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Follicular Fluid Hormone Concentrations After Ovarian Stimulation Using Gonadotropin Preparations with Different FSH/LH Ratios. I. Comparison of an FSH-Dominant and a Purified FSH Preparation

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ABSTRACT: **Objective**—A small amount of LH is necessary for 17β -estradiol production in the ovarian follicle. Human menopausal gonadotropin (hMG) contains equal amounts of FSH and LH activity, whereas recombinant FSH is a gonadotropin preparation without LH. The aim of the present randomized study was to investigate whether ovarian stimulation treatment with recombinant FSH or hMG resulted in different steroidal composition of follicular fluid. **Methods**—Antral fluid from mature follicles was collected in in vitro fertilization cycles and concentrations of testosterone, androstenedione, estrone, estradiol, progesterone, FSH, and LH were determined. Seven patients (27 samples) were treated with hMG, 6 patients (22 samples) with recombinant FSH. **Results**—Androgen, estrogen, progesterone, and FSH concentrations in follicular fluid tended to be lower in the group treated with recombinant FSH, but the variation was large and differences were statistically not significant. **Conclusion**—Treatment with a gonadotropin preparation containing no LH resulted in adequate androgen and estrogen levels in antral fluid of the ovarian follicle in women with normal endocrine profiles, even during pituitary suppression by a GnRH agonist. Apparently, the amount of endogenous LH was sufficient for steroid production within the follicle. *Int J Fertil* 42(5):306–310, 1997

KEY WORDS: follicular fluid, hormone, IVF, gonadotropins, recombinant FSH

INTRODUCTION

RECOMBINANT HUMAN FSH (recFSH) is produced by a Chinese hamster ovary cell line transfected with the human FSH α - and β -subunit genes [1]. It is a pure FSH preparation, completely devoid of LH activity, in contrast with purified urinary FSH preparations. The development of recFSH has offered an opportunity to test the two-cell two-gonadotropin hypothesis. This hypothesis means that LH stimulates the production of androgens in the theca cells of the ovarian follicle, while FSH stimulates aromatization of the androgens into estrogens in the granulosa cells. The estrogens produced are then secreted into follicular fluid and into the blood circulation.

The two-cell two-gonadotropin theory has been confirmed by studies in hypophysectomized immature rats [2]. RecFSH treatment augmented ovarian weight, while serum 17β -estradiol concentrations remained low. A clinical study on a gonadotropin-deficient woman showed that recFSH administration stimulated multiple follicular growth, without a concomitant increase in serum estradiol levels [3]. Concentrations of androgens and estrogens in follicular fluid were extremely low. Thus, LH appeared to be necessary for adequate ovarian stimulation with correspondingly high serum estradiol concentrations.

Devroey et al [4] showed that in *in vitro* fertilization (IVF) cycles, during pituitary suppression with GnRH agonists (GnRHa), recFSH administration resulted in multiple follicular growth with adequate serum estradiol levels. So the low amount of endogenous LH, still present despite pituitary suppression by a GnRHa, apparently was sufficient for stimulation of the theca cells to produce androgens, which were aromatized into estradiol. Still, lower LH stimulation of the ovary might result in a decrease of androgen production by the thecal cells, lower estradiol production by the granulosa cells, and lower follicular fluid estradiol concentrations. In the present randomized study in IVF cycles, we compared steroid levels in the antral fluid of mature follicles after recFSH and hMG treatment. Because follicular fluid is the endocrine environment of the oocyte, differences in the steroidal composition might affect oocyte quality.

METHODS

Patients

Thirteen women undergoing IVF treatment for

tubal pathology or unexplained infertility participated in the study. The inclusion criteria were: age between 20 and 40 years; normal endocrine serum profile in the early follicular phase of the cycle (FSH concentration below 8 IU/L, LH/FSH ratio less than 3, testosterone concentration below 2.5 nmol/L, prolactin concentration below 700 mIU/L and thyroxin concentration between 58 and 148 nmol/L); no hormonal medication for at least two menstrual cycles prior to the study; no endometriosis observed on laparoscopy; both ovaries present; body weight between 80% and 130% of the ideal weight (according to the Metropolitan Height and Weight Tables); and no severe male subfertility of the partner (at least 20×10^6 sperm/mL, at least 30% motile sperm, motility grade at least fairly good, at least 40% morphologically normal sperm and absence of anti-sperm antibodies as confirmed by the direct immunobead test, or a fertilization rate of at least 50% if the patient had had IVF before).

The study was approved by the hospital's Ethical Committee, and all the women gave their informed consent.

Treatment Protocol

The women were randomly divided into two groups: 7 women were treated with hMG (Humegon, NV Organon, Oss, The Netherlands): Group A; 6 women received recFSH (Puregon, NV Organon): Group B. Both preparations were administered intramuscularly (i.m.) in a fixed daily dose of 150 IU.

In each group gonadotropin administration was combined with buserelin (Suprecur, Hoechst AG, Frankfurt, Germany) treatment which was started on day 1 of the menstrual cycle, in a daily dose of 600 μ g intranasally. On the fourteenth day of buserelin treatment the serum estradiol concentration was measured by time-resolved fluoroimmunoassay (DELFLIA, Wallac Oy, Turku, Finland). If estradiol was below 200 pmol/L, gonadotropin administration was started the next day. If the estradiol concentration was still higher than 200 pmol/L, administration of gonadotropins was postponed until the estradiol level was below 200 pmol/L. Buserelin administration was continued until the day of human chorionic gonadotropin (hCG) administration.

Transvaginal ultrasonography was performed every other day during the stimulation phase of the IVF cycle to measure follicular growth and endometrial thickness. The administration of gonadotropins was discontinued as soon as at least three follicles had a (largest) diameter ≥ 15 mm, the mean diameter of the largest follicle was ≥ 20 mm, and the endometrial thickness was ≥ 9 mm. On the day these criteria were fulfilled, 10,000 IU of hCG were administered i.m. between 11 P.M. and 12 midnight.

Transvaginal ultrasound-guided oocyte retrieval was performed 35 hours after the hCG injection. Follicular fluid from the four largest follicles was collected separately. In three patients, the fluid from only three follicles could be collected. The follicular fluid was not diluted with flushing medium or fluid from another follicle. The fluid was centrifuged and stored at -20°C until assayed. Embryo transfer was performed on the third day after oocyte retrieval. The luteal phase of the cycle was supported with progesterone, administered intravaginally.

Assays

Concentrations of estradiol and progesterone in follicular fluid were determined by RIA, as described by Thomas et al [5]. Concentrations of estrone [6], testosterone [7], and androstenedione [8] were determined by RIAs developed in this laboratory. Concentrations of FSH and LH were measured by IRMA procedures [9].

Statistics

Results are expressed as median values with tenth and ninetieth percentiles (P_{10} , P_{90}). Statistical analysis was performed using the two-sample Wilcoxon test (rank sum test). Correlations were detected by Spearman's rank correlation test. Differences with $P \leq .01$ were considered significant.

RESULTS

Characteristics of the IVF cycles are listed in Table I. Median serum LH concentrations on the day of hCG administration were equal in the two groups, so administration of exogenous LH in the case of

TABLE I
Cycle characteristics of the study groups.

	Group A	Group B
Treatment	Humegon	Puregon
No. patients	7	6
No. treatment days	11 (10, 11)	10 (9, 11)
No. follicles ≥ 15 mm*	9 (5, 15)	12 (10, 17)
Serum estradiol (pmol/L)*	6,100 (3,660, 15,400)	7,550 (5,400, 11,450)
Serum LH (IU/L)*	1.4 (1.3, 1.8)	1.4 (0.9, 2.8)
No. oocytes	7 (2, 18)	11 (9, 18)
Fertilization rate (%)	86 (43, 100)	73 (62, 89)

Median values, tenth, and ninetieth percentiles in parentheses.

*On the day of hCG administration.

Group A did not result in an increase in serum LH levels of this group as compared to the Puregon group. The total number of follicular fluid samples was 27 in Group A and 22 in Group B. Median values of the hormone concentrations in the three or four collected follicular fluid samples were calculated for each patient. The median value of these individual median concentrations was determined

TABLE II
Median values of individual median concentrations of hormones in follicular fluid after ovarian stimulation treatment with Humegon (7 women) or Puregon (6 women).

	Humegon	Puregon
No. of samples	27	22
Testosterone (nmol/L)	14.5 (9.4, 23.5)	11.0 (6.1, 20.0)
Androstenedione (nmol/L)	33 (16, 80)	19 (14, 77)
Estradiol ($\mu\text{mol/L}$)	2.4 (1.3, 3.1)	1.4 (0.7, 3.2)
Estrone (nmol/L)	145 (66, 240)	125 (54, 245)
Progesterone ($\mu\text{mol/L}$)	40 (18, 57)	31 (20, 39)
LH (IU/L)	<1.0 (<1.0, <1.0)	<1.0 (<1.0, <1.0)
FSH (IU/L)	2.2 (1.9, 3.5)	1.7 (1.2, 4.5)

Tenth and ninetieth percentiles are given in parentheses.

in each group. The results are listed in Table II.

In both groups, high levels of androgens and estrogens were present in follicular fluid. Testosterone, androstenedione, estradiol, estrone, progesterone, and FSH levels tended to be lower in Group B than in Group A, but inter- and intra-individual variations were rather large, and the observed differences were not statistically significant. LH concentrations in follicular fluid were undetectably low in both groups, so administration of exogenous LH did not result in measurable LH immunoreactivity in antral fluid. No significant correlations were detected between the individual fertilization rates and median concentrations of testosterone, androstenedione, androgens (testosterone plus androstenedione), estradiol, estrone, estrogens (estradiol plus estrone), progesterone, the androgen/estrogen ratio, or the progesterone/estradiol ratio in follicular fluid.

DISCUSSION

Administration of exogenous LH did not result in an increase of serum LH levels in the group treated with hMG, probably because of the short half-life of LH contained in this preparation [10]. However, binding to the LH receptors in the theca interna might occur before elimination from the circulation has occurred. Differences in LH binding in the ovary might influence the steroidal composition of follicular fluid, and subsequently affect oocyte quality.

Theoretically, one would expect that more LH stimulation of the ovary would lead to an increase of the estradiol concentration in follicular fluid for several reasons. First, LH stimulates androstenedione production in the theca interna [11]. Androstenedione is converted to testosterone or to estrone and then to estradiol in the granulosa cells by 17 β -hydroxy-steroid dehydrogenase and aromatase. Next, estradiol is secreted into the follicular fluid [12]. Thus, an increase in androstenedione concentrations would lead to higher follicular fluid estradiol levels. Secondly, LH stimulates aromatase activity of granulosa cells from mature follicles [13]. Higher testosterone and estradiol concentrations also effect FSH enhancement of aromatase activity [14,15], which would further increase estradiol production by the granulosa cells and thus estradiol levels in follicular fluid. Estradiol in turn enhances the LH stimulation of androgen pro-

duction [16]. All described effects of LH point in the same direction: more LH stimulation of the ovary invariably leads to higher estradiol concentrations in follicular fluid. LH also increases progesterone production in mature granulosa cells [13], which will result in higher progesterone concentrations in follicular fluid. The progesterone produced will serve as a potential precursor for androgen synthesis by the theca cells, and the androgens in turn are further aromatized to estrogens by the granulosa cells [17].

In the group treated with recFSH (Group B), androstenedione, estrone, estradiol, and progesterone concentrations in follicular fluid tended to be lower than in the group treated with hMG (Group A). This supports the theory of shortness of LH activity leading to a decrease of androgen, estrogen and progesterone production. However, there appears to be a large inter- and intra-individual variation, and the differences are statistically not significant. FSH concentrations in follicular fluid were slightly lower in Group B than in Group A. FSH stimulates aromatase activity, so lower FSH levels will result in a decrease in estradiol production [18]. Furthermore, FSH induces granulosa cell receptors for LH [19,20], making granulosa cells more responsive to LH stimulation of aromatase activity. The lower FSH levels in follicular fluid in Group B might, therefore, also be partly responsible for the slightly lower follicular fluid estrogen concentrations in Group B.

In conclusion, of women with normal endocrine profiles, treatment with recFSH resulted in adequate androgen and estrogen levels in antral fluid of the ovarian follicle, even during pituitary suppression by a GnRH-agonist. In gonadotropin-deficient women, androgen and estrogen levels in follicular fluid remained very low during recFSH treatment, due to the absence of LH [3]. In our study group, however, endogenous LH levels appeared to be sufficient to stimulate androgen production by the theca cells. Androgen and estrogen concentrations in follicular fluid were only slightly lower after recFSH treatment than after hMG treatment, so the endocrine environment of the oocyte will be quite similar. The observed minor differences were not statistically significant. It is proposed to conduct a larger study to provide evidence whether androgen and estrogen concentrations were indeed lower when no exogenous LH was administered.

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