

Serum and follicular fluid leptin levels in patients undergoing controlled ovarian hyperstimulation for in vitro fertilization cycle

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Summary

Objective: To determine serum and follicular fluid leptin levels in patients undergoing controlled ovarian hyperstimulation (COH) for an in vitro fertilization-embryo transfer (IVF-ET) cycle and their possible correlation to COH variables.

Setting: Large university-based IVF unit.

Patients: 16 consecutive patients undergoing our routine IVF long gonadotrophin-releasing hormone-analog protocol.

Interventions and main outcome measures: Blood was drawn three times during the COH cycle: 1) day on which adequate suppression was obtained (Day-S); 2) day of or prior to human chorionic gonadotrophin (hCG) administration (Day-hCG); and 3) day of ovum pick-up (Day-OPU). Levels of sex steroids and serum and follicular fluid leptin were compared among the three time points. Serum leptin was measured with a commercial two-site immunoradiometric assay.

Results: Results showed significantly higher levels of serum leptin on Day-OPU and Day-hCG than on Day-S, and significantly higher follicular than serum leptin levels on Day-OPU. Though a significant correlation was observed between serum leptin and body mass index (BMI), no correlations were found between serum or follicular fluid leptin and serum sex-steroid levels or IVF treatment variables.

Conclusion: While serum leptin increases during COH for IVF, there is apparently no correlation of serum and follicular leptin levels with sex-steroid levels or IVF outcome.

Key words: Leptin; Follicular fluid; Ovulation induction; Sex steroids; BMI.

Introduction

Leptin is a hormone product of the *obese (ob)* gene. It is secreted by adipose tissue [1] and its level is positively correlated with the amount of total body fat mass [2, 3]. Leptin plays a central role in the regulation of body weight and energy homeostasis and in signalling the brain that adequate energy stores are available for reproduction [4]. It is also expressed in several reproductive tissues and also modulates various reproductive functions which are mediated mainly through the hypothalamic-pituitary-gonadal axis [5-7]. Although, recent observations indicate that leptin may have direct intraovarian actions, its role in the control of reproductive physiology is still not well understood.

The aim of the present prospective study was to longitudinally investigate serum and follicular fluid leptin levels during controlled ovarian hyperstimulation (COH) and to examine whether they correlate with serum sex-steroid levels or other COH variables.

Patients and Methods

The study population consisted of 16 consecutive patients attending the in vitro fertilization (IVF) unit of our department for

treatment of infertility (anovulatory-4, male factor-5, unexplained-5, mechanical-2). The study required no modification of our routine IVF protocols. Briefly, patients were pretreated with gonadotrophin-releasing hormone agonist (GnRH-a) in the mid-luteal phase using a long protocol. Fifteen days later, when adequate suppression was obtained, the patients underwent ovarian stimulation with human menopausal gonadotrophin (hMG) (Pergonal, Teva, Petah Tiqva, Israel). The gonadotrophin dosage was adjusted individually according to serum estradiol (E_2) levels and vaginal ultrasound measurements of follicular diameter, obtained every one or two days. Human chorionic gonadotrophin (hCG) (Chorigon, Teva, Petah Tiqva, Israel), 10,000 IU, was administered when the leading follicles reached a minimum of 18 mm in diameter, with a peak serum E_2 level of > 2000 pmol/l. Oocytes were aspirated by the transvaginal ultrasonographic route approximately 34 hours after hCG injection. Clear follicular fluid was collected, centrifuged for 10 min at 1000 g, and then stored in aliquots at -20°C until assayed.

For the purpose of the study, in addition to the routine monitoring during the COH cycle, blood samples were drawn to determine the hormonal profile (E_2 , progesterone and hCG) and serum leptin levels at three time points: 1) day on which adequate suppression was obtained (Day-S); 2) day of or prior to hCG administration (Day-hCG); and 3) day of ovum pick-up (Day-OPU).

For serum leptin determination, blood samples were centrifuged for 10 min at 1000 g, and the plasma was stored in aliquots at -20°C until assayed. Serum leptin was measured in duplicate with a commercial two-site immunoradiometric assay (Diagnostic Systems Laboratories, Inc. Texas, USA). All samples were assayed at one time to avoid inter-assay variations. The

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minimal sensitivity of the assay was < 0.10 ng/ml and the intra- and interassay variability were 4.9% and 6.6%, respectively. Blanks and controls were included in all experiments.

Informed consent was obtained from all patients before participation in the study, and the study was approved by the Clinical Research Committee.

Data were analyzed with the SPSS for Windows (SPSS version 10.0) statistical package. The results are expressed as means \pm standard deviations or rates. Comparisons between groups were performed with the Wilcoxon test or the Friedman test for several related samples (repeated measures) for continuous data, and chi-square Fisher's exact test for categorical data. Simple and multiple linear regression and stepwise analyses were performed to evaluate the correlation between variables and to calculate the prediction equation. Log transformation of the data was performed when values had a skewed distribution. A *p* value of 0.05 or less was considered significant.

Results

Mean age of the 16 patients was 35 ± 5.2 years, and mean body mass index (BMI) was 25.4 ± 4.1 . Mean number of gonadotropin ampoules used during the COH cycle was 37.2 ± 14.4 , mean number of oocytes retrieved 13.9 ± 8.9 and mean fertilization rate, $59 \pm 24\%$. Pregnancy rate was 31.25%.

Mean serum E_2 , progesterone and serum leptin levels on Day-S, Day-hCG and Day-OPU, and follicular fluid leptin level on Day-OPU are presented in Table 1. As expected, serum E_2 and progesterone levels were significantly higher on Day-OPU than Day-S ($p < 0.01$ for both). Serum E_2 level was significantly higher on Day-hCG than Day-OPU ($p < 0.01$), whereas serum progesterone was significantly lower ($p < 0.01$).

Serum leptin levels were significantly higher on Day-OPU and Day-hCG than Day-S ($p < 0.013$) (Table 1). Furthermore, follicular fluid leptin level was significantly higher than serum leptin level on Day-OPU ($p < 0.007$). Figure 1 presents the E_2 , progesterone and serum and follicular fluid leptin levels during the cycle. No significant correlations were observed between serum and follicular fluid leptin and E_2 or progesterone levels.

BMI significantly correlated with serum leptin levels on Day-S (Pearson correlation, $R^2 = 0.432$, $p < 0.006$), Day-hCG ($R^2 = 0.453$, $p < 0.004$), and Day-OPU ($R^2 = 0.555$, $p < 0.001$), and with follicular fluid leptin level on Day-OPU ($R^2 = 0.523$, $p < 0.001$) (Figure 2).

There were no significant correlations of serum leptin level or ratios of serum leptin to BMI ratio with patient

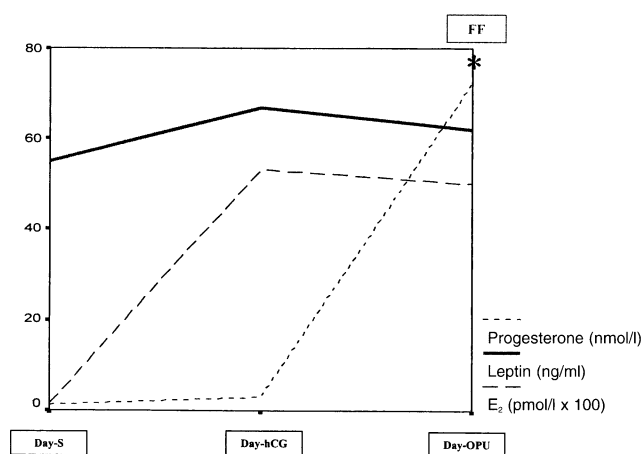


Figure 1. — Serum sex steroids and serum and follicular fluid leptin levels during COH.

age, cause of infertility, amount of menotropin used, number of oocytes retrieved, or fertilization or pregnancy rates. In a linear regression model and stepwise analysis controlling for patient age, BMI, E_2 and leptin levels as independent variables, only E_2 level on Day-hCG was independently correlated with the number of oocytes retrieved ($R^2 = 0.587$, $p < 0.004$).

Discussion

The present study shows that serum leptin level is significantly increased during COH until peak E_2 is reached, with no significant difference after hCG administration. Furthermore, follicular fluid leptin levels were significantly higher than serum levels. While serum leptin level significantly correlated, as expected with patients' BMI, there was no correlation between serum leptin and IVF treatment variables or pregnancy rate.

The well-established correlation between serum leptin and BMI demonstrated in our study, was also reported recently by Urbancsek *et al.* [8], who found no correlation between area under the curve (AUC) levels of leptin and ovarian hormones. Our finding of a lack of correlation between leptin and sex-steroid levels is supported by the study of Tsai *et al.* [9] which examined the relationship between estradiol concentrations and leptin levels at the time of hCG injection and oocyte retrieval. Stock *et al.* [10] also noted a positive correlation between BMI and leptin levels, an increase in leptin levels throughout the treatment cycle, and no correlation between leptin and progesterone levels. Butzow *et al.* [11] reported a 20-fold increase in serum estradiol concentrations during IVF, but could not demonstrate any significant correlation to changes in leptin concentrations. In contrast, Zhao *et al.* [12] demonstrated that serum leptin concentrations during stimulated cycles correlated positively with both BMI and estradiol concentrations.

The increase in serum leptin level during ovarian hyperstimulation noted here, is consistent with previous reports by other groups [10-18]. Although the exact

Table 1. — Hormonal profile and soluble L-selectin levels of the study patients.

	Day-S	Day-hCG	Day-OPU
Estradiol (pmol/l)	149 \pm 55	5315 \pm 2172	4997 \pm 3381
Progesterone (mmol/l)	1.37 \pm 0.72	1.42 \pm 0.8	72.3 \pm 39.5
Serum leptin (ng/ml)	54.6 \pm 27.7	66.9 \pm 35.0	66.8 \pm 38.2
FF leptin (ng/ml)			75.7 \pm 38.3

All values are mean \pm SD. Day-S = day in which adequate suppression was achieved; Day-hCG = day of or day prior to human chorionic gonadotropin administration; Day-OPU = day of oocyte pick-up; FF = Follicular fluid.

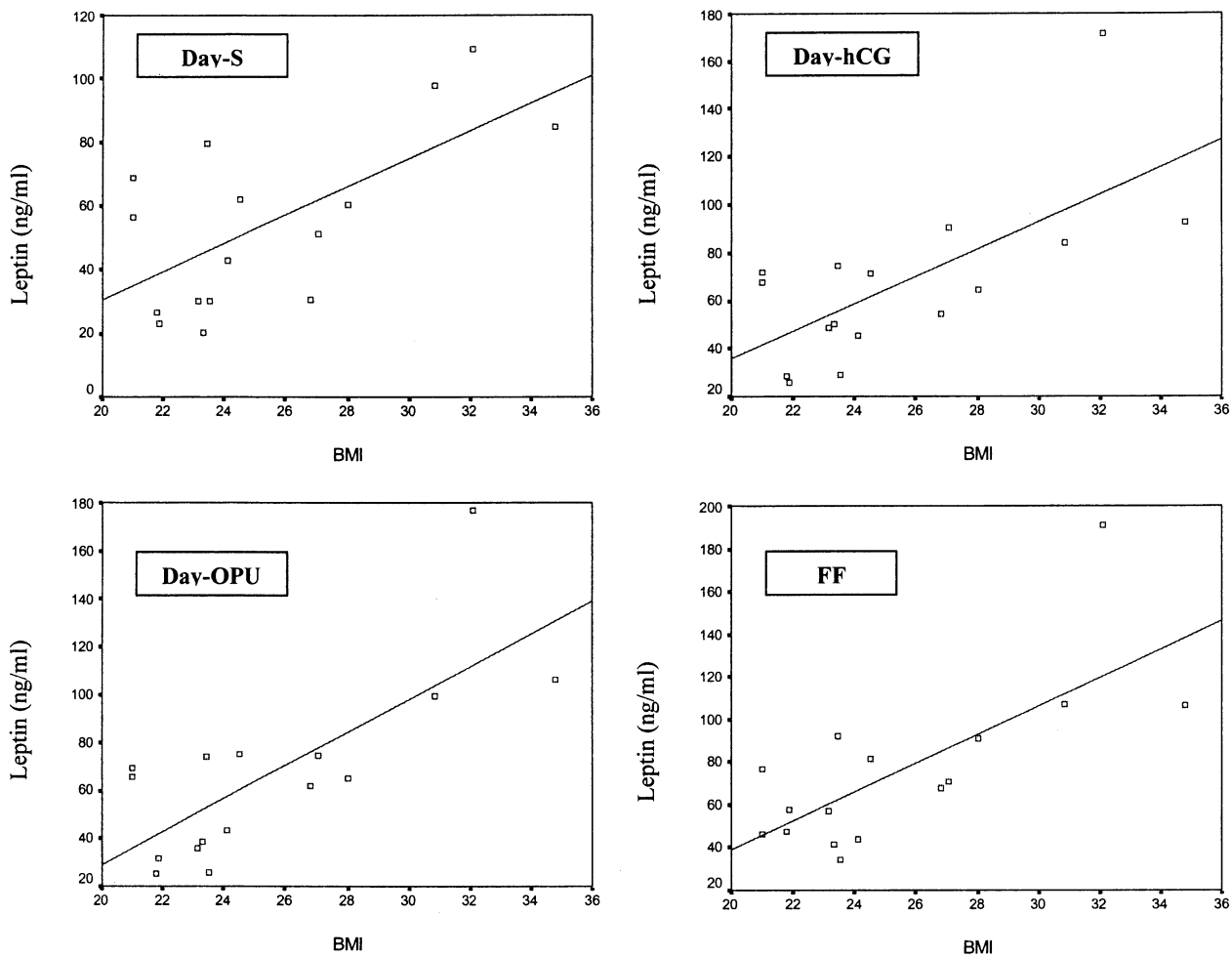


Figure 2. — Correlation between patients' BMI and serum leptin levels on Day-S, Day-hCG and Day-OPU and follicular fluid leptin level on Day-OPU.

mechanism of the elevated leptin level is still unknown, the rise in estrogen may be involved. In addition, higher follicular fluid than serum leptin levels may point to an intraovarian source of leptin. Accordingly, Lindheim *et al.* [16] speculated that leptin might be involved in follicular growth or maturation. Butzow *et al.* [11], however, found similar follicular fluid and serum leptin levels, which may suggest that leptin plays no substantial intraovarian role.

Tsai *et al.* [9] demonstrated that leptin levels at the time of hCG injection were significantly lower in pregnant than non-pregnant women, supporting the earlier study by Brannian *et al.* [19], wherein, multiple logistic regression analyses showed a correlation between serum leptin and pregnancy success, but not between pregnancy and BMI. However, the ratio of serum leptin to BMI was more strongly correlated with pregnancy success than leptin alone, and women with a low ratio had significantly better quality embryos on day 3 after retrieval and a higher implantation rate than those with a high ratio. Mantzoros *et al.* [20], in a study of patients with poly-

cystic ovary syndrome, noted that after adjustment for age and BMI, women who became pregnant tended to have lower mean follicular fluid leptin concentrations than women who did not. Butzow *et al.* [11] also found that the relative serum leptin increase was negatively associated with the ovarian response to hyperstimulation measured by the number of follicles and oocytes retrieved. This relationship was also maintained when the cumulative dose of follicle-stimulating hormone (FSH) was included as a covariable. The reduced ovarian response was not a function of BMI, basal leptin levels, or insulin concentrations and fasting serum insulin concentrations remained unchanged in response to IVF, but were positively correlated to serum leptin concentrations; Contrarily, our study yielded no correlation of serum or follicular leptin levels or leptin-to-BMI ratio, with IVF treatment variables or pregnancy outcome. Further increasing the confusion is the study by Unkila-Kallio *et al.* [17] which demonstrates an opposite trend, namely, higher concentrations of leptin at 12 days after embryo transfer in women who achieved successful pregnancies

than in women who had miscarriages or failed to become pregnant. The solution may lie in the known negative impact of obesity on patient response to COH or pregnancy outcome [21]. That is, the leptin level may act as a confounding factor which positively correlates with obesity, rather than an independent variable affecting ovarian response to COH. We therefore conclude that while leptin significantly correlates with BMI and increases during COH, it probably has no direct effect on follicular growth and maturation or ovarian response to COH.

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