Addition of estradiol to progesterone for luteal supplementation in patients stimulated with GnRH antagonist/rFSH for IVF: a randomized controlled trial

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BACKGROUND: The role of progesterone for luteal support in stimulated cycles for IVF is well established. However, controversy still surrounds the benefit of additional supplementation with estradiol (E2) in GnRH agonist (GnRHa) cycles, while no such data are available for GnRH antagonists. The aim of this randomized controlled trial (RCT) was to compare ongoing pregnancy rates in patients stimulated with recombinant FSH (rFSH) and GnRH antagonist for IVF, who received micronized progesterone for luteal phase supplementation, with or without the addition of E2.

METHODS: Two hundred and one patients underwent ovarian stimulation with a fixed dose of 200 IU rFSH and GnRH antagonist. Patients were randomized to receive, for luteal phase supplementation, either 600 mg of micronized progesterone vaginally (n = 100, progesterone group) or 600 mg of micronized progesterone and 4 mg of E2 valerate orally (n = 101, progesterone/E2 group). The main outcome measure was ongoing pregnancy at 12 weeks per patient randomized.

RESULTS: Demographics, stimulation parameters and embryological data were comparable for the two groups compared. Twenty-six ongoing pregnancies were achieved in the progesterone (26%) and 30 in the progesterone/E2 group (29.7%). (Difference: 3.7 and 95%, CI: –15.8 to 8.6%). CONCLUSION: It appears that the addition of E2 to progesterone in the luteal phase after stimulation with rFSH and GnRH antagonist does not enhance the probability of pregnancy.

Key words: estradiol/GnRH antagonists/luteal phase support

Introduction

The luteal phase is defined as the period from occurrence of ovulation until the establishment of a pregnancy or the resumption of menses 2 weeks later. In the context of assisted reproduction techniques, luteal phase support is the term used to describe the administration of medication with the aim to support the process of implantation.

The ‘ideal’ scheme of luteal phase support in stimulated cycles has been a matter for debate since the early days of IVF. Edwards and Steptoe (1980) were the first to postulate luteal phase inadequacy resulting from ovarian stimulation as a cause of IVF failure. Although the exact mechanism with which luteal phase support enhances implantation is not clear, it is well accepted that luteal phase supplementation is crucial during the time between the disappearance of exogenous HCG administered for final oocyte maturation and the rise in endogenous HCG during early implantation (Nyboe et al., 2002).

The role of progesterone as luteal support in stimulated cycles is well established (Maslar, 1988). However, controversy surrounds the benefit of additional supplementation with estradiol (E2). (Ludwig and Diedrich, 2001). Smitz et al. (1993), in a prospective randomized study, evaluated the advantage of adding E2 valerate 6 mg per os daily to intravaginal micronized progesterone (600 mg daily) as luteal supplementation in 378 infertile women superovulated with a GnRH agonist (GnRHa) and HMG for IVF. The clinical pregnancy rate was similar whether or not E2 valerate was added to intravaginal progesterone.

The meta-analysis by Pritts and Atwood (2002) suggested that progesterone in combination with E2 is the best luteal support in long and short agonist protocols. A beneficial effect of E2 might be related to the dose in which it is used. Lukaszuk
et al. (2005), in a prospective randomized study, recently evaluated the effect of different E2 supplementation doses during the luteal phase on implantation and pregnancy rates in women undergoing ICSI in agonist cycles \((n=231)\). All subjects received luteal phase support with natural micronized progesterone (Utrogestan, Laboratories Besins-Iscovesco, Paris, France), 600 mg/day vaginally in three divided doses, starting on the day of oocyte pickup. Women were randomly allocated to daily doses of 0, 2 or 6 mg of E2 during the entire luteal phase. It was shown that the addition of a high dose of E2 to daily progesterone supplementation significantly improved the probability of pregnancy in women treated with a long GnRHa analogue protocol for controlled ovarian stimulation (COS). Similarly, Fahri et al. (2000), in a prospective randomized study, evaluated the effect of adding E2 to progesterin supplementation during the luteal phase in 271 patients undergoing IVF, who had E2 levels of higher than 2500 pg/dl at the day of HCG administration. All patients received progesterone supplementation at a dosage of 150 mg/day divided between 50-mg i.m. injections and 50-mg vaginal tablets starting on the day after oocyte retrieval. Patients were randomized to receive 2 mg of E2 (Estrophem; Novo Nordisk, Bagsvaerd, Denmark), given orally, starting on day 7 after embryo transfer, or without any exogenous E2 supplementation during the luteal phase.

It was shown that for patients who are treated with the long GnRHa protocol for COS, the addition of E2 to the progesterin-supplement regimen has a beneficial effect on pregnancy and implantation rates. However, such an effect could not be shown in the short GnRHa protocol.

Currently, no data exist concerning the effect of co-administering E2 with progesterone in GnRH antagonist cycles on the probability of pregnancy. The aim of this randomized controlled trial (RCT) was to compare ongoing pregnancy rates between two different modes of luteal supplementation, using progesterone with and without the addition of E2 in patients stimulated with recombinant FSH (rFSH) and GnRH antagonists.

### Materials and methods

**Patient population**

Between October 2004 and October 2005, 201 infertile patients were randomized to receive progesterone or progesterone plus E2 for luteal phase support, according to a computer-generated not concealed randomization list prior to initiation of stimulation. The inclusion criteria were age ≤39 years, BMI between 18 and 29 kg/m², presence of both ovaries, basal levels of E2 (≤80 pg/ml), progesterone (≤1.6 mg/ml) and FSH levels <10 IU/l at initiation of stimulation, fewer than three prior cycles (agonist or antagonist cycles). Patients could enter the study only once. The exclusion criteria were presence of polycystic ovarian syndrome (The Rotterdam ESHRE/ASRM-Sponsored PCOS consensus workshop group, 2004), presence of endometriosis American Fertility Society (AFS) classification stage >2, azoospermia testicular sperm extraction (TESE) and need for preimplantation genetic diagnosis (PGD).

The research project was approved by our Institutional Review Board.

**Ovarian stimulation and IVF/ICSI procedures**

Recombinant FSH (Puregon®, NV Organon, Oss, the Netherlands) was started in the afternoon of day 2 of the cycle at 200 IU. The dose of rFSH remained unchanged during stimulation until day 10 of the cycle. To inhibit premature LH surge, daily GnRH antagonist (Orgalutran® 0.25 mg, NV Organon) was used from the morning of day 6 of stimulation. Final oocyte maturation was achieved by administration of 10,000 IU of HCG (Pregnyl®, NV Organon) as soon as ≥3 follicles of ≥17 mm were present. Oocyte retrieval was carried out 36 h after HCG administration. Previous studies have described ICSI and IVF procedures in detail (Van Steirteghem et al., 1993; Devroey et al., 1995; Devroey and Van Steirteghem, 2004; Van Landuyt et al., 2005).

Embryos were transferred on day 3 after oocyte retrieval. Either one or two embryos were transferred per patient. Patients were stratified by the number of embryos transferred.

### Luteal supplementation

**Progesterone one group**

Luteal phase supplementation with vaginal administration of 600 mg natural micronized progesterone in three separate doses (Utrogestan®, Besins-Iscovesco, 100 mg 3 × 2/day) was applied, starting 1 day after oocyte retrieval and continued until 7 weeks of gestation if pregnancy was achieved.

**Progesterone/E2 group**

The same treatment as in the previous group was applied with the addition of E2 valerate (Progynova®, Schering N.V., S.A B-1831 Diegem, Belgium) 2 × 2mg/day per os, also starting 1 day after oocyte retrieval and continued until 7 weeks of gestation if pregnancy was achieved. Progynova® 2 mg was administered once in the morning and once in the evening.

### Hormonal measurements

Hormonal assessment was performed at initiation of stimulation and on the day of HCG administration. Additional blood samples were taken as necessary between antagonist initiation and HCG administration. The serum HCG test was performed on days 16 and 18 after the administration of HCG. Serum LH, FSH, HCG, E2 and progesterone were measured with the automated Elecsys immunoanalyzer (Roche Diagnostics, Mannheim, Germany). Intra-assay and inter-assay coefficients of variation (CVs) were <3% and <4% for LH, <3% and <6% for FSH, <5% and <7% for HCG, <5% and <10% for E2 and <3% and <5% for progesterone, respectively.

### Ultrasound assessment

Ultrasound was performed on day 6 of stimulation and thereafter as necessary to ensure that HCG was injected on the first day that the patient had ≥3 follicles of ≥17 mm. For that purpose, a follicular growth of 2 mm per day was assumed to be present (Kolibianakis et al., 2004).

### Outcome measures

The main outcome measure was ongoing pregnancy per randomized patient. Secondary outcome measure was early pregnancy loss. Ongoing pregnancy was defined as pregnancy developing beyond 12 weeks, while early pregnancy loss was defined as the proportion of patients with initially positive HCG in whom pregnancy failed to develop before 12 weeks of gestation.

### Statistical analysis

Sample sizes of 1417 in each group achieve 80% power at a 5% significance level to detect a difference of 5%, considered as clinically important, assuming a 35% ongoing pregnancy rate in the treatment group and a 30% ongoing pregnancy rate in the control group. This is not feasible for a single centre study. Thus, embarking on the current study, the aim was to provide data on the effect of E2 addition to micronized progesterone on ongoing pregnancy rates on a relatively
large patient population, which could be included in a future meta-analysis on this issue. An arbitrary choice was made to perform an analysis when 100 patients had been included in each arm. To the best of our knowledge, this is the first RCT evaluating E2 addition to micronized progesterone for luteal support in GnRH antagonist cycles.

Normally distributed metric variables were analysed using the independent sample t-test, while not normally distributed variables were analysed using the Mann–Whitney U-test. Nominal variables were analysed in the form of frequency tables using the Fisher’s exact test. All tests were two-tailed with a confidence level of 95% (P < 0.05). Values are expressed as mean ± SD, unless otherwise stated.

**Results**

Two hundred and one patients undergoing IVF were randomized, prior to initiation of stimulation, to receive the two different modes of luteal support. The flowchart of the patients included in the study is shown in Figure 1. No significant differences were observed between the two groups for the mean age at initiation of stimulation, number of previous IVF trials, number of cumulus oocyte complexes retrieved, number of embryos transferred, number of embryos frozen on day 3 of culture, BMI, days of stimulation and rFSH units used for the stimulation. Moreover, the cause of infertility in the two groups was also similar (Table I).

Similarly, no significant differences were observed regarding hormonal values in the follicular phase on day 1 and on the day of HCG administration between the two groups compared (Table II). The number of developing follicles and the endometrial thickness on the day of HCG were also similar.

There was no significant difference either in the type of treatment (IVF versus ICSI) performed in the two groups compared (29% IVF versus 71% ICSI in the progesterone group and 28.7% IVF versus 71.3% ICSI in the progesterone/E2 group) or in the number of embryos transferred. Single embryo transfer versus double embryo transfer: 71 versus 29% in the progesterone group and 71.3 versus 28.7% in the progesterone/E2 group, respectively.

The implantation rate per embryo transfer was 37.8% in the progesterone group versus 42.4% in the progesterone/E2 group, respectively.

The early pregnancy losses, which included biochemical pregnancies, ectopic pregnancies and first trimester abortions, were also similar in the two groups (23.5 versus 23.1%, respectively). The pregnancy per patient randomized was 26% in the progesterone group versus 29.7% in the progesterone/E2 group (P = 0.548, not significant).

Ongoing pregnancy per patient randomized was 26% in the progesterone group versus 29.7% in the progesterone/E2 group (P = 0.637). Also, there were no significant differences between the ongoing pregnancy rates per oocyte retrieval and per embryo transfer in the two groups (36.8 versus 30.3% and 32.6 versus 28.9%, respectively). The early pregnancy losses, which included biochemical pregnancies, ectopic pregnancies and first trimester abortions, were also similar in the two groups (23.5 versus 23.1%, respectively) (Table III).

**Discussion**

To the best of our knowledge, this is the first study performed so far in GnRH antagonist cycles comparing two different luteal phase support schemes. The current study suggests that addition of 4 mg E2 to progesterone does not increase the probability of pregnancy.

To minimize possible sources of bias in the current study, a fixed dose of rFSH and a fixed protocol of GnRH antagonist administration were used. Moreover, all embryos were transferred on day 3 of culture while randomization was stratified for the number of embryos transferred.

No studies currently exist to evaluate the role of E2 for luteal support in patients stimulated with GnRH antagonists, though; such an intervention has been evaluated in GnRHa cycles. The effect of E2 addition to progesterone for luteal phase support is, however, debatable. The studies by Lukaszuk et al. (2005) and Fahri et al. (2000) favoured the use of progesterone plus E2 as providing better luteal support than progesterone only.

On the contrary, other authors failed to observe any beneficial effect from adding E2 to progesterone as luteal supplementation.

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**Table I. Patient and stimulation characteristics in the progesterone and the progesterone/E2 group**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Progesterone</th>
<th>Progesterone/E2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (mean ± SD)</td>
<td>32.05 ± 3.66</td>
<td>32.03 ± 3.55</td>
</tr>
<tr>
<td>BMI (mean ± SD)</td>
<td>22.70 ± 2.76</td>
<td>22.01 ± 2.82</td>
</tr>
<tr>
<td>Number of previous trials (mean ± SD)</td>
<td>1.53 ± 0.73</td>
<td>1.34 ± 0.61</td>
</tr>
<tr>
<td>Aetiology of infertility in the two groups (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Andrological</td>
<td>61</td>
<td>62.4</td>
</tr>
<tr>
<td>Endometriosis</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Idiopathic</td>
<td>13</td>
<td>13.9</td>
</tr>
<tr>
<td>Tubal</td>
<td>22</td>
<td>19.8</td>
</tr>
<tr>
<td>Days of stimulation (mean ± SD)</td>
<td>8.99 ± 1.50</td>
<td>9.04 ± 1.61</td>
</tr>
<tr>
<td>FSH units (mean ± SD)</td>
<td>1796 ± 500.81</td>
<td>1807.92 ± 322.39</td>
</tr>
<tr>
<td>Cumulus oocyte complex (mean ± SD)</td>
<td>12.33 ± 7.40</td>
<td>11.91 ± 6.15</td>
</tr>
<tr>
<td>Number of embryo’s transferred (mean ± SD)</td>
<td>1.29 ± 0.46</td>
<td>1.27 ± 0.45</td>
</tr>
<tr>
<td>Number of embryo’s frozen at day 3 (mean ± SD)</td>
<td>3.05 ± 3.47</td>
<td>2.88 ± 3.36</td>
</tr>
</tbody>
</table>

No significant differences were observed between the groups compared.

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**Figure 1.** Flowchart of the patients included in the study. OPU, oocyte pick up; ET, embryo transfer.
Table II. Hormonal measurements on day 1 and on the day of HCG administration in patients who received progesterone or progesterone/E2 for luteal phase supplementation

<table>
<thead>
<tr>
<th></th>
<th>Progesterone group day 1</th>
<th>Progesterone/E2 group day 1</th>
<th>Progesterone group day HCG</th>
<th>Progesterone/E2 group day HCG</th>
</tr>
</thead>
<tbody>
<tr>
<td>LH IU/l (mean ± SD)</td>
<td>5.45 ± 2.46</td>
<td>5.45 ± 2.46</td>
<td>2.05 ± 2.27</td>
<td>1.91 ± 1.73</td>
</tr>
<tr>
<td>E2 pg/ml (mean ± SD)</td>
<td>38.45 ± 14.50</td>
<td>41.25 ± 17.75</td>
<td>1997.78 ± 1323.69</td>
<td>1890.45 ± 996.69</td>
</tr>
<tr>
<td>FSH IU/l (mean ± SD)</td>
<td>8.04 ± 3.40</td>
<td>8.36 ± 4.10</td>
<td>14.91 ± 3.62</td>
<td>15.68 ± 4.10</td>
</tr>
<tr>
<td>Progesterone pg/ml (mean ± SD)</td>
<td>0.75 ± 0.48</td>
<td>0.75 ± 0.37</td>
<td>1.24 ± 0.62</td>
<td>1.30 ± 0.64</td>
</tr>
</tbody>
</table>

No significant differences were observed between the groups compared.

Table III. Achievement of pregnancy in the two groups randomized

<table>
<thead>
<tr>
<th></th>
<th>Progesterone group (%)</th>
<th>Progesterone/E2 group (%)</th>
<th>P value</th>
<th>Difference in ongoing pregnancy rate (%) (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ongoing pregnancy/patient randomized</td>
<td>26 (26/100)</td>
<td>29.7 (30/101)</td>
<td>0.64</td>
<td>3.7 (−15.8 to +8.6)</td>
</tr>
<tr>
<td>Ongoing pregnancy per oocyte retrieval</td>
<td>26.8 (26/97)</td>
<td>30.3 (30/99)</td>
<td>0.64</td>
<td>3.5 (−15.9 to +9.1)</td>
</tr>
<tr>
<td>Ongoing pregnancy per embryo transfer</td>
<td>28.9 (26/90)</td>
<td>32.6 (30/92)</td>
<td>0.63</td>
<td>7 (−16.8 to +9.6)</td>
</tr>
<tr>
<td>Early pregnancy loss*</td>
<td>23.4 (8/34)</td>
<td>23.1 (9/39)</td>
<td>1</td>
<td>0.4 (−18.4 to +19.9)</td>
</tr>
</tbody>
</table>

No significant differences were observed between the groups compared.

*Biochemical pregnancies, ectopic pregnancies and first trimester abortions.

in cycles stimulated with HMG according to a long protocol (Smits et al., 1993; Lewin et al., 1994).

The choice of 4 mg of E2 supplementation in the current study was arbitrary, because no dose-finding studies have been performed so far regarding this issue in antagonist cycles. Fahri et al. (2000) in GnRHa cycles suggested that a beneficial effect of E2 supplementation is present when a dose of 2 mg of E2/day was co-administered with progesterin support, while Smits et al. (1993), in agonist cycles, could not confirm such an effect using a higher E2 dose supplementation (6 mg/day). Although addition of E2 appears not to be beneficial in the current study using a dose of 4 mg, the probability that a beneficial effect might be present after supplementations with higher E2 doses cannot be excluded.

The role of E2 in the follicular phase is well documented. E2 is indispensable for endometrial priming and is also responsible for the proliferation of surface epithelium, glands, stroma and blood vessels in endometrium. So far, however, the exact role of E2 in the luteal phase has not been determined (Younis et al., 1994). In the luteal phase of an IVF cycle, serum E2 and progesterone often decrease to low levels if no hormonal support is provided. The luteal decline in sex steroid levels is associated with reduced implantation and pregnancy rates. (Hutchinson-Williams et al., 1989).

The main causes of luteal decline in sex steroid levels (luteal phase defect) are most probably related to the initial high concentrations of E2 in the early luteal phase (De Ziegler et al., 1992). E2 is involved in the regulation of LH secretion (Nippoldt et al., 1989) and might cause extremely low LH concentrations in the luteal phase as a result of a strong negative feedback mechanism (Beckers et al., 2003). On the contrary, high progesterone levels, in the absence of E2 in the luteal phase, fail to suppress plasma gonadotrophins (Nippoldt et al., 1989; De Ziegler et al., 1992).

To maintain adequate levels of sex steroids, different regimens of luteal support have been described. (Nosarka et al., 2005) However, the current study finding does not support the addition of E2 to progesterone in GnRH antagonist cycles.

Given the fact that the number and quality of the embryos transferred were similar for the two groups compared, the similarity in pregnancy rates observed might reflect an adequate endometrial preparation in the two groups.

Interestingly, luteal E2 depletion in the human does not seem to adversely affect the morphological developmental capacity of the endometrium. (Younis et al., 1994). Moreover, De Ziegler et al. (1992) showed that E2 is not necessary for the endometrial action of secretion and that, regarding the morphological appearance of the endometrium in the luteal phase, it cannot be suggested that E2 conveys a positive endometrial effect (Bourgain et al., 1994).

In the future, it might be interesting to study the effect of the addition of E2 in subgroups of patients stimulated for IVF by GnRHa or antagonist with low levels of E2 during the luteal phase. Moreover, the effect of different E2 doses on the probability of pregnancy warrants further investigation.

In conclusion, the addition of E2 to progesterone in the luteal phase after stimulation with rFSH and GnRH antagonist does not appear to increase the probability of pregnancy.

References


De Ziegler D, Bergeron C, Cornel C, Medalie D, Massal M, Milgrom E, Frydman R and Bouchard P (1992) Effects of luteal estradiol on the secretory action of secretion and that, regarding the morphological developmental capacity of the endometrium. (Younis et al., 1994). Moreover, De Ziegler et al. (1992) showed that E2 is not necessary for the endometrial action of secretion and that, regarding the morphological appearance of the endometrium in the luteal phase, it cannot be suggested that E2 conveys a positive endometrial effect (Bourgain et al., 1994).

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References


