Estradiol and leptin as conditional prognostic IVF markers

G Anifandis¹, E Koutselini¹, K Louridas¹, V Liakopoulos¹, K Leivaditis¹, T Mantzavinós¹, D Sioutopoulou¹ and N Vamvakopoulos²

¹Department of Biology & Genetics of Larissa, University of Thessalia Medical School, 22 Papakyriazi, Larissa 41222, Larissa, Greece and ²2nd Department of Obstetrics and Gynecology of Athens, University of Athens, Areataieio hospital, 76 V. Sofias Ave, 11528, Athens, Greece

Correspondence should be addressed to Anifandis Georgios; Email: ganif@med.uth.gr

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.

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 concen-
trations were also measured that day. Oocyte retrieval was
scheduled for 36–38 h after hCG injection and performed
under light sedation. If was aspirated, centrifuged at
1500 r.p.m. and frozen at −20°C for future analysis. Stan-
dard luteal-phase progesterone support (Utrogestan®,
Faran S. A. Greece) was given and 15 days after ET, serum
β-hCG concentrations were measured. If a viable preg-
nancy 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 normal
rFSH responders (>500–4000 pg/ml estradiol) the
optimum concentration range of estradiol conferring optim-
al 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 blasto-
meres (<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 inse-
mination. 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 blasto-
meres. 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 sig-
nificant. Pearson’s test was used for the correlations
between the variables.

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

Table 1
Classification of rFSH-stimulated women in four groups according to their normal peak estradiol concentrations. Implantation rate was
defined as the number of live births divided by the total number of transferred embryos. Pregnancy rate was defined as the percentage ratio of
women with live births to total rFSH-treated women and is directly related to IVF outcome, which is defined by the prognostic IVF value of
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</thead>
<tbody>
<tr>
<td>No. of women</td>
<td>61</td>
<td>53</td>
<td>41</td>
<td>45</td>
<td></td>
</tr>
<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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<tr>
<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>
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<tr>
<td>Leptin (on the HCG day; ng/ml)</td>
<td>57.14±7.9h</td>
<td>46.5±8.4h</td>
<td>66.7±8.4h</td>
<td>60.3±5.8h</td>
<td>0.03</td>
</tr>
<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>
</tr>
<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>
</tr>
<tr>
<td>Embryo quality</td>
<td></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>
</tr>
<tr>
<td>Grade B with ≥5 blastomeres</td>
<td>0.14±0.007h</td>
<td>0.97±0.21h</td>
<td>0.55±0.21l</td>
<td>0.11±0.111h</td>
<td>&lt;0.05</td>
</tr>
<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±0.13h</td>
<td>0.93±0.27h</td>
<td>0.64±0.28h</td>
<td>0.53±0.31h</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Good embryo quality</td>
<td>58.4±6.9h</td>
<td>73.6±6.9h</td>
<td>64.6±6.9h</td>
<td>70%</td>
<td>NS</td>
</tr>
<tr>
<td>Poor embryo quality</td>
<td>31.2±6.9h</td>
<td>12.4±6.9h</td>
<td>25.7±6.9h</td>
<td>28.4±6.9h</td>
<td>NS</td>
</tr>
</tbody>
</table>

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.
Estradiol and leptin as IVF markers

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 ng/ml) and ff (52 ± 9.8 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 pg/ml) and high (>4000 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 mediation 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.

ff 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 ng/ml (serum) and >63.8 ± 9.8 ng/ml (ff)), 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 ng/ml) in women with peak estradiol of 1001–2000 pg/ml (Table 1) and lower at high serum leptin levels (60.3 ± 8.8 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).

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Table 1 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 only leptin and not BMI values differ significantly between four patient groups studied. It is interesting to note that women with peak estradiol levels above 3000 pg/ml.

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.
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 (fif)). 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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