Does the estradiol level on the day of human chorionic gonadotrophin administration have an impact on pregnancy rates in patients treated with rec-FSH/GnRH antagonist?

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BACKGROUND: The purpose of this prospective observational study was to evaluate the association between estradiol (E2) levels on the day of human chorionic gonadotrophin (hCG) administration and pregnancy rates in a recombinant FSH (rec-FSH) antagonist fixed protocol.

METHODS: A group of 207 patients (≤39 years of age), treated by IVF/ICSI, received 200 IU/day rec-FSH from Day 2 of the cycle and daily GnRH antagonist starting on Day 6 of stimulation. The criteria for hCG administration included only the presence of ≥2 follicles of ≥17 mm diameter. One to two embryos were transferred on Day 3 after oocyte retrieval.

RESULTS: The area under the curve (AUC) for E2 on the day of hCG could not distinguish between pregnant and non-pregnant women (AUC:0.5; 95% confidence interval (CI): 0.42–0.59). No significant difference was observed between the three percentile groups of E2 values on the day of hCG administration [group 1, lower 25th percentile (<1142 pg/ml); group 2, medium 50th percentile (1142–2446 pg/ml) and group 3, higher 75th percentile (>2446 pg/ml)] for the ongoing pregnancy rates (P = 0.52). On the contrary, the linear regression model showed that higher E2 values on the day of hCG administration significantly improved the scores of transferred embryos (P = 0.01) as well as the total embryo score (P = 0.02). Yet subgroup analysis only in this high responders group revealed lower E2 and progesterone levels on the day of hCG in pregnant women compared with the non-pregnant (P = 0.01).

CONCLUSIONS: E2 concentrations on the day of hCG administration in GnRH antagonist cycles are not associated with pregnancy rates. A potential deleterious impact of estradiol on endometrial receptivity is shown for the high responders who have high E2 levels and improved embryo quality without a concomitant rise in pregnancy rate.

Key words: estradiol / GnRH antagonist / IVF / pregnancy rate / implantation

Introduction

Supraphysiological serum estradiol levels are inextricably related to the ovarian stimulation that is necessary for multiple follicular developments. The E2 levels can be increased more than 10-fold compared with those found during spontaneous cycles (Pittaway and Wentz, 1983). On the basis of the fact that cyclic changes in the endometrium are regulated by ovarian steroid hormones, the increased E2 levels due to controlled ovarian hyperstimulation may compromise endometrial receptivity for embryo implantation (Garcia et al., 1984; Hadi et al., 1994). Assessment of the association between estradiol levels on the day of human chorionic gonadotrophin (hCG) administration and pregnancy achievement in in-vitro fertilization (IVF) cycles has been the focus of interest for many years. Adverse effects of supraphysiological serum E2 can include alteration in both endometrial receptivity and oocyte/embryo quality. However, the detrimental effect of
high E₂ levels on uterine receptivity has been subjected to dispute. Simon et al. (1995) has suggested that high E₂ levels are responsible for cases of impaired endometrial receptivity without affecting embryo quality in oocyte donation cycles, although Sharara and McClamrock (1999) have failed to find a detrimental effect on pregnancy outcome of high E₂ levels on the day of hCG administration. To investigate a deleterious effect of E₂ on embryonic implantation, an established in-vitro model for embryonic adhesion was used by Valbuena et al. (2001). Higher E₂ concentration affected embryonic adhesion although a direct toxic effect on the embryo seemed to occur at the cleavage stage.

The systematic review by Kosmas et al. (2004) has shown that E₂ levels do not affect treatment outcome in GnRH agonist down-regulated IVF/ICSI cycles. However, the role of E₂ levels on the day of hCG administration in pregnancy achievement has only been evaluated retrospectively.

The purpose of this prospective observational study was to evaluate the association of E₂ levels on the day of hCG with pregnancy rates and embryo quality in a recombinant FSH (rec-FSH) antagonist fixed protocol. The criteria for hCG administration did not include E₂ levels and were only based on the sonographically confirmed presence of ≥3 follicles of ≥17 mm diameter (Kolibianakis et al., 2004). In order to exclude the effects of a prolonged culture period on the embryo quality (Chen et al., 2003) and to investigate the E₂ effect on the outcome, one to two embryos were transferred on Day 3.

Materials and Methods

Patient population

There were 230 patients, treated by IVF/ICSI at the Centre for Reproductive Medicine of the Dutch-Speaking Brussels Free University between October 2007 and December 2008, included in the study. Patients could enter the study only once. Inclusion criteria were age ≤39 years, body mass index (BMI) between 18 and 29 kg/m², presence of both ovaries and basal levels of estradiol (≤80 pg/ml) and progesterone (≤1.6 ng/ml) on Day 1 of the cycle. Exclusion criteria were the presence of endometriosis stage ≥3 (American Fertility Society), polycystic ovarian syndrome (Rotterdam criteria), the need for preimplantation genetic diagnosis and azoospermia testicular sperm extraction.

Ovarian stimulation

Recombinant FSH (Puregon; NV Organon, Oss, The Netherlands) was started on Day 2 of the menstrual cycle at a dose of 200 IU/day in all patients. The dose of rec-FSH remained unchanged during stimulation until Day 10 of the cycle. If it was necessary to increase the dose of rec-FSH after 10 days of stimulation, or to decrease the dose of rec-FSH due to a risk of Ovarian Hyperstimulation Syndrome (OHSS), the patient was dropped out of the study. To inhibit a premature LH surge, daily GnRH—antagonist (Orgalutran; NV Organon) was used from the morning of Day 6 of stimulation.

Final oocyte maturation was achieved with 10 000 IU of hCG (Pregnyl; Organon) as soon as three follicles ≥17 mm were present on ultrasound (US) scan. Oocyte retrieval was carried out 36 h after hCG administration by transvaginal US-guided puncture of follicles.

Conventional IVF or ICSI was carried out as described by Van Landuyt et al. (2005). Oocytes and embryos were cultured in sequential media at 37°C in an atmosphere of 6% CO₂, 5% O₂ and 89% N₂.

The results of the study by Chen et al. (2003) support that increasing E₂ levels on the day of hCG administration are associated with improved pregnancy rates when embryo transfer is performed on Day 5. The increase in pregnancy rates could be potentially favoured by other factors such as a more synchronized supportive uterine environment. One or two embryos were therefore transferred on Day 3 after oocyte retrieval.

Embryo selection for transfer or freezing was done in the morning of the day of transfer. Embryos were classified as top quality (score 3), medium quality (score 2) or poor quality (score 1) embryos. Supernumerary top or medium quality embryos were frozen on Day 3. Top quality embryos on Day 3 were defined as embryos with at least eight blastomeres on that morning and with <10% fragmentation. Medium quality embryos were embryos with at least 8 cells and 10–30% fragmentation or 6–7-cell embryos with 0–20% fragmentation. Poor quality embryos were defined as embryos with 6–7 cells and 20–50% fragmentation or 4–5-cell embryos up to 50% fragmentation.

Luteal supplementation

The luteal phase was supplemented with vaginal administration of 600 mg natural micronized progesterone in three separate doses (Utrogestan; Pitee, Brussels, Belgium) starting 1 day after oocyte retrieval and continuing for 14 days.

Hormonal measurements

Hormonal assessment was performed at the initiation of stimulation, on Day 6 of rec-FSH stimulation, on Day 8 and on the day of hCG administration. Additional blood samples were taken as necessary between antagonist initiation and hCG administration. Serum LH, FSH, E₂, P and hCG were measured by means of the automated Elecsys immunoanalyzer (Roche Diagnostics, Mannheim, Germany). Intra-assay and interassay coefficients of variation (CVs) were <3% and <4% for LH, <3 and <6% for FSH, <5 and <10% for E₂, <3% and 5% for P and <5 and <7% for hCG, respectively.

US assessment

US was performed on Day 6 of stimulation and thereafter as necessary in order to ensure that hCG would be injected on the first day that the patient had ≥3 follicles of ≥17 mm. At 7 weeks after embryo transfer, a US was performed and the presence of an intrauterine gestational sac confirmed a clinical pregnancy. An US at 12 weeks of gestation defined the ongoing pregnancy.

Outcome measures

The primary outcome measure was to assess the predictive value of E₂ on the ongoing pregnancy rate and secondary outcome measures were the number of COCs retrieved, embryo quality and the implantation rate (as defined from dividing the total number of fetal hearts observed by US by the number of embryos transferred).

Power analysis

No data were available in rec-FSH/GnRH antagonist cycles to accurately estimate sample size for the present study. As a consequence, a power analysis could not be performed in this prospective observational study. It was arbitrarily decided that an analysis would be planned as soon as 230 patients had been included.
Statistical analysis

Nominal variables were analyzed in the form of a frequency table by the use of the χ² test. Normally distributed metric variables were analyzed by the independent sample t-test. Receiver operating characteristic (ROC) curve analysis was performed to assess the predictive value of E₂ day hCG on pregnancy achievement. Tukey’s Hinges percentile analysis used to define groups of E₂ on hCG day. One-way analysis of variance (ANOVA) with least significant difference post hoc test was used to compare the three percentile groups. Multiple linear and logistic regressions were used to identify the variables that may contribute to the outcomes whereas adjusting for confounding variables (the parameters could enter the model only if they were significantly different between pregnant and non-pregnant patients). All tests were two-tailed, with a confidence level of 95% (P < 0.05). Values are expressed as mean (SD), unless stated otherwise.

Results

There were 230 patients started stimulation. For seven patients, due to a lack of response, an increase in the dose of rec-FSH was necessary and therefore they were excluded from the study. Additionally, 16 patients dropped out because a decrease in the dose of rec-FSH was necessary due to risk of OHSS. Finally, 207 patients were further analysed.

The women included in this study were aged 32.9 ± 3.7 years. The serum E₂ concentrations on the day of hCG administration were on average 1972.8 ± 1237.2 pg/ml and the mean number of oocytes obtained was 11.77 ± 6.9. The ongoing pregnancy rate was 29.5% (61/207); 55 were singleton and six were twin pregnancies.

Table I summarizes the patient characteristics, stimulation and treatment outcome parameters of the pregnant compared with non-pregnant women. There was no significant difference in E₂ levels on the day of hCG between pregnant and non-pregnant women (P = 0.40). Pregnant patients had significantly lower FSH and LH levels on the day of hCG administration compared with the non-pregnant patients (13.8 versus 15.3, P = 0.01; 1.5 versus 2.4, P = 0.01).

Logistic regression using as the dependent variable the achievement of ongoing pregnancy and as independent variables the basal FSH, and LH, progesterone and FSH on the day of hCG is shown in Table II. A significant effect on the achievement of ongoing pregnancy was observed for the basal FSH (0.84, 95% confidence interval (CI): 0.72–0.97) and the progesterone level on day hCG (0.52, 95% CI: 0.23–0.92). The lower the level of FSH at the initiation of the stimulation and the lower the level of progesterone on the day of hCG administration, the higher the probability of achievement of ongoing pregnancy. The lack of an effect of the E₂ levels on the hCG day on the pregnancy rate was confirmed with logistic regression.

To assess the predictive value of serum E₂ level on the day of hCG administration on pregnancy achievement, a ROC curve analysis was performed (Fig. 1). The area under the curve (AUC) calculated could not distinguish between pregnant and non-pregnant women (AUC:0.50; 95% CI: 0.42–0.59). Percentile analysis to define percentile groups of E₂ on the day hCG was performed. Table III shows patient characteristics and outcomes according to three groups of estradiol values on the day of hCG as follows: group 1, lower 25th percentile (<1142 pg/ml); group 2, medium 50th percentile (1142–2446 pg/ml) and group 3, higher 75th percentile (>2446 pg/ml). There were no statistically significant differences between the three groups in the implantation rates, ongoing pregnancy rates and the number of pregnancies. A post-hoc analysis revealed that significant difference existed between medium (1142–2446 pg/ml) and higher (>2446 pg/ml) percentile in the duration of the stimulation, the amount of FSH administered, the duration of antagonists treatment and the serum progesterone on the day of hCG. The basal E₂ was different only between the lower (<1142 pg/ml) and medium (1142–2446 pg/ml) percentile. Although the difference in the scores of the embryos transferred...
Pregnancy rates and E2 levels on day of hCG

**Table II Logistic regression on achievement of ongoing pregnancy after embryo transfer**

<table>
<thead>
<tr>
<th>Dependent variable: achievement of ongoing pregnancy</th>
<th>P-value</th>
<th>Odds ratio</th>
<th>95% CI Lower</th>
<th>95% CI Upper</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal FSH</td>
<td>0.02</td>
<td>0.84</td>
<td>0.72</td>
<td>0.97</td>
</tr>
<tr>
<td>LH on hCG day</td>
<td>0.14</td>
<td>0.87</td>
<td>0.72</td>
<td>1.04</td>
</tr>
<tr>
<td>P on hCG day</td>
<td>0.02</td>
<td>0.52</td>
<td>0.23</td>
<td>0.92</td>
</tr>
<tr>
<td>FSH on hCG day</td>
<td>0.13</td>
<td>0.94</td>
<td>0.86</td>
<td>1.02</td>
</tr>
<tr>
<td>Model P = 0.01</td>
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</tr>
</tbody>
</table>

CI = confidence interval.

**Figure I** ROC curve for AUC E2 on hCG day with dependent parameter the achievement of ongoing pregnancy.

correlation only with the embryo quality of the transferred embryos ($r^2 = 0.02, P = 0.001$).

**Discussion**

To the best of our knowledge, this is the first study to prospectively investigate the association of E2 levels on the day of hCG and pregnancy achievement in GnRH antagonist down-regulated IVF/ICSI treatment cycles, since previously reported studies had been conducted retrospectively in GnRH agonist cycles.

According to our findings, low basal FSH and low progesterone level on the day of hCG are independent predictors of pregnancy achievement. In addition, our results have shown that the concentration of estradiol on the day of hCG administration does not interfere with the occurrence of ongoing pregnancies.

Regarding the FSH levels, our results based on the logistic regression analysis are in agreement with the only study of GnRH antagonist cycles by Jurema et al. (2003), which has shown that lower basal levels of FSH are correlated with improved ovarian response and pregnancy rates in IVF cycles. Basal FSH levels are one of the most commonly used tests for predicting success in IVF treatment as first described by Muasher et al. (1988). Numerous studies have shown that an elevated basal FSH levels are associated with lower pregnancy rates in ART (Scott et al., 1989; Martin et al., 1996; Sharif et al., 1998).

Additionally, a systematic review of tests predicting IVF outcome by Broekmans et al. (2006) has shown that the measurement basal FSH in regularly cycling women is accurate in the prediction of non-pregnancy only at very high threshold levels. The authors recommend its use as a screening test for counselling purposes.

Our data show that a rise in progesterone on the day of hCG administration impairs pregnancy outcome. Premature luteinization refers to a rise in serum progesterone levels on the day of hCG administration above a threshold level, and its incidence is between 5 and 30% in IVF patients (Schoolcraft et al., 1991; Silverberg et al., 1991; Fanchin et al., 1993; Ubaldi et al., 1995). A recent systematic review and meta-analysis by Venetis et al. (2007) on this issue analysed mostly GnRH agonist cycles (GnRH agonists: n = 109, GnRH antagonists: n = 2624). The results suggest that progesterone elevation, on the day of hCG administration for final oocyte maturation, does not appear to be associated with the probability of pregnancy in women undergoing ovarian stimulation with GnRH analogues and gonadotrophins for IVF. A more recent publication by our group (Papanikolaou et al., 2009) after using a GnRH antagonist/rec-FSH protocol has shown that even modest rises of progesterone in the follicular phase have detrimental effect on the implantation potential of a good-quality cleavage stage embryo.

In the case of estradiol, our results showed no association between E2 levels and pregnancy achievement, which is in agreement with the meta-analysis by Kosmas et al. (2004). The studies included in this meta-analysis differ in their conclusions: two studies (Chenet et al., 1990; Gelety and Buyalos, 1995) show a positive association, two studies show a negative association of E2 and pregnancy rates (Simon et al., 1995; Ng, 2000), and the majority of studies show no association (Dor et al., 1992; Sharara and McClamrock, 1999; Papageorgiou et al., 2002; Chen et al., 2003). The controversy of this issue might be partially explained by the retrospective design of
the studies, small sample sizes, as well as methodological issues such as the inclusion of more than one cycle for analysis of the same patients in more than three studies (Kosmas et al., 2004).

E2 levels are routinely assessed during ovarian stimulation by a number of clinics, even though they are not used as a criterion for hCG administration. The decision on timing for inducing final oocyte maturation is mostly based on sonographic evaluation of the number and size of the follicles. The use of E2 levels to distinguish poor, normal and high responders in order to predict treatment outcome is still controversial (Papageorgiou et al., 2002). Different E2 cut-off levels (Simon et al., 1995, 1998; Ng, 2000) make interpretation of data cumbersome. Despite the fact that percentile curves present a more objective tool, there are still no uniformly accepted E2 percentiles. Papageorgiou et al. (2002) have used 10th and 90th percentiles to define the type of response, with normal responders having E2 between the 10th and 90th percentile. In the present study, we have used percentile analysis to divide the patients in three groups according to E2 levels on day of HCG administration (1142, 1142–2446, >2446 pg/ml). Our results show that in the higher 75th percentile (E2 levels >2446 pg/ml), the pregnancy rates remained the same as compared with the medium and lower percentile group, although the embryo score was higher than the two other groups. These patients had significantly higher progesterone levels on the day of hCG, significantly more oocytes were retrieved than from the other two groups and their embryos, both transferred and frozen, had higher scores. The association between the high E2 and premature progesterone elevation suggest that at least one of the mechanisms that play a role in the premature increase of plasma progesterone is

| Table III Percentile groups of E2 on the day of hCG administration and their effects on different parameters and outcomes |
|-------------------------------------------------|-------------------------------------------------|-------------------------------------------------|------------------|
| Lower 25th percentile (<1142 pg/ml) (n = 51)     | Medium 50th percentile (1142–2446 pg/ml) (n = 105) | Higher 75th percentile (>2446 pg/ml) (n = 51) | P                |
| Age (years)                                     | 32.6(3.7)                                       | 33.4 (3.5)                                      | 32.41 (4.1)       | 0.21 |
| BMI (kg/m²)                                     | 23.6 (3.7)                                      | 23 (3.2)                                       | 23.5 (3.3)        | 0.49 |
| No. of trials                                   | 1.2 (0.539)                                     | 1.1 (0.6)                                      | 1.1 (0.38)        | 0.72 |
| Cause of infertility (%)                        |                                                 |                                                 |                  |
| Male                                           | 56.9                                            | 67.6                                            | 62.7             | 0.16 |
| Ovulatory                                      | 0                                               | 1.9                                             | 3.9              |      |
| Tubal                                          | 3.9                                             | 11.4                                            | 9.8              |      |
| Idiopathic                                     | 35.3                                            | 17.1                                            | 23.5             |      |
| Lesbian                                        | 3.9                                             | 1.9                                             | 0                |      |
| Treatment (%)                                   |                                                 |                                                 |                  |
| IVF                                            | 23                                              | 17.1                                            | 17.6             | 0.61 |
| ICSI                                           | 76.5                                            | 82.9                                            | 82.4             |      |
| Duration of stimulation (days)                  | 9.3 (1.7)                                       | 9.1 (1.5)                                       | 9.8 (1.4)        | 0.02** |
| Total FSH administered (IU)                     | 1870.6 (346)                                    | 1807.6 (293.4)                                  | 1952.9 (291)     | 0.02** |
| Duration of antagonist treatment (days)         | 5.3 (1.7)                                       | 5.1 (1.5)                                       | 5.7 (1.4)        | 0.04** |
| Basal FSH (IU/L)                                | 8.5 (3.6)                                       | 7.9 (2.3)                                       | 6.9 (2)          | 0.07  |
| Basal E2 (pg/ml)                                | 34.2 (16.5)                                     | 42.2 (18.4)                                     | 40.4 (11.8)      | 0.02* |
| Basal LH (IU/L)                                 | 5 (2.9)                                         | 5.2 (2.1)                                       | 6.1 (2.2)        | 0.04  |
| Progesterone on hCG day (ng/mL)                 | 1.2 (0.8)                                       | 1.2 (0.6)                                       | 1.4 (0.5)        | 0.04** |
| Endometrium on day hCG                         | 9 (1.5)                                         | 9.5 (2.1)                                       | 9.6 (2)          | 0.30  |
| No. COCs retrieved                             | 7.7 (4.8)                                       | 11.1 (5.5)                                      | 17.2 (8)         | 0.01**** |
| No of embryos transferred                      | 1.4 (0.5)                                       | 1.5 (0.5)                                       | 1.6 (0.5)        | 0.06  |
| Score of embryos transferred                   | 3.2 (1.5)                                       | 3.9 (1.6)                                       | 4.1 (1.5)        | 0.01*** |
| Score of embryos frozen                        | 3 (4.4)                                         | 4.8 (7.1)                                       | 7.9 (12.3)       | 0.05  |
| Total embryo score                             | 5.8 (4.9)                                       | 8.7 (7.6)                                       | 12 (12.6)        | 0.02*** |
| Implantation rate (%)                          | 23.5                                            | 23.8                                            | 25.5             | 0.96  |
| Ongoing pregnancy rate (%)                     | 23.5                                            | 32.4                                            | 29.4             | 0.52  |
| No of pregnancies (%)                          |                                                 |                                                 |                  |
| Singletons                                      | 83.3                                            | 90.9                                            | 93.8             | 0.64  |
| Twins                                          | 16.7                                            | 9.1                                             | 6.3              |      |

Data are presented as mean (SD). P value correspond on post-hoc analysis between lower and medium percentile. P** value correspond on post-hoc analysis between medium and upper percentile. P*** value correspond on post-hoc analysis between lower and upper percentile. P**** correspond on post-hoc analysis between the three percentiles.
linked to the high response of the ovary to ovarian stimulation. An excess in the number of follicles and consecutively an excess of proliferating granulosa cells can lead to an increased progesterone production.

The fact that the embryos were of better quality in the high-responder group, with progesterone elevation, makes the theory of impaired oocyte/embryo quality unlikely. Instead, the high follicular progesterone can advance into the endometrium and therefore the replacement of a Day 3 embryo (earlier than when it happens naturally) in an asynchronous endometrium may result in implantation failure (Papanikolaou et al., 2009).

In combination with the premature elevation of progesterone, the high E2 levels had a negative effect, since transfer of high quality embryos in cycles with high E2 on day of hCG did not result in pregnancy achievement.

Chen et al. (2007) have evaluated 1196 GnRH agonist cycles grouped by peak E2 percentile distribution into three groups: normal responder between percentile 25–75: 1199–3047 pg/ml, n = 595 cycles; moderate high responders P75–P90, E2 between 3048–4127 pg/ml, n = 180 cycles; and for the high response group, the E2 cut-off concentration was set as P90 and above E2 > 4128 pg/ml, n = 119 cycles. The high response group showed decreased trends in implantation and pregnancy rates compared with normal responders, but statistical significance was reached only for the difference in implantation rates. High serum estrogen levels were detrimental to implantation, but not to the quality of oocytes, which may be due to an adverse effect on endometrial receptivity in ovarian stimulation cycles.

On the basis of our results and the findings by Chen et al. (2007), in order to improve pregnancy rates in the case of high estradiol levels at the end of the ovarian stimulation, a proposed strategy could be the selection for embryo transfer on Day 5. Transfer of blastocysts may offer the endometrium time to recover from any negative effects caused by the exposure to high peak E2 levels.

Ovarian stimulation induces supraphysiological levels of steroids during the follicular phase resulting in advanced endometrial

<table>
<thead>
<tr>
<th>Table IV  Comparison between different parameters for pregnant and non-pregnant women in high responders group (E2 &gt; 2446 pg/ml)</th>
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</thead>
<tbody>
<tr>
<td>Pregnant (n = 15)</td>
</tr>
<tr>
<td>Age (years)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
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<tr>
<td>No. of trials</td>
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<tr>
<td>Cause of infertility (%)</td>
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<tr>
<td>Male</td>
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<td>Ovulatory</td>
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<td>Tubal</td>
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<td>Idiopathic</td>
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<tr>
<td>Lesbian</td>
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<tr>
<td>Treatment (%)</td>
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<tr>
<td>IVF</td>
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<td>ICSI</td>
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<td>Duration of stimulation (days)</td>
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<tr>
<td>Total FSH administered (IU)</td>
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<tr>
<td>Duration of antagonist treatment (days)</td>
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<tr>
<td>Basal FSH (IU/l)</td>
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<td>Basal E2 (pg/ml)</td>
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<td>E2 on Day 6 (pg/ml)</td>
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<td>E2 on Day 8 (pg/ml)</td>
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<td>LH on day of hCG (IU/L)</td>
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<td>Progesterone on day of hCG (ng/mL)</td>
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<tr>
<td>FSH on hCG day (IU/l)</td>
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<tr>
<td>Treatment outcome</td>
</tr>
<tr>
<td>No. COCs retrieved</td>
</tr>
<tr>
<td>No. embryos transferred</td>
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<tr>
<td>Quality score of transferred embryos</td>
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</tbody>
</table>

Values are means (standard deviation).
development regardless of the type of GnRH analogue used. In GnRH-agonist cycles, endometrial biopsies taken in the pre-ovulatory phase prior to hCG injection have shown accentuated proliferative aspects and early secretory changes even before the rise in progesterone occurs (Marchini et al., 1991). Biopsies taken on the day of oocyte retrieval show endometrial advancement in more than 90% of the cases, with no pregnancy occurring if the advancement is exceeded by 3 days (Ubaldi et al., 1997). These findings have been confirmed in GnRH-antagonist cycles (Kolibianakis et al., 2002). A study by Van Vaerenbergh et al. (2009) on endometrial gene expression on the day of oocyte retrieval in antagonist cycles with embryo replacement on Day 3 also confirmed the histologically advanced endometrial maturation. A lower endometrial receptivity was observed in patients with histological dating exceeding 3 days advancement.

Increased sensitivity to progesterone resulting in secretory advancement could be induced by elevated estrogen concentration (Jacobs et al., 1987). In GnRH-agonist stimulated cycles, moderate responders exhibit less desynchrony in endometrial glandular and stromal maturation compared with high responders (Basir et al., 2001). However, very little data are available on the role of E2 on the endometrial receptivity in GnRH-agonist stimulated cycles. Detti et al. (2008a) found no correlation in endometrial US dynamics, E2 concentrations and pregnancy outcome. Decreased endometrial thickness in these cycles was related to miscarriage rate but not to estradiol concentrations (Detti et al., 2008b).

In our study the absolute number of oocytes obtained was proportional to the E2 on hCG day, with reduced numbers of COCs from the higher to the lower percentiles for E2 levels. However, as the number of oocytes retrieved increased, the implantation and pregnancy rates remained unchanged, no decreases were observed, in contradiction with previous studies (Forman et al., 1988; Pellicer et al., 1989). An explanation for the lack of effect on implantation and pregnancy rates could be the low number of very high responder women in the study.

In conclusion, although improved embryo quality has been observed in the presence of high E2 levels, no subsequent increase in pregnancy rate was noticed. This observation suggests that elevated E2 concentrations could have a deleterious effect on endometrial receptivity. An increase in progesterone during the follicular phase, which is also a reflection of a good ovarian response, can also have a negative impact on the endometrium. The results of this study render further molecular and clinical research to establish the degree of endometrial advancement with the regard to the follicular estradiol levels in the GnRH antagonist cycles.

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