Implantation is apparently unaffected by the dopamine agonist Cabergoline when administered to prevent ovarian hyperstimulation syndrome in women undergoing assisted reproduction treatment: a pilot study

Claudio Álvarez, Isabel Alonso-Muriel, Gabriela García, Juana Crespo, José Bellver, Carlos Simón and Antonio Pellicer

Instituto Valenciano de Infertilidad (IVI), University of Valencia, Plaza de la Policía Local, 3, 46015 Valencia, Spain

BACKGROUND: Ovarian hyperstimulation syndrome (OHSS) is a result of ovarian overexpression of vascular endothelial growth factor (VEGF) and its receptor 2 (VEGFR2). VEGF/VEGFR2 binding disrupts cellular junctions and increases vascular permeability (VP), a characteristic of OHSS, but enhances angiogenesis, which is a fundamental step in implantation. In animals, the dopamine agonist Cabergoline (Cb2) prevents VP without affecting angiogenesis. In humans, Cb2 averts OHSS, but a possible detrimental effect on angiogenesis and implantation has not been explored. A pilot study was designed to analyze whether or not Cb2 administration, as a procedure for preventing OHSS, affects the outcome of assisted reproduction treatment (ART).

METHODS: A retrospective study with endpoints of implantation and ongoing/term pregnancy rates. Women (n = 35) at risk of OHSS (20–30 follicles developed and >20 oocytes collected) took a daily oral dose of 0.5 mg Cb2 for 8 days, beginning on the day of hCG. They were matched with controls treated during the same period and who were similar with respect to age, number and quality of the embryos replaced, embryonic stage at transfer and sperm quality. RESULTS: No difference was detected between the groups in fertilization, implantation or pregnancy rates. A total of 14 ongoing (beyond 32 weeks) or full term pregnancies were registered in each group. No major problem was detected during pregnancy or after delivery in any of these babies. CONCLUSIONS: Administration of Cb2 in order to prevent OHSS is safe and does not appear to affect ART outcome.

Keywords: ovarian hyperstimulation syndrome; dopamine agonists; Cabergoline; assisted reproduction treatment outcome; implantation

Introduction

Severe forms of ovarian hyperstimulation syndrome (OHSS) arise in 0.5–5% of assisted reproduction treatment (ART) cycles (Delvigne and Rozenberg, 2002), and have been known to cause maternal death (Cluroe and Synek, 1995; Semba et al., 2000). Despite being a potentially life-threatening iatrogenic complication, this syndrome has been managed expectantly until now, due to its pathophysiology being unknown.

Development of OHSS in women undergoing ART depends on the presence of ovaries and the administration of hCG, as the syndrome subsides or fails to develop when an oophorectomy is performed (Amarin, 2003), when women become pregnant following oocyte donation (Pau et al., 2006), or when hCG administration is withheld during controlled ovarian hyperstimulation (COH) with gonadotrophins (Schenker, 1993; Aboulghar and Mansour, 2003). As hCG has no vasoactive activities, its actions are mediated by one or more of the angiogenic substances released by the ovaries in response to this gonadotrophin.

Vascular endothelial growth factor (VEGF) is the most important mediator of hCG-dependent ovarian angiogenesis. It is known that VEGF is expressed in human ovaries (Yan et al., 1993) and that VEGF mRNA levels in granulosa cells increase after hCG administration (Neulen et al., 1995; Wang et al., 2002). Protein levels in serum, plasma and peritoneal fluids are also elevated in women at risk of developing OHSS (Pellicer et al., 1999). VEGF not only stimulates new blood vessel development but also induces vascular hyperpermeability (McClure et al., 1994; Bates and Harper, 2002) by interacting with its VEGF receptor 2 (VEGFR-2) (Gille et al., 2001).
VEGFR-2 is up-regulated by ovarian steroids and hCG in through VEGF expression (Kapiteijn et al., 1999). This suggests that VEGF is implicated in the pathophysiology of OHSS. However, due to its side effects (thromboembolism and vomiting) (Glade-Bender et al., 2003; Kuenen et al., 2003), this type of drug is unsuitable for the treatment of OHSS.

There are, however, other important issues concerning VEGF targeting. The early stages of pregnancy are highly dependent on ovarian steroids (Wulff et al., 2001; Zimmermann et al., 2001a,b, 2003; Pauli et al., 2005) and uterine (Rockwell et al., 2002; Heryanto et al., 2003) angiogenesis. It is well known that VEGF is a key factor in endometrial angiogenesis. The entire VEGF system, including ligands and receptors, is expressed in the human endometrium (Girling and Rogers, 2005; Panyadeera et al., 2006). The expression of VEGF and VEGFR-2 is up-regulated by ovarian steroids and hCG in different mammalian species (Berndt et al., 2006; Herve et al., 2006; Ngan et al., 2006; Licht et al., 2007). Similarly, the implanting embryo stimulates endometrial angiogenesis through VEGF expression (Kapiteijn et al., 2006). Thus, it is assumed that VEGF-mediated angiogenesis is a fundamental step in mammalian embryo implantation. In fact, pregnancy can be intercepted by targeting VEGF with monoclonal antibodies (Ghosh and Sengupta, 2005; Sharkey et al., 2005). Consequently, the use of compounds such as SU-5416 may affect implantation in humans.

In an attempt to solve these problems, we investigated other compounds that may have similar effects on VP, but which do not exert the undesirable side effects of the aforementioned antibody. Dopamine agonist Cabergoline (Cb2), when administered to immature rats at low doses simultaneously with hCG, prevented an increase in VP and did not affect angiogenesis (Gomez et al., 2006). We also demonstrated that the effects of Cb2 were attributable to VEGFR-2 dephosphorylation, and concluded that Cb2 may provide a new, specific and non-toxic approach to the treatment of OHSS, by which the increased VP, which is mediated by the VEGF/VEGR2 pathway, is blocked without altering angiogenesis. This could be of great clinical interest to the field of ART.

Given the available knowledge, we decided to run a trial in humans. A prospective, randomized, placebo-controlled study was carried out with oocyte donors receiving 0.5 mg/day of Cb2 for 8 days, starting on the day of hCG administration (Alvarez et al., 2007). We demonstrated that Cb2 reduced the amount of ascites accumulating in the pelvis, and both hemoconcentration and the incidence of moderate–severe OHSS were seen to diminish (Alvarez et al., 2007). Moreover, by employing magnetic resonance and evaluating several pharmacokinetic parameters, we showed that VP in human ovaries subjected to COH increased after hCG administration, and that this effect was prevented by Cb2. However, this study did not address the possible dual effect of Cb2 on VP and angiogenesis, as was the case in our study with animals (Gomez et al., 2006).

It is of particular relevance to know whether or not Cb2 affects angiogenesis in the human endometrium. If our approach for preventing OHSS affects endometrial angiogenesis and implantation, it would be of limited interest to reproductive medicine, as it could only be employed in those women at risk of OHSS who are not undergoing embryo replacement. The best marker of adequate angiogenesis in the endometrium is the ability of a replaced embryo to successfully implant. Thus, to address the possible effect of Cb2 administration on implantation, we designed a pilot retrospective study in which the outcome of ART was compared in two groups of patients. One of the groups was formed of women at risk of developing OHSS and receiving Cb2, whereas the other group was composed of women without risk of OHSS.

Materials and Methods

This study was performed in patients undergoing ART at the IVI Valencia clinic between January 2005 and March 2006. The protocol for COH employed either GnRH agonists or antagonists. When agonists were used, pituitary desensitization was initiated with a daily s.c. administration of 0.1 mg of triptorelin (Decapeptyl 0.1; Ipn, Farma, Barcelona, Spain), which began in the luteal phase of the menstrual cycle. This dose was continued until ovarian quiescence was confirmed by vaginal ultrasound following menstruation. When antagonists were employed, patients received oral contraceptive pills during the cycle prior to that of ovarian stimulation. Later, gonadotrophin administration was initiated on Day 2 of menses and the antagonist (Cetrotide, Serono Laboratories, Madrid; or Ganirelix, Organon Laboratories, Barcelona, Spain) was subsequently added at a dose of 0.25 mg/day when the leading follicle reached a diameter of 14 mm. Stimulation of the ovaries included recombinant FSH (Gonal F; Serono; or Puregon; Organon) and recombinant LH (Luvieris, Serono) or hMG (Menopur, Ferring Laboratories, Madrid, Spain), which were individually adjusted according to the patient’s age, number of antral follicles in basal conditions, response in previous cycles, basal FSH levels and body mass index (BMI). Recombinant hCG (250 mg, Ovitre; Serono) was administered when the mean diameter of at least three leading follicles reached 18 mm. Serum estradiol levels were measured with a microparticle enzyme immunoassay Axsym System (Abbott Cientifico S.A., Madrid, Spain) during controlled ovarian stimulation, obtaining the last sample on the day of hCG administration. Injection of the GnRh analog and gonadotrophins was discontinued on the day of hCG administration. Transvaginal oocyte retrieval under general sedation was scheduled 36 h after the hCG injection.

A total of 35 patients at risk of developing OHSS were included in the study group. Only patients living in the city of Valencia were asked to participate in the study so that subsequent side effects could be closely monitored. Furthermore, women undergoing preimplantation genetic screening were excluded. 'At risk’ was defined as the development of 20–30 follicles >12 mm and >20 retrieved oocytes. Immediately after the decision to administer hCG was taken and a written consent was signed, patients (n = 35) received one 0.5 mg tablet of Cb2 per day for 8 days. The control group consisted of 35 women undergoing ART during the same period of time, but who were not at risk of developing OHSS. They were matched with study subjects by age, number and quality of replaced embryos, embryonic stage at transfer and sperm quality. In the treatment group, the total number of embryo replacements performed was 18 on Day 3, 12 on Day 5 and 5 on Day 6, and similar numbers were matched in the controls.
Statistical analyses were performed using the Statistical Package for Social Science version 12.0 (SPSS Inc., Chicago, IL, USA). Categorical data were expressed as number and percentage, and numerical data as mean ± SD. Student’s t-test, χ^2 test and Fisher’s exact test were used when appropriate. Significance was defined as P < 0.05.

**Results**

Table I compares the epidemiological data of the patient and the control groups. As far as can be ascertained, there was no difference between the subjects in age, BMI, years of infertility or cause of infertility. The stimulation strategies are analyzed in Table II. Women receiving Cb2 underwent protocols with GnRH agonists in 77.1% of cases, significantly (P = 0.01) more than those in the control group (42.9%). GnRH antagonist protocols were significantly (P = 0.01) more employed in the control group (57.1%) than in women receiving Cb2 (22.8%). Moreover, peak estradiol values on the day of hCG administration were significantly (P = 0.01) more in the Cb2 group than among controls.

As expected, none of the patients in the control group displayed OHSS. In the group of women receiving Cb2, only one patient suffered hemoconcentration and needed evacuating culdocentesis.

**Table I.** Epidemiological data of the study groups.

<table>
<thead>
<tr>
<th></th>
<th>Cb2 group (n = 35)</th>
<th>Control group (n = 35)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)a</td>
<td>32.4 ± 4.0</td>
<td>32.4 ± 4.0</td>
<td>NS</td>
</tr>
<tr>
<td>Body mass indexa</td>
<td>23.1 ± 3.3</td>
<td>23.2 ± 4.0</td>
<td>NS</td>
</tr>
<tr>
<td>Infertility yearsa</td>
<td>2.9 ± 2.1</td>
<td>3.3 ± 2.6</td>
<td>NS</td>
</tr>
<tr>
<td>Unexplained infertility</td>
<td>2</td>
<td>2</td>
<td>NS</td>
</tr>
<tr>
<td>Tubal factor</td>
<td>2</td>
<td>2</td>
<td>NS</td>
</tr>
<tr>
<td>Polycystic ovary</td>
<td>10</td>
<td>10</td>
<td>NS</td>
</tr>
<tr>
<td>Endometriosis</td>
<td>1</td>
<td>1</td>
<td>NS</td>
</tr>
<tr>
<td>Recurrent abortion</td>
<td>2</td>
<td>2</td>
<td>NS</td>
</tr>
<tr>
<td>Male factor</td>
<td>15</td>
<td>15</td>
<td>NS</td>
</tr>
<tr>
<td>Mixed factor</td>
<td>3</td>
<td>3</td>
<td>NS</td>
</tr>
</tbody>
</table>

*Values expressed as mean ± SD.

**Table II.** COH data for the two groups.

<table>
<thead>
<tr>
<th></th>
<th>Cb2 Group (n = 35)</th>
<th>Control group (n = 35)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>GnRH agonist COH (%)</td>
<td>27 (77.1)</td>
<td>15 (42.9)</td>
<td>0.01</td>
</tr>
<tr>
<td>GnRH antagonist COH (%)</td>
<td>8 (22.8)</td>
<td>20 (57.1)</td>
<td>0.01</td>
</tr>
<tr>
<td>FSH total doses (IU)a</td>
<td>1462.0 ± 676.9</td>
<td>1707.3 ± 867.0</td>
<td>NS</td>
</tr>
<tr>
<td>HMG/LH total doses (IU)a</td>
<td>351.4 ± 491.7</td>
<td>505.6 ± 688.5</td>
<td>NS</td>
</tr>
<tr>
<td>Estradiol on hCG day (pg/ml)a</td>
<td>3716.9 ± 947.4</td>
<td>1981.8 ± 1212.9</td>
<td>0.0001</td>
</tr>
<tr>
<td>COH (days)a</td>
<td>10.7 ± 1.5</td>
<td>10.5 ± 1.7</td>
<td>NS</td>
</tr>
</tbody>
</table>

aValues expressed as mean ± SD.

The outcome of ART is shown in Table III. The number of oocytes retrieved was significantly (P = 0.0001) higher in women who received Cb2. As a consequence, the number of embryos obtained was significantly (P = 0.0001) higher, but they were of a considerably lower quality (P = 0.002). Implantation and live birth rates did not differ between the two groups when a mean of 1.8 ± 0.4 embryos were replaced. Six single-embryo replacements were recorded in each group, which led to two pregnancies in each group. One spontaneous abortion was registered in week 18 in the Cb2-treated group, but no fetal malformation was reported. Moreover, no minor or major malformation has been observed in any of the 28 babies born to date.

**Discussion**

This study suggests that Cb2 can be employed safely in ART to prevent OHSS, without compromising outcome. The data provide additional information to that of our recent publication of a randomized placebo-controlled study in which Cb2 reduced ovarian VP and the severity of OHSS in women subjected to COH (Álvarez et al., 2007). We feel that, considered together, the two reports present information of considerable interest with respect to procedures in which COH is employed in patients at risk of developing OHSS. Initially, we provided ‘proof of concept’ that dopamine agonists, and specifically Cb2, reduce VP in women, as human ovarian cells contain dopamine type 2 receptors (Álvarez et al., 2007). We also showed that the amount of ascites in the abdomen and occurrence of hemoconcentration were reduced after Cb2 administration (Álvarez et al., 2007). The data in the present report suggest that the aforementioned strategy does not compromise implantation or ongoing pregnancy, which is the major goal of ART treatments. We are currently carrying out a prospective, randomized, placebo-controlled study to confirm these data.

The fundamental role played by VEGF in endometrial angiogenesis and implantation (Neulen et al., 1995; Licht et al., 2007) causes concern with respect to the use of any substance that may interfere with the VEGF/VEGFR-2 pathway, as both VP and angiogenesis could be affected. Administration of high doses of dopamine agonists may influence three processes.
namely angiogenesis, VP and prolactin secretion (Basu et al., 2001). We first evaluated the use of Cb2 in a rat model, and showed that the specific actions of Cb2 on VP and angiogenesis were dose-dependent (Gómez et al., 2006). On the other hand, administration of low doses, such as those applied in humans who suffer from hyperprolactinemia, decreases prolactin levels without affecting physiologic angiogenesis, as the latter occurs in reproductive organs (Mornex et al., 1978; Bigazzi et al., 1979; Robert et al., 1996; Ciccarelli et al., 1997; Liu and Tyrrell, 2001). The dose of 0.5 mg/day (total dose 4 mg) of Cb2 established for this and the previous study (Alvarez et al., 2007) was based on experience gained through our rodent studies, and after consulting the literature for the average dose employed to treat prolactinomas (Biller et al., 1996; Colao et al., 1997). Some published studies have used twice the dose administered to our patients (Di Sarno et al., 2001). Moreover, the potential adverse and teratogenic effects of Cb2 administration in early pregnancy in humans have failed to manifest themselves with doses as high as 7 mg per week (Verhelst et al., 1999; Ricci et al., 2002). The data presented herein show that endometrial angiogenesis was not greatly altered, if at all, by the dose of Cb2 employed since neither implantation nor overall ART outcome was affected.

We also found that the retrieval of more oocytes was associated with a lower quality of the resulting embryos. This finding confirmed our expectations, since it has recently been demonstrated by Baart et al. (2007) that reducing the dose of gonadotrophins administered to ART patients produces a substantial decrease in the rate of aneuploidies in the embryos. In this way, outcome in the study group could have been negatively influenced by the quality of the embryos, although this effect should have been balanced by the fact that, in these women, there were a higher number of embryos available for selection for transfer.

We have also observed that fewer antagonists were employed in women at risk of OHSS. The effects of agonists and antagonists on implantation have been a matter of controversy for years. However, the clinical data currently available do not substantiate a negative effect of either treatment on implantation (Al-Inany et al., 2007). Moreover, our studies of the endometrium during the implantation window have failed to demonstrate a difference between agonists and antagonists on several molecular and morphologic markers of endometrial receptivity (Simón et al., 2005). Thus, the different percentage of patients receiving agonists or antagonists of GnRH in each group should not influence outcome.

Funding

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Submitted on June 5, 2007; resubmitted on August 11, 2007; accepted on September 6, 2007.