two groups.

Table II Outcome of ovarian response, oocyte retrieval, fertilization, and pregnancy related to serum LH concentrations on day of hCG administration

	Concentration of LH (IU/L) on day of hCG administration	
	<5	≧5
No. of cycles	45	29
Total FSH (IU)[a]	2908.3±173.8	2697.1±182.3
Duration of FSH (d)[a]	10.1±0.3	9.5±0.3
Estradiol (pg/mL, hCG day)[a]	3124.7±328.9	2656.5±342.7
No. ≧15 mm follicle[a]	6.1±0.51[b]	4.2±0.6
No. oocytes retrieved per cycle[a]	10.0±1.3	6.5±1.0
No. of fertilized oocytes per cycle	7.6±1.0[b]	4.6±0.8
Rate of fertilization (%)[a]	76.4±2.8	72.8±5.4
Embryos transferred[a]	3.5±0.2	2.9±0.3
Implantation rate (%)[a]	8.8±2.4	7.9±3.8
No. of clinical pregnancies (% per cycle)	12 (27%)	6 (22%)

[a]values are given as the mean±SEM.
[b]vs. LH ≧5 group, P<0.05

DISCUSSION

The ovarian responses to FSH and LH occur in a sequential manner to produce follicular maturation from the small antral follicle stage to the pre-ovulatory follicle stage. At the beginning of each monthly menstrual cycle, follicle recruitment and growth depends on the serum level of FSH, below which follicular development will stop[8,9]. Although follicular growth can be induced by FSH to the pre-ovulatory stage in the total absence of LH, the resulting follicles have developmental deficiencies, such as abnormally low estradiol and inability to luteinize and rupture in response to hCG[10]. Optimal follicular development is therefore also dependent on a minimal exposure to LH or a LH threshold[6]. A dose finding study in patients with severe deficiency in LH and FSH has shown that the serum LH levels of >1.2 IU/L are necessary to provide adequate LH support to FSH-induced follicular development[11].

Several reports that LH was either absent or completely inactive have given important clues to our understanding of the role of LH in follicular development. In women with long-standing hypogonadotrophic hypogonadonism who were administered FSH alone without LH showed lower serum and follicular fluid estradiol concentrations, decreased endometrial thickness, reduced occurrence of ovulation, reduced oocyte fertilization rates, and lower embryo cryosurvival rates when compared with hMG treatment[12,13].

An LH surge was considered when the serum LH level > 10 IU/L. It is generally accepted that very high levels of LH in the follicular phase (>10 IU/L) are detrimental[6]. Some clinical studies have revealed correlations with prematurely increased LH concentrations, implantation failure, and early miscarriages[14,15].

In the present study, only two patients (2.7%) had LH concentrations < 1.0 IU/L, suggesting that GnRH-agonist treatment with the short protocol does not lead to profound suppression of LH. In the LH < 5 IU/L group, the number of follicles≧15 mm increased significantly compared with the LH≧5 IU/L group, suggesting LH levels affect the ovarian response. However, the consumption of exogenous FSH, the duration of gonadotropin stimulation and the level of estradiol on the day of hCG administration did not differ between the two groups.

Although our data showed the number of retrieved oocytes was greater in the group with a LH < 5 IU/L compared to the group with a LH≧5 IU/L, this difference was not significant between the two groups. The quality of the retrieved oocytes seemed to be affected by the LH levels on the day of hCG administration. The number of fertilized oocytes by which they underwent fertilization was significantly correlated to the LH level; the group with a LH < 5 IU/L performed significantly better than the group with a LH≧5.

The present study was unable to demonstrate differences in pregnancy outcomes when the LH cutoff volume of 5 IU/L on the day of hCG administration was used to create two groups. This is in contrast to the findings of Liu *et al*.[6], who found superior clinical pregnancy and implantation rates in the group of women with a LH day 8 < 5 IU/L compared with the ≧5 IU/L group. This discrepancy might be due to different time points (cycle day 8 vs. hCG day), a fewer number of cases (270 vs. 74) in our study, and a single measurement of LH on the day of hCG administration. A more detailed LH monitoring during the day and over time could provide more detailed information, since it cannot be excluded that short-term, intra-patient variations of LH secretion occur and that LH levels may vary during FSH treatment[4]. A Study by Nakagawa *et al*.[16], who used the ratio of late-follicular to mid-follicular phase LH concentrations, found a relative decrease in the LH concentrations (LH<1.0) during ovarian stimulation with recombinant FSH in a GnRH-agonist long protocol negatively affected ART outcomes. Further, it should be stressed that our study was not able to show whether LH is the mediator of pregnancy outcome or a marker.

In conclusion, the present study suggests that circulating levels of LH on the day of hCG administration have a significant impact on the ovarian response and further fertilization of oocytes. During the course of ovarian stimulation with GnRH-agonist administration with the short protocol, the LH concentrations should not be too high to obtain the best response of ovarian stimulation and fertilization, which in turn secure the best chance for successful treatment.

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