Estradiol and leptin as conditional prognostic IVF markers

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Abstract

We studied the concentration of serum estradiol and serum and follicular fluid leptin in 200 women undergoing their first in vitro fertilization with embryo transfer (IVF-ET) program at the time of human chorionic gonadotrophin administration and oocyte retrieval, in an attempt to assess their concerted role on embryo quality and the prognosis of IVF outcome. Low serum (46.49 ± 8.4 ng/ml) and follicular fluid (52 ± 9.8 ng/ml) leptin levels were associated with a high number of ‘good-quality’ embryos (73.6%) and high implantation (11.2%) and pregnancy (35.8%) rates and were observed in women with normal peak estradiol levels of between 1000 and 2000 pg/ml. It appears that leptin and estradiol interact coordinately in a concentration-dependent manner to control IVF outcome. Further studies will be required to substantiate and clarify the mechanism of proposed conditional interaction between the two hormonal systems.

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Introduction

The role of estradiol in in vitro fertilization (IVF) is well known up to the fertilization stage, while its role beyond that stage remains controversial. Some groups report that high estradiol levels on the day of human chorionic gonadotrophin (hCG) administration are associated with major oocyte production, which is accompanied by suppression of implantation and endometrial receptivity and decreased pregnancy rates (Chenette et al. 1990, Sharara & McClamrock 1999), while others report no influence of estradiol in these processes (Simon et al. 1995, Pellicer et al. 1996).

The quality of transferred embryos plays a crucial role in implantation and pregnancy success, although the factors affecting embryo quality are not well known. Among those, estradiol has an important role, although its value as a single prognostic IVF marker is relatively weak. Additional parameters are therefore needed to improve the prognosis of IVF outcome. The role of leptin in human reproduction and IVF with embryo transfer (IVF-ET) (Moschos et al. 2002), which is mediated through membrane receptors in the ovaries and follicles (Karlsson et al. 1997, Loffler et al. 2001), may justify its potential involvement in the control of embryo quality. In agreement with this possibility previous reports indicated that the pregnancy rates of women with high circulating serum and follicular fluid (ff) leptin levels were low (Mantzoros et al. 2000) and together with high vascular epidermal growth factor (VEGF) and NO were associated with ‘poor’ embryo quality (Barroso et al. 1999).

A primary aim of the present study was to assess the role of leptin on estradiol-dependent embryo quality and elucidate its prognostic value as an additional marker of IVF outcome. Our findings suggest that estradiol and leptin interact coordinately to conditionally modulate IVF outcome at the level of embryo quality through concentration-dependent mechanisms.

Materials and Methods

The ethics committee of the 2nd Department of Obstetrics and Gynecology of the University of Athens, Greece, approved our submitted IVF protocol in the context of this prospective study. The women who signed a written informed consent form were enrolled in the study. Two hundred of these women with basal follicle-stimulating hormone (FSH) levels of <8.5 IU/l underwent their first IVF cycle between January 2003 and January 2004 using a long stimulation protocol with 1 mg daily s.c. gonadotrophin-releasing hormone-a leuprolide acetate (LA) administration, starting from the midluteal phase of the previous cycle. The dose was not modified for at least 10 days or until estradiol levels dropped to <50 pg/ml and no follicular activity was...
noted by transvaginal ultrasound examination. At that time, 3–6 ampoules of recombinant FSH (rFSH; Puregon, Organon Organon Pharmaceuticals, USA Inc. Roseland, NJ, USA; and Gonal F, Serono Inc. Rockland, MA, USA) were administered daily according to the ovarian response of every woman and the LA dose decreased to 0.5 mg/day s.c. rFSH administration and serum estradiol determination continued until at least two follicles a reached diameter of 18–22 mm or estradiol levels increased above 500 pg/ml. At that time hCG (10 000 IU; Pregnyl®, Organon) was administered and LA injections were discontinued. The peak estradiol level was defined as the level of estradiol on the day of hCG administration. Serum leptin concentrations were also measured that day. Oocyte retrieval was scheduled for 36–38 h after hCG injection and performed under light sedation. ff was aspirated, centrifuged at 1500 r.p.m. and frozen at −20 °C for future analysis. Standard luteal-phase progesterone support (Utrogestan®), Faran S. A. Greece) was given and 15 days after ET, serum β-hFSH concentrations were measured. If a viable pregnancy was confirmed, progesterone supplementation was continued until 8–10 weeks of gestation.

The women studied were categorized according to their peak estradiol levels in four groups: 500–1000, 1001–2000, 2001–3000, and 3001–4000 pg/ml. The biological rationale for this classification was to identify among normal rFSH responders (>500–4000 pg/ml estradiol) the optimum concentration range of estradiol conferring optimal embryo quality, implantation and pregnancy rate. Women with ovarian hyperstimulation and polycystic ovarian syndromes, which alter pregnancy rate, were identified by their elevated estradiol (>4000 pg/ml) and leptin levels and were excluded from the study.

Embryo quality was defined as the number of blastomeres (<5 and ≥5) and grade of fragmentation (on a scale of 1–4 with 3–4 = highest quality = A and 1–2 = poorest quality = B) in the second or third day after insemination. For statistical purposes embryo quality was defined as good when the majority of transferred embryos were grade A or B with five or more blastomeres and poor when they were grade A or B with less than five blastomeres. Embryos were referred as equal quality, when the same number of good- and poor-quality embryos was transferred.

The data were expressed as means± S.E.M. Kruskal–Wallis, Mann–Whitney and χ² tests were used where appropriate and P < 0.05 was considered statistically significant. Pearson’s test was used for the correlations between the variables.

Estradiol and leptin determinations were performed with commercial RIA and IRMA diagnostic kits (Diagnostic Systems Laboratories, Webster, TX, USA).

### Results

Table 1 shows the characteristics of normal rFSH-responding women categorized according to their peak estradiol levels in four groups. The serum and ff leptin levels of women with peak estradiol levels 3001–4000 pg/ml correlated negatively with good-quality embryos, of grade A and with ≥5 blastomeres (r = −0.744, P = 0.05 and r = −0.733, P = 0.048, respectively), and positively with

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<tbody>
<tr>
<td>No. of women</td>
<td>61</td>
<td>53</td>
<td>41</td>
<td>45</td>
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<tr>
<td>Age (years)</td>
<td>36.95±3.73</td>
<td>35.3±5.06</td>
<td>33.8±3.93</td>
<td>33.4±5.07</td>
<td>0.01</td>
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<td>Body mass index (kg/m²)</td>
<td>22.7±3.04</td>
<td>23.4±3.65</td>
<td>24.8±5.63</td>
<td>22.3±2.8</td>
<td>NS</td>
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<td>Leptin (on the HCG day; ng/ml)</td>
<td>57.14±7.9d</td>
<td>46.5±8.4h</td>
<td>66.7±8.4b</td>
<td>60.3±5.8d</td>
<td>0.03</td>
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<tr>
<td>No. of rFSH ampoules</td>
<td>35.4±2.82</td>
<td>28.3±2.41</td>
<td>29.1±4.19</td>
<td>18.7±1.89</td>
<td>NS</td>
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<tr>
<td>Stimulation days</td>
<td>6.61±0.36</td>
<td>6.26±0.35</td>
<td>6.00±0.47</td>
<td>5±0.31</td>
<td>NS</td>
</tr>
<tr>
<td>No. of retrieved oocytes</td>
<td>3.74±1.94</td>
<td>6.43±2.61</td>
<td>8.54±4.13</td>
<td>9.03±4.4</td>
<td>&lt;0.001</td>
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<td>Phase II oocytes</td>
<td>2.81±0.38</td>
<td>5.75±0.36</td>
<td>8.36±1.39</td>
<td>8.77±1.67</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>No. of transferred embryos</td>
<td>1.96±1.07 (n = 120)</td>
<td>3.94±1.53 (n = 209)</td>
<td>4.07±1.72 (n = 167)</td>
<td>3.94±1.41 (n = 178)</td>
<td>&lt;0.001</td>
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<td>Embryo quality</td>
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<tr>
<td>Grade A with ≥5 blastomeres</td>
<td>1±0.26</td>
<td>1.93±0.34</td>
<td>2.18±0.63</td>
<td>2.65±0.45</td>
<td>&lt;0.001</td>
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<td>Grade B with ≥5 blastomeres</td>
<td>0.14±0.002m</td>
<td>0.97±0.21k</td>
<td>0.55±0.21l</td>
<td>0.11±0.11m</td>
<td>&lt;0.05</td>
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<tr>
<td>Grade A with &lt;5 blastomeres</td>
<td>0.41±0.13</td>
<td>0.14±0.006</td>
<td>0.73±0.38</td>
<td>0.71±0.52</td>
<td>NS</td>
</tr>
<tr>
<td>Grade B with &lt;5 blastomeres</td>
<td>0.41±0.13m</td>
<td>0.93±0.27h</td>
<td>0.64±0.28i</td>
<td>0.53±0.31m</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Good embryo quality</td>
<td>58.4±4b</td>
<td>73.6±4a</td>
<td>64.6±4a</td>
<td>70%</td>
<td>NS</td>
</tr>
<tr>
<td>Poor embryo quality</td>
<td>31.2±4a</td>
<td>12.4±4a</td>
<td>25.7±4a</td>
<td>28.4±4a</td>
<td>NS</td>
</tr>
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P values among the four groups was estimated with the Kruskal–Wallis test. Superscript letters: a was significantly lower (P < 0.05) than b, c and d, and k was significantly higher (P < 0.05) than l, m and n with the Mann–Whitney test. For embryo quality, e was significantly higher (P < 0.05) than h, and p was significantly lower (P < 0.05) than q, r and s with the χ² test. NS, not significant.
poor-quality embryos, of grade B and with <5 blastomeres \((r = 0.974, P = 0.02)\) and \(r = 0.916, P = 0.05\), respectively. We observed a negative correlation \((r = -0.23, P = 0.048)\) between estradiol and leptin in women with peak estradiol levels above 3000 pg/ml.

Table 1 also shows both BMI and leptin values in all four patient groups studied. It is interesting to note that only leptin and not BMI values differ significantly between the various groups. This finding clearly differentiates the prognostic IVF value of leptin from that of BMI.

Figure 1 shows the association between low ff leptin and high pregnancy and implantation rate, expressed in percentages. The range of optimal hormonal conditions needed to achieve maximum implantation and pregnancy rates is clearly defined in the figure.

**Discussion**

Pregnancy success in IVF programs depends on endometrial receptivity (Devroey et al. 2004), which is a function of embryo quality and implantation ability. Estradiol has a positive influence on IVF up to the fertilization stage, while in the subsequent implantation and pregnancy stages its action is controversial (Chenette et al. 1990, Simon et al. 1995, Pellicer et al. 1996, Sharara & McClamrock 1999). Recent data suggest that high ff leptin concentrations result in pregnancy failure (Mantzoros et al. 2000), while leptin/body mass index ratio has a higher predictive IVF value than leptin alone (Brannian et al. 2001). In the present study pregnancy success was maximal when estradiol and leptin levels were between 1001 and 2000–pg/ml and 46.49 ± 8.4 ng/ml (serum) and 52 ± 9.8 ng/ml (ff), respectively, suggesting that both parameters have higher prognostic IVF value than either one alone (Table 1). Furthermore, the association observed in Table 1 between low serum \((46.49 ± 8.4 \text{ ng/ml})\) and ff \((52 ± 9.8 \text{ ng/ml})\) leptin levels and high numbers of good-quality embryos \((73.6\%)\) suggests a pivotal role of leptin in estradiol-dependent embryo quality and their subsequent implantation ability.

Serum estradiol was used to monitor rFSH stimulation. Both low \((<500 \text{ pg/ml})\) and high \((>4000 \text{ pg/ml})\) serum estradiol concentrations, on the day of hCG administration, resulted in undesirably low and high oocyte yields, respectively. Our study confirmed the known linear correlation between high estradiol level and oocyte yield, decreased implantation and pregnancy rate (Table 1). It also revealed that the highest numbers of good-quality embryos were produced by women with peak estradiol levels of between 1000 and 2000 pg/ml rather than between 3000 and 4000 pg/ml (Table 1). This may result from oocyte immaturity or increased leptin production, which may reduce embryo implantation and endometrial receptivity. It appears that the medication of high oocyte yield and implantation and pregnancy failure by high but not low ff leptin levels (Table 1) negatively affected ovarian steroidogenesis, follicle maturation and embryo development. The negative correlation observed between a certain normal range of estradiol and leptin levels suggested that under such conditions these hormones exert a negative influence on embryo quality. In that sense, leptin may be a valuable marker of functional IVF staging (Nomikos & Vamvakopoulos 2001) at the level of embryo quality.

If leptin, VEGF and NO have been used as markers of IVF outcome (Barroso et al. 1999). Furthermore, the elevated ratio of serum leptin to body mass index has been correlated with fewer good-quality embryos and lower implantation and pregnancy rates (Brannian et al. 2001). In our study, high leptin levels \((>57.14 ± 7.9 \text{ ng/ml})\) correlated with fewer good-quality embryos and lower implantation and pregnancy rates (Brannian et al. 2001). In our study, high leptin levels \((>57.14 ± 7.9 \text{ ng/ml})\) affected negatively the quality of transferred embryos. This finding was consistent with the negative correlation observed between good-quality embryos and serum and ff leptin levels and the significantly elevated leptin of women producing high numbers of poor-quality embryos (Table 1). We observed strong positive correlation between serum and ff leptin levels in all the groups of women examined \((P < 0.001)\). This finding may indicate a role of leptin in subfertility via correlation with poor-quality embryos by reducing ovarian responsiveness to gonadotrophins, which may result from down-regulation of rFSH-induced estradiol production.

Higher implantation and pregnancy rates were observed at low serum leptin levels \((46.49 ± 8.4 \text{ ng/ml})\) in women with peak estradiol of 1001–2000 pg/ml (Table 1) and lower at high serum leptin levels \((60.3 ± 5.8 \text{ ng/ml})\) in women with peak estradiol of 3001–4000 pg/ml (Table 1 and Fig. 1). It is possible that high serum and ff leptin concentrations reduce endometrial receptivity and implantation ability through the leptin receptors of the endometrium (Gonzalez et al. 2000, Kitawake et al. 2000).
We conclude that the highest implantation and pregnancy rates observed in our study group resulted from the concerted action of estradiol (1001–2000 pg/ml, serum) and leptin (46.49 ± 8.4 ng/ml (serum) and 52 ± 9.8 ng/ml (ff)). Outside this concentration range these hormones exert negative influence on embryo quality and IVF outcome. By modulating estradiol-dependent embryo quality, leptin appears to constitute an additional prognostic indicator of IVF outcome. The conditional hormonal IVF effects observed suggest that estradiol and leptin interact coordinately in a concentration-dependent manner. Further studies are needed to substantiate and clarify the mechanism of proposed interaction between the two hormonal systems.

Acknowledgements
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