The significance of premature luteinization in an oocyte-donation programme

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BACKGROUND: Several evidences indicate that premature luteinization (PL) may affect IVF outcome. The primary end-point of the present study was to verify the effect of PL on the pregnancy rate (PR) of our oocyte-donation programme. METHODS: PL was defined as serum progesterone ≥1.2 ng/ml on the day of HCG. We analysed retrospectively 240 oocyte-donation cycles in which 120 women donated twice, with PL in the first donation cycle and no PL in the following one, acting as its own control. Recipients (n = 240) were divided in two groups according to the presence of PL (n = 120) or not (n = 120). Both groups were compared regarding donor cycle parameters and recipient cycle outcome. RESULTS: There was no difference in PR between the groups (55.7 versus 54.4%, respectively). The number of total oocytes (18.2 ± 0.6 versus 18.7 ± 0.6; P = 0.003) and the number of mature oocytes retrieved (16.9 ± 0.6 versus 19.4 ± 0.6; P = 0.005) were different among donors with progesterone <1.2 ng/ml and PL, respectively. There were no differences between the oocyte recipients in fertilization, cleavage, embryo division on day 3, blastocyst development or fragmentation rates. The number of embryos transferred, number of embryos cryopreserved, and implantation and miscarriage rates were similar between the groups. CONCLUSION: PL does not appear to have a negative impact on ongoing PR in our oocyte-donation programme.

Key words: controlled ovarian hyperstimulation/embryo quality/GnRH agonist/oocyte-donation programme/premature luteinization

Introduction

Despite the suppression of endogenous gonadotrophins by a GnRH agonist, 5 to 30% of controlled ovarian hyperstimulation (COH) cycles are marked by a subtle rise in serum progesterone levels before HCG administration (Edelstein et al., 1990; Schoolcraft et al., 1991; Silverberg et al., 1991; Fanchin et al., 1993; Givens et al., 1994; Ubaldi et al., 1995). Although several authors did not find any negative effect of this on IVF outcome (Howles et al., 1988; Mahadevan et al., 1988; Ben-Nun et al., 1990; Hassiakos et al., 1990), pregnancy rate (PR) has been reported to be inversely related to serum progesterone levels on the day of HCG administration (Hamori et al., 1987; Edelstein et al., 1990; Schoolcraft et al., 1991; Silverberg et al., 1991; Kagawa et al., 1992; Mio et al., 1992; Fanchin et al., 1993; Check et al., 1994; Givens et al., 1994; Mio and Terakawa, 1995; Bosch et al., 2003; Ozcakir et al., 2004).

The origin and consequences of this premature elevation of serum progesterone, defined as premature luteinization (PL), on IVF outcome remain controversial (Fanchin et al., 1995, 1997b; Shulman et al., 1996). It has been described that it may affect it as an ovarian event, with adverse effects on oocyte maturation, fertilization or early cleavage (Fanchimont et al., 1989; Schoolcraft et al., 1991; Silverberg et al., 1991; Fanchin et al., 1993, 1997a). On the other hand, poorer embryo quality was not found in a number of studies (Legro et al., 1993; Hofmann et al., 1993, 1996; Check et al., 1994; Silverberg et al., 1994; Bustillo et al., 1995; Yovel et al., 1995; Fanchin et al., 1996). These findings suggest that PL may influence the endometrium, adversely affecting implantation and subsequent embryo development due to premature decidualization. This hypotheses was sustained by others groups (Garcia et al., 1984, 1989; Forman et al., 1989; Sharma et al., 1990; Silverberg et al., 1991; Mio et al., 1992; Burns et al., 1994; Borman et al., 2004).

In order to discriminate the endometrial factor from the quality of the oocyte cohort, some reports have used the oocyte-donation model (Hofmann et al., 1993; Legro et al., 1993; Check et al., 1994; Fanchin et al., 1996) or embryo cryopreservation (Silverberg et al., 1991, 1994) to assess separately the respective influences of PL on IVF outcome. In the present study, for the first time, the effect of PL was studied in the same oocyte donor, acting as its own control, undergoing COH cycle in two consecutive cycles, to clarify the eventual effects of PL, avoiding an inter-patient variability. The aim of this study was to verify the effect of PL on the PR of our oocyte-donation programme.
Materials and methods

Institutional approval
This project was approved by the Institutional Review Board on the use of human subjects in research at the Instituto Valenciano de Infertility and complies with the Spanish Law of Assisted Reproductive Technologies (35/1988).

Design and definition of PL
We retrospectively assessed our files from 2384 oocyte-donation cycles performed in our Institution from January 2003 to December 2004. Of them, 525 showed PL (22%) and only 240 (45.7%) accomplished the inclusion criteria.

The donor included in this study showed in her first cycle serum progesterone ≥1.2 ng/ml and in the next cycle serum progesterone <1.2 ng/ml on the HCG day. In this way, they worked as their own controls.

Several different cut-offs have been used in the literature (Fanchimont et al., 1989; Schoolcraft et al., 1991; Silverberg et al., 1991; Fanchin et al., 1993, 1997a). In this study, the value of 1.2 ng/ml was chosen as a cut-off value, based on previous studies that have reported poorer IVF outcome associated with increasing serum progesterone (Legro et al., 1993; Bosch et al., 2003). Nevertheless, in order to avoid the arbitrary effect of this cut-off value, we correlated P-values directly with numerical embryo parameters.

Oocyte recipients
Oocyte recipients (n = 240) were included in our oocyte-donation programme because of low response, 80 (33.3%); premature ovarian failure, 72 (30%); menopause, 36 (15%); female advanced age, 34 (14.2%); and genetic or chromosomal disorders, 18 (7.5%). Cases with uterine pathology (submucous or >2-cm intramural fibroids, polyps, adhesions, adenomyosis, or Müllerian defects), recurrent miscarriage or severe male infertility (<5 million fresh spermatozoa/mm³ and <5% normal forms and/or non-obstructive azoospermia) were not included in the present study.

The protocol for HRT was described previously (Remohí et al., 1995). Briefly, a baseline transvaginal scan was carried out prior to down-regulation to ensure that the uterus and ovaries were normal. For all recipients who were still cycling, down-regulation was performed using an i.m. dose of 3.75 mg of Triptorelin (Decapeptyl®; Ipsen Pharma, Barcelona, Spain) beginning in the midluteal phase of the previous cycle. HRT was initiated on day 1–3 of the following cycle, and doses of oestradiol valerate (Progynova®; Schering Spain, Madrid, Spain) were increased as follows: 2 mg/day for the first 8 days of treatment, 4 mg/day for the following 3 days, and, at least, 6 mg/day until the pregnancy test. On day 15, a scan was performed to evaluate endometrial growth. On the day after the donation, 800 mg/day of micronized intravaginal progesterone (Progeffik®; Effik Laboratories, Madrid, Spain) was added. Embryo transfer was performed on days 2, 3 or 5 of embryo development under ultrasound guidance.

Embryos were classified according to cell number, symmetry and degree of fragmentation (Alikani et al., 2000). Serum β-human chorionic gonadotrophin (β-HCG) was measured in the recipients 16 days after donor’s oocyte retrieval. Ongoing pregnancy was confirmed 2 weeks later if the existence of a embryo with a heart beat was confirmed by transvaginal scan. Ongoing pregnancy rate (OPR) is the percentage of patients undergoing embryo transfer, who were shown to have one or more embryos with positive heart beat on scan evaluation. Implantation rate (IR) was obtained by dividing the number of gestational sacs seen in scan by the number of replaced embryos. Miscarriage rate (MR) was defined as the percentage of pregnancies that terminated before the completion of the twentieth week of gestation after previous scan detection of embryo’s heart beat (Soares et al., 2005).

Oocyte donors
All donors were included in our oocyte-donation programme after being thoroughly informed and having fulfilled our inclusion criteria (Soares et al., 2005). Briefly, subjects were aged between 18 and 35 years old, and we had access to their complete medical history, which considered current or past exposure to radiation or hazardous chemical substances, i.v. drug use, and reproductive history. All subjects were shown to be normal in a physical and gynaecological examination, had no family history of hereditary or chromosomal diseases, had a normal karyotype and tested negative in a screening for sexually transmitted diseases (Garrido et al., 2002).

One hundred and twenty donors who underwent 240 oocyte-donation cycles (two cycles/donor) were included in the study. In the first cycle, the donor showed serum progesterone ≥1.2 ng/ml on the day of HCG administration. To compare the IVF outcome, we chose the next cycle of the same donor in which serum progesterone was <1.2 ng/ml on the HCG day. According to this categorization, recipients (n = 240) were divided into two groups: Group I (n = 120) – patients who received oocytes from donors with progesterone <1.2 ng/ml, and Group II (n = 120) – patients who received oocytes from donors with progesterone ≥1.2 ng/ml. Both groups were compared regarding donor cycle parameters (gonadotrophin doses, stimulation days, serum estradiol (E₂) levels on the HCG day, number of oocytes per retrieval and mature oocytes retrieved) and recipient’s IVF outcome (fertilization, cleavage, embryo division on the days 2 and 3, blastocyst development, fragmentation degree, number of embryos transferred and cryopreserved, IR, OPR, and MR.

For COH, only GnRH agonist protocols were used as previously described (Garrido et al., 2004). Briefly, patients started administration of 0.1 mg of leuprolide acetate (Procrin®; Abbott, Madrid, Spain) in the midluteal phase of the previous cycle, until negative vaginal ultrasound defined ovarian quiescence. The dose of GnRH agonist was then decreased to 0.05 mg until the day of HCG administration. The starting dose varied from 150 to 300 IU/day of FSH (Gonal-F®; Serono, Madrid, Spain; or Puregon®; Organon, Madrid, Spain) and/or human menopausal gonadotrophin (Menopur®; Ferring Pharmaceuticals, Madrid, Spain) for the first 2–5 days, according to age, body mass index and response to previous COH, when serum E₂ was assessed and gonadotrophin dose adjusted according to a step-up or step-down protocol.

When three or more follicles reached 18 mm in diameter, HCG (Ovitrelle®, 250 µg; Serono, Madrid, Spain) was administered and oocyte retrieval was scheduled 36 h later. Serum E₂ and progesterone levels were measured in the morning of the HCG administration. Samples were tested with a microparticle enzyme immunoassay Assym System (Abbott Científico SA, Madrid, Spain). The serum E₂ kit had a sensitivity of 28 pg/ml and intraobserver and interobserver variation coefficients of 6.6 and 7.7%, respectively. The serum progesterone kit had a sensitivity of 0.2 ng/ml, with the intraobserver and interobserver variation coefficients of 9.6 and 3.9%, respectively.

Statistical analysis
Statistical analysis was performed by χ²-test and Student’s t-test for categorical comparisons. A P-value of <0.05 was considered significant. Statistical analysis was performed using the Statistical Package for the Social Sciences for Windows, version 11.0 (SPSS, Chicago, IL, USA) and MedCalc Software (Ghent, Mariakerke, Belgium).

Significance was defined as P < 0.05.

Results
Statistical calculus showed that 200 cases (100 women/group) would be necessary to detect an anticipated decrease of 15% in PR with 80% power and 5% significance level.
Both groups were compared regarding donor cycle parameters (age, gonadotrophin dose, stimulation days, E₂ levels on day of HCG, number of oocytes retrieved, and mature oocytes retrieved). There were no statistical differences with respect to all the abovementioned parameters (Table I), except for the number of oocytes retrieved (18.2 ± 0.6 versus 20.8 ± 0.6; \( P = 0.003 \)) and number of mature oocytes retrieved (16.9 ± 0.6 versus 19.4 ± 0.6; \( P = 0.005 \)).

Recipient’s characteristics (age, waiting time with HRT until donation, endometrial thickness and male factor) did not differ between groups. The results are resumed in Table II. The number of oocytes received per recipient (Group I – 11.4 ± 0.3 versus Group II – 11.7 ± 0.3) and semen parameters were comparable between groups (data not shown). Moreover, there were no differences between Group I and Group II in fertilization, cleavage rates, embryo division on day 3, blastocyst development or fragmentation rates. The number of embryos transferred, number of embryos cryopreserved, implantation, ongoing pregnancy and MR were similar between groups (Table II). We performed embryo transfer on blastocyst stage (day 5) in only 9 patients in Group I and 11 patients in Group II.

### Table I. Oocyte donor parameters depending on progesterone levels on the HCG day; Group I (<1.2 ng/ml) and Group II (≥1.2 ng/ml)

<table>
<thead>
<tr>
<th>Progesterone level</th>
<th>( P )-value</th>
</tr>
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<tbody>
<tr>
<td>&lt;1.2 ng/ml (( n = 120 ))</td>
<td>≥1.2 ng/ml (( n = 120 ))</td>
</tr>
<tr>
<td>Gonadotrophins (IU)</td>
<td>2250 ± 82</td>
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<tr>
<td>Stimulation days</td>
<td>10.7 ± 0.1</td>
</tr>
<tr>
<td>Estradiol on the day of HCG (pg/ml)</td>
<td>2630.4 ± 100.4</td>
</tr>
<tr>
<td>Number of oocytes retrieved</td>
<td>18.2 ± 0.6</td>
</tr>
<tr>
<td>Number of mature oocytes retrieved</td>
<td>16.9 ± 0.6</td>
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</tbody>
</table>

### Table II. Oocyte donation outcome parameters depending on progesterone levels on the HCG day; Group I (<1.2 ng/ml) and Group II (≥1.2 ng/ml)

<table>
<thead>
<tr>
<th>Progesterone level</th>
<th>( P )-value</th>
</tr>
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<tbody>
<tr>
<td>&lt;1.2 ng/ml (( n = 120 ))</td>
<td>≥1.2 ng/ml (( n = 120 ))</td>
</tr>
<tr>
<td>Oocytes received</td>
<td>11.4 ± 0.3</td>
</tr>
<tr>
<td>Fertilization (%)</td>
<td>69.23 ± 2.1</td>
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<tr>
<td>Cleavage (%)</td>
<td>90.6 ± 2.4</td>
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<tr>
<td>Embryo fragmentation (%)</td>
<td>7.95 ± 0.6</td>
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<tr>
<td>Blastocyst development (%)</td>
<td>65.9 ± 13.3</td>
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<tr>
<td>Number of embryos transferred</td>
<td>1.93 ± 0.02</td>
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<tr>
<td>Number of embryos cryopreserved</td>
<td>1.45 ± 0.2</td>
</tr>
<tr>
<td>Implantation (%)</td>
<td>26.6</td>
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<tr>
<td>Pregnancy (%)</td>
<td>65/120 (54.4)</td>
</tr>
<tr>
<td>Miscarriage (%)</td>
<td>5/65 (7.5)</td>
</tr>
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\( P \)-value represents the significance value of the studied comparison; \( P < 0.05 \) = significant.

### Discussion

In standard IVF patients, it is difficult to separate the potential deleterious effects of PL on the oocyte from that on the endometrium, since both are simultaneously exposed to progesterone. Oocyte donation offers a powerful tool to discriminate both factors. Choosing a donor patient helps reduce the variability in oocyte quality that infertility may introduce. Oocyte donors are young and fertile and trend to respond to ovarian stimulation with a large number of high-quality oocytes. Moreover, in a donor oocyte programme, the recipient endometrium is spared from any potentially harmful side effects of ovarian stimulation, including PL.

The present study offers more cases to resolve these conflicting views, since the number of cycles studied reaches the 80% power required for drawing definite conclusions on PR. Moreover, we analysed outcome on donors who repeated donation cycles presenting PL or not, working as their own controls, a novel model to study the effect of progesterone in oocyte and embryo quality. Our results cannot find a detrimental effect on the oocyte performance in terms of fertilization or cleavage rates. Moreover, no adverse effect on the embryo quality was found, when we compared embryo fragmentation and blastocyst development rates, as well as number of transferred and cryopreserved embryos per cycle.

The presence of elevated serum progesterone during follicular phase is not always prevented by LH suppression promoted by GnRH agonists and is seen in up to 30% of COH for IVF (Edelstein et al., 1990; Schoolcraft et al., 1991; Silverberg et al., 1991; Fanchin et al., 1993; Givens et al., 1994). Several hypotheses may be considered to explain this phenomenon: the rising oestradiol levels may induce increased LH secretion sufficient to stimulate granulose cells to produce progesterone but inadequate to trigger ovulation (Peluso, 1990; Ubaldi et al., 1995); the highest sensitivity of the granulose cells to FSH is due to the increased oestradiol levels as well as due to the increased number of follicles with 17 mm or more (Peluso, 1990; Filicori et al., 2003; Glamoclija et al., 2005), and a possible LH effect from exogenous gonadotrophins (Peluso, 1990). In this study, we observed a significantly higher number of total and mature oocytes retrieved in donors who showed PL. These findings in our oocyte donor programme are similar to those found in the above studies. However, we did not find a relationship between serum E₂ and progesterone on the day of HCG. Moreover, we did not verify that gonadotrophin dose was associated to serum progesterone levels, once the mean doses administered per cycle were not different between groups, as others authors previously observed (Mio et al., 1992; Burns et al., 1994). Interestingly, despite more follicles, more oocytes and more mature oocytes in the PL group, there were no differences regarding serum E₂ between the groups. This was consistent with the findings observed in a previous study (Bosch et al., 2003). We think that it must be caused by the premature process of luteinization of the granulose cells. In these cases, we verified more follicles, but lower E₂ production by each one, a possible direct effect of the progesterone on the ovarian ambient, a finding supported by Glamoclija et al. (2005).
Many groups have reported significantly declining PR with increasing serum progesterone values on the day of HCG administration (Hamori et al., 1987; Edelstein et al., 1990; Schoolcraft et al., 1991; Silverberg et al., 1991; Kagawa et al., 1992; Mio et al., 1992; Fanchin et al., 1993; Check et al., 1994; Givens et al., 1994; Mio and Terakawa, 1995; Bosch et al., 2003; Ozcakir et al., 2004). Although a significant inverse relationship between serum progesterone on the day of HCG and the success of IVF is established in many programmes, the involved endocrinologic mechanism is unclear. Forman et al. (1989), Sharma et al. (1990) and Silverberg et al. (1991) suggested that the mechanism of deleterious effect of an elevated progesterone was abnormally accelerated endometrial maturations leading to impaired endometrial receptivity. However, several clinical trials have been performed in which progesterone on the day of HCG administration, without any negative impact on PR due to a deleterious effect on the endometrium, suggesting that there is no negative impact of the PL on IVF outcome (Howles et al., 1988; Mahadevan et al., 1988; Ben-Nun et al., 1990; Hassiakos et al., 1990).

To study a possible impact of the PL on the oocyte quality, Hofmann et al. (1993) studied 68 cycles of oocyte donation from donors with and without PL. They concluded that the negative impact of high levels of progesterone on OPR in IVF cycles from young women is not due to an adverse effect of PL on oocyte and embryo quality. Legro et al. (1993) analysed 114 consecutive oocyte-donation cycles and found higher PR in recipients who received oocytes from donors with PL. They suggested that PL in oocyte donors, as detected by elevated serum progesterone concentration on the day of HCG administration, may simply be a clinical marker of the reproductively fit ovary. These authors affirmed that the term ‘premature luteinization’ may even be an inaccurate designation. If serum progesterone represents a cumulative effect due to low-level production by multiple follicles, then PL, at least at the level of the individual follicle, may not occur. Fanchin et al. (1996) analysed 162 cycles of oocyte donation from 102 fertile donors and observed the same results. However, Schoolcraft et al. (1991) reported a diminished serum E2 on the day after HCG administration, a finding that was interpreted by the authors as suggesting that progesterone was a marker of impaired follicle/oocyte quality due to post maturity.

Hereby, the premature elevation of the serum progesterone on the day of HCG administration during COH does not appear to have a negative impact on OPR in our oocyte-donation programme.

References


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