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# Follicular Fluid Hormone Concentrations During Controlled Ovarian Hyperstimulation 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**—The aim of the present randomized study was to investigate whether ovarian stimulation treatment with gonadotropin preparations containing different amounts of LH activity resulted in variations of steroidal composition of follicular fluid. A different endocrine milieu within the follicle might influence oocyte quality. **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. A comparison was made between treatment with a purified FSH preparation (nine patients, 35 follicular fluid samples) and a FSH-dominant human menopausal gonadotropin (hMG) preparation (nine patients, 34 samples). **Results**—No differences in any of the hormone levels could be detected between the two groups. **Conclusion**—Treatment with gonadotropin preparations containing different FSH/LH ratios did not result in different androgen, estrogen and progesterone levels in follicular fluid. *Int J Fertil* 42(6):426-430, 1997

**KEY WORDS:** follicular fluid, hormone, IVF, gonadotropins, hMG

## INTRODUCTION

**T**HE GENERALLY ACCEPTED THEORY for the mechanism of estradiol production by the ovary is the two-cell two-gonadotropin concept [1]. This means that LH stimulates androgen synthesis in cells of the theca interna around the ovarian follicle [2]. Thecal androgens are transported to the granulosa cells, where they are aromatized into estrogens [3]. FSH stimulates the necessary aromatase activity in the granulosa cells [4,5]. The estrogens are secreted into the follicular fluid and the blood circulation. As the follicle matures, the estradiol concentration in follicular fluid increases significantly.

If ovarian hyperstimulation treatment is applied, for instance in in vitro fertilization (IVF) cycles, different stimulation protocols can be used. In general, the administration of a gonadotropin preparation is preceded by and combined with administration of a GnRH agonist (GnRHa). Several gonadotropin preparations containing FSH and LH activity in various amounts can be applied for ovarian stimulation. In the present study, we investigated whether treatment with gonadotropic preparations containing different amounts of LH activity resulted in a different steroid composition in the antral fluid of mature follicles. Thus, we tested whether lower amounts of exogenous LH led to a decrease in androgen production by the thecal cells, to lower estradiol production by the granulosa cells, and to lower follicular fluid estradiol concentrations. Differences in the endocrine milieu of the oocyte might influence oocyte quality. In a randomized study in IVF patients, treatment with purified urinary FSH was compared with a gonadotropic preparation comprising FSH and LH activity in a ratio of 3:1. Concentrations of androgens, estrogens, progesterone, FSH, and LH in follicular fluid were compared.

## METHODS

### Patients

Twenty 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 concen-

tration 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, and no male subfertility (at least  $20 \times 10^6$  sperm/mL, at least 50% motile sperm, motility grade at least fairly good, at least 60% 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: one group of ten women was treated with purified urinary FSH (ratio FSH/LH activity >60, Metrodin, Serono, Pharma-Import BV, Haarlem, The Netherlands): Group A; the other group of ten women was treated with a preparation containing FSH and LH activity in a ratio of 3:1 (Normegon, NV Organon, Oss, The Netherlands): Group B. Each preparation was administered intramuscularly (i.m.) in a fixed daily dose of 150 IU. Details about IVF results and serum hormone levels were published previously [6].

Gonadotropin administration was combined with buserelin (Suprefact, Hoechst AG, Frankfurt, Germany) treatment, which started on day 21 of the menstrual cycle, in a daily dose of 900  $\mu$ g intranasally. On the tenth day of buserelin treatment, the serum estradiol concentration was determined by time-resolved fluoroimmunoassay (DELFA, Wallac Oy, Turku, Finland). If the estradiol concentration was below 500 pmol/L, administration of gonadotropins was started the next day; if the serum estradiol concentration was still higher than 500 pmol/L, the administration of gonadotropins was postponed until it was below 500 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. Serum

estradiol concentrations were determined by the time-resolved fluoroimmunoassay procedure. The administration of gonadotropins was discontinued as soon as at least three follicles were present with a (largest) diameter  $\geq 15$  mm, one of which had a diameter  $\geq 20$  mm, and the serum estradiol concentration was approximately 1,000 pmol/L per large follicle ( $\geq 15$  mm). In one patient of each group the IVF treatment had to be canceled before oocyte retrieval because of a significant drop in the estradiol level.

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, 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 for 10 minutes at 2,000  $\times g$  and stored at  $-20^{\circ}\text{C}$  until assayed. Embryo transfer was performed on the third day after oocyte retrieval. The luteal phase of the cycle was supported by i.m. hCG injections.

### Assays

Concentrations of estradiol and progesterone in follicular fluid were determined by radioimmunoassays (RIA), as described by Thomas et al [7]. Concentrations of estrone [8], testosterone [9], and androstenedione [10] were determined by RIAs developed in this laboratory. Concentrations of FSH and LH were measured by immunoradiometric assay (IRMA) procedures [11].

### Statistics

Results are expressed as median values with tenth and ninetieth percentiles. 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. The two canceled cycles were not included in the evaluation.

### RESULTS

Table I lists the cycle characteristics of the two groups. Serum LH concentrations were higher in

**TABLE I**  
Cycle characteristics of the study groups.

	Group A	Group B
Treatment	Metrodin	Normegon
No. of patients	9	9
No. of treatment days	9 (9, 15)*	8 (7, 10)*
No. follicles $\geq 15$ mm <sup>†</sup>	6 (5, 11)	9 (4, 19)
Serum estradiol (pmol/L) <sup>†</sup>	5,700 (2,200, 9,800)	7,500 (4,240, 13,400)
Serum LH (IU/L) <sup>†</sup>	1.6 (1.1, 2.9)*	3.2 (1.7, 4.0)*
No. oocytes	8 (6, 11)	13 (5, 21)
Fertilization rate (%)	67 (22, 100)	63 (14, 100)

Median values, tenth, and ninetieth percentiles in parentheses.

\*.01 <  $P$  < .05

<sup>†</sup>On the day of hCG administration.

Group B than in Group A on the day of hCG administration (.01 <  $P$  < .05), which might be an effect of administration of exogenous LH in Group B. This observation is rather unexpected, since the half-life of LH is very short [12]. The total number of follicular fluid samples was 35 in Group A and 34 in Group B. Median values of the hormone concentrations in the three or four follicular fluid samples collected were calculated for each patient. The median value of these individual median concen-

**TABLE II**  
Median values of individual median concentrations of hormones in follicular fluid after ovarian stimulation treatment with Metrodin (9 women) or Normegon (9 women).

	Metrodin	Normegon
Number of samples	35	34
Testosterone (nmol/L)	13.5 (8.0, 19.5)	13.0 (8.2, 15.0)
Androstenedione (nmol/L)	57 (23, 144)	42 (26, 153)
Estradiol ( $\mu\text{mol/L}$ )	2.6 (1.4, 3.8)	2.6 (1.5, 3.7)
Estrone (nmol/L)	150 (104, 204)	160 (120, 263)
Progesterone ( $\mu\text{mol/L}$ )	33 (23, 52)	32 (19, 53)
LH (IU/L)	<1.0	<1.0
FSH (IU/L)	1.6 (1.3, 2.2)	1.7 (1.0, 2.7)

Tenth and ninetieth percentiles are given in parentheses.

trations was determined in each group. The results are listed in Table II. There were no significant differences between the two groups for any of the hormones determined.

Follicular fluid steroid levels were considerably higher than serum steroid levels. Androstenedione and testosterone concentrations were about ten times higher than the normal serum concentrations. Estrone, estradiol, and progesterone concentrations were about one thousand times higher than serum levels. The elevated progesterone levels indicated that the process of luteinization following hCG administration had already started. Concentrations of FSH were lower than in serum, and LH concentrations in follicular fluid were undetectably low.

No significant correlations were found between the individual fertilization rates (percentage fertilized oocytes) and median concentrations of testosterone, androstenedione, estrone, estradiol, androgens (testosterone plus androstenedione), estrogens (estrone plus estradiol), the androgen/estrogen ratio, and the progesterone/estradiol ratio. A positive correlation existed between the fertilization rate and progesterone concentrations in Group B ( $P < .01$ ), but not in Group A.

## DISCUSSION

The two gonadotropic preparations used in the present study contain different amounts of LH activity. Reducing the amount of exogenous LH may result in less LH stimulation of the ovary. This might affect the rate of androgen production by the theca cells and subsequent estrogen production in the granulosa cells. Lower amounts of exogenous LH may, therefore, result in a decrease of androgen and estrogen levels in follicular fluid.

In both study groups, high androgen and estrogen levels were present in the follicular fluid. Comparing treatment with purified FSH (Group A) and a FSH-dominant hMG preparation (Group B), no differences were seen in the median concentrations of steroids and of FSH and LH. No evidence for a decrease in follicular fluid estradiol concentrations after treatment with the preparation with the lowest LH content was found. Thus, the endocrine milieu of the oocyte appeared to be similar in the two groups.

However, a comparison between the two groups can only be made with certain reservations. One of

the criteria for hCG administration was a serum estradiol concentration of 1,000 pmol/L per large follicle. If, as we already stated, the estradiol production was indeed lower in the group with the lower exogenous LH administration (Group A), serum estradiol levels would also be lower. Since the serum estradiol level was required to reach a certain minimum before hCG was administered, this would mean that oocyte retrieval would be postponed, and the follicles would be more mature at the time of ovum pickup. Indeed, the number of treatment days was higher in Group A than in Group B. Since maturation of the follicle has an impact on the steroidal composition of the follicular fluid [13], this could mean that the two groups were not completely comparable.

Several studies on the steroidal composition of follicular fluid in IVF cycles have been reported [14–17]. Generally, in comparison with our results, similar values of estradiol, androstenedione, testosterone, and progesterone were found. Only minor differences between the results of the studies were observed, but these may be caused by the use of different RIAs and by variations in the stimulation protocol. Suchanek et al [16] reported mean FSH levels in follicular fluid (10.6 IU/L) that were higher than those in our study. The discrepancy may be explained by the assay used: they applied a RIA, while we used an IRMA.

In summary, in the present study, follicular fluid steroid concentrations were similar after ovarian stimulation with either a FSH-dominant or a purified FSH preparation.

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