Pituitary down-regulation in IVF/ICSI: consequences for treatment regimens
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Citation for published version (APA):

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Timing luteal phase support in GnRH agonist down-regulated IVF/embryo transfer cycles

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Madelon Van Wely
Fulco van der Veen

*Human Reproduction 2006; 21: 905-8*
Background

The aim of this study was to compare the effect of three different times of onset of luteal phase support on ongoing pregnancy rate in infertile patients undergoing treatment with GnRH down-regulated IVF and embryo transfer (IVF/ET).

Materials and Methods: All consecutive eligible patients planned to undergo their first IVF treatment cycle were randomly allocated to receive vaginal progesterone as luteal support at three different time points, that is, after HCG administration for final oocyte maturation (HCG group), at the day of oocyte retrieval (OR group) or at the day of ET (ET group). The primary endpoint of this study was ongoing pregnancy rate.

Results: A total of 385 women were randomized, 130 were allocated to the HCG group, 128 to the OR group and 127 to the ET group. An ongoing pregnancy rate of 20.8% was found in the HCG group versus 22.7 and 23.6% in the OR group and ET group, respectively. The mean number and quality of the retrieved oocytes and the transferred embryos did not differ.

Conclusion: Based on this data, an 18% difference in ongoing pregnancy rate between the three different times of onset of luteal phase support in GnRH agonist down-regulated IVF/ET cycles can be refuted. Smaller clinically meaningful differences may be present.
Introduction

The use of GnRH agonists for preventing premature LH surges in controlled ovarian hyperstimulation in IVF/embryo transfer (IVF/ET) has greatly improved the planning of oocyte retrieval (OR). This pituitary suppression, however, still blocks the output of LH for at least 10 days after cessation of the agonist, causing a luteal phase deficiency characterized by a decline in serum estradiol (E2) and progesterone 8 days after HCG administration for oocyte maturation (Smitz et al., 1987). This decline in steroids was thought to have a negative effect on pregnancy rate and exogenous supplementation of progesterone or HCG, i.e. luteal phase support, proved indeed to be mandatory (Pritts and Atwood, 2002; Daya and Gunby, 2004).

In all studies included in the above-mentioned meta-analyses, we have seen that the time of onset of administration of luteal phase support ranged randomly from the day before OR to 4 days after ET. Only three randomized studies have been performed to assess the impact of the moment of starting luteal phase support on pregnancy rate in GnRH agonist down-regulated IVF cycles. In the first study, a decrease of 12% in pregnancy rate was seen when luteal phase support was started 12 h before OR compared to 12 h after OR (Sohn et al., 1999). In the second study, a decrease of 24% was seen when luteal phase support was delayed until 6 days after OR compared to 3 days after OR (Williams et al., 2001). In the third study, no difference was found when luteal phase support was started at OR compared to starting at ET (Baruffi et al., 2003).

The chosen time points of the start of luteal support assessed in these randomized studies, however, did not cover the complete implantation window, which includes the day of HCG administration until the day of ET. The aim of this study, therefore, was to assess the impact of the onset of luteal phase support on ongoing pregnancy rate, in which all the different possible moments to start luteal support are represented. We compared administration of vaginal progesterone starting before OR, i.e. at the time of HCG administration for final oocyte maturation (HCG group), at OR (OR group) and at ET (ET group) in a randomized clinical trial, in infertile patients undergoing treatment with GnRH agonist down-regulated IVF/ET.
Patients and methods

All consecutive patients between January 1993 and December 1997 scheduled for their first IVF treatment in the Center of Reproductive Medicine at the Academic Medical Center in Amsterdam, the Netherlands, were asked to participate in this study. The study was approved by the Institutional Review Board. After informed consent, patients were assigned to three different luteal phase support groups and received 400 mg micronized progesterone vaginally in two daily doses starting at the evening of HCG administration for final oocyte maturation (HCG group) or at the evening after OR (OR group) or at the evening after ET (three days after OR) (ET group). Progesterone treatment was continued until the onset of menstruation, or until 18 days following OR. The randomization was performed at a baseline visit by fertility doctors by opening a sealed opaque envelope containing the arm of treatment. The envelopes were prepared and numbered by the main investigator.

The IVF treatment was performed according to a local standard protocol described earlier (Repping et al., 2002). Venous blood samples for serum E₂ (nmol/l) and progesterone (nmol/l) were drawn at OR and on the 3rd, 6th, 9th, 12th and 18th day after OR and were assessed with a radioimmunoassay assay (DPC, Los Angeles, CA, USA) for E₂ and (Orion Diagnostica, Espoo, Finland) for progesterone. The intra-assay variation was 5 or 6% for E₂ and progesterone, respectively.

Retrieved oocytes were classified as Score I, II, III and IV (atretic oocytes) for IVF patients (Veeck, 1988). Embryos were classified according to fixed criteria (Veeck, 1988): Score I as excellent quality with no fragmentation, Score II as good with 1–10% fragmentation, Score III as fair with 21–50% fragmentation and Score IV as poor with more than 50% fragmentation.

Biochemical pregnancies were defined as an increase in serum HCG >2 IU/ml or a positive pregnancy test assesses at the 18th day after oocyte retrieval (Tandem ICON test; Hybritech, San Diego, CA, USA). Clinical pregnancies were defined as a gestational sac seen by transvaginal ultrasound at the 35th day after oocyte retrieval. Ongoing pregnancies were defined as a positive fetal heartbeat by transvaginal ultrasound 10 weeks after OR.

Analysis
The primary endpoint in this study was ongoing pregnancy rates, expressed as a rate ratio with corresponding 95% confidence intervals (CI). Secondary
endpoints were oocyte quality, fertilization rate, number and quality of obtained, transferred and frozen embryos, biochemical, clinical pregnancies and live birth rates. In order to prove a two-sided difference of 18% in ongoing pregnancy rate between the HCG group or the ET group over a control rate of 30% in the OR group, 115 patients were needed per study arm (Sohn et al., 1999; Williams et al., 2001). We required at least 380 patients, taking into account a withdrawal of 10%. The study was designed as a superiority trial, with a power of 80% and an alpha of 0.05. The analysis of all outcomes was on an intention-to-treat basis. Pregnancy outcomes were expressed as a relative risks (RR) with 95% CI. Comparison of hormonal values was performed by one-way analysis of variance. The Kruskal–Wallis test was used to compare the number and quality of retrieved oocytes and embryos. A significance level of $P < 0.05$ (two-sided) was used for all tests.

**Results**

A total of 385 consecutive patients, with an indication for IVF and scheduled for their first treatment cycle, were asked to participate in this study. A flow chart of inclusion, randomization, discontinuation and treatment is shown in Figure 1. Of the 385 randomized patients, 30 left the study before the start of the IVF treatment. Reasons for this discontinuation were a change in personal circumstances (12), incorrect use of the pretreatment (pituitary down-regulation) medication (6), occurrence of spontaneous pregnancies (2), failure to produce sperm (2), withdrawn informed consent (4), active hepatitis B infection (1), ovarian cysts (2) or unknown reasons (3).
Pituitary down-regulation in IVF/ICSI

Figure 1.

Table I summarizes patients’ characteristics for all treatment groups. Age, parity, indication for IVF and the total motile sperm count were equally divided between the three groups.

The IVF results are summarized in Table II. No difference was found in mean number of stimulation days, mean number or quality of retrieved oocytes, the mean number or quality of transferred embryos or the total number of frozen embryos.
Timing luteal phase support

Table I. Baseline characteristics

<table>
<thead>
<tr>
<th></th>
<th>HCG-group</th>
<th>OR-group</th>
<th>ET-group</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean Age in years (SD)</td>
<td>34.4 (3.9)</td>
<td>33.7 (4.5)</td>
<td>33.6 (4.1)</td>
<td>NS</td>
</tr>
<tr>
<td>Primary infertility (%)</td>
<td>57</td>
<td>55</td>
<td>64</td>
<td>NS</td>
</tr>
<tr>
<td>Indication IVF (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male factor</td>
<td>28</td>
<td>28</td>
<td>30</td>
<td>NS</td>
</tr>
<tr>
<td>Tubal factor</td>
<td>31</td>
<td>31</td>
<td>32</td>
<td>NS</td>
</tr>
<tr>
<td>Unexplained</td>
<td>31.5</td>
<td>27.5</td>
<td>30</td>
<td>NS</td>
</tr>
<tr>
<td>Other</td>
<td>9.5</td>
<td>13.5</td>
<td>8</td>
<td>NS</td>
</tr>
<tr>
<td>Total Motile Sperm</td>
<td>67.2 (87.5)</td>
<td>51.6 (72.1)</td>
<td>76.1 (105.8)</td>
<td>NS</td>
</tr>
<tr>
<td>Count (SD)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

NS = Not Significant

Table II IVF Results

<table>
<thead>
<tr>
<th></th>
<th>HCG-group</th>
<th>OR-group</th>
<th>ET-group</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stimulation treatment days mean (SD)</td>
<td>10.0 (2.5)</td>
<td>10.1 (2.8)</td>
<td>9.7 (2.3)</td>
<td>NS</td>
</tr>
<tr>
<td>no. of retrieved oocytes, mean (SD)*</td>
<td>10.5 (6.3)</td>
<td>9.7 (6.8)</td>
<td>10.7 (8.3)</td>
<td>NS</td>
</tr>
<tr>
<td>Quality of oocytes: mean (SD)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Score I</td>
<td>6.7 (4.3)</td>
<td>6.2 (5.0)</td>
<td>7.0 (5.0)</td>
<td>NS</td>
</tr>
<tr>
<td>Score II</td>
<td>0.3 (1.0)</td>
<td>0.2 (0.5)</td>
<td>0.2 (0.5)</td>
<td>NS</td>
</tr>
<tr>
<td>Score III</td>
<td>3.5 (3.8)</td>
<td>3.3 (3.9)</td>
<td>3.8 (4.3)</td>
<td>NS</td>
</tr>
<tr>
<td>Score IV</td>
<td>0.01 (0.9)</td>
<td>0.05 (0.2)</td>
<td>0.06 (0.2)</td>
<td>NS</td>
</tr>
<tr>
<td>Quality of embryo's mean (SD)*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Score 1</td>
<td>0.6 (1.7)</td>
<td>0.6 (1.4)</td>
<td>0.6 (1.1)</td>
<td>NS</td>
</tr>
<tr>
<td>Score II</td>
<td>2.5 (2.5)</td>
<td>2.6 (3.5)</td>
<td>2.7 (3.3)</td>
<td>NS</td>
</tr>
<tr>
<td>Score III</td>
<td>2.4 (2.7)</td>
<td>2.1 (2.4)</td>
<td>2.2 (2.9)</td>
<td>NS</td>
</tr>
<tr>
<td>Score IV</td>
<td>0.4 (0.7)</td>
<td>0.5 (0.9)</td>
<td>0.5 (1.2)</td>
<td>NS</td>
</tr>
<tr>
<td>no. of embryo's transferred, mean (SD)</td>
<td>2.2 (0.8)</td>
<td>2.2 (0.7)</td>
<td>2.1 (0.7)</td>
<td>NS</td>
</tr>
<tr>
<td>no. of embryo's frozen mean (SD)</td>
<td>1.5 (2.5)</td>
<td>2.1 (3.9)</td>
<td>1.7 (3.2)</td>
<td>NS</td>
</tr>
</tbody>
</table>

*per oocyte retrieval
Pituitary down-regulation in IVF/ICSI

Mean serum E₂ levels did not differ between the three groups (Figure 2). With the exception of the day of OR, where a significantly higher mean serum progesterone level was seen in the HCG group (P < 0.001), no differences were seen in mean serum progesterone levels during the remainder of the luteal phase (Figure 3).

**Figure 2.**

Mean serum estradiol values (nmol/L) and 95% CI at the day of oocyte retrieval (OR), 3, 6, 9, 12 and 18 days thereafter (OR + 3, OR + etc) (closed circles = HCG-group, triangles = OR-group, closed squares = ET-group)

**Figure 3.**

Mean serum progesterone values (nmol/L) and 95% CI at the day of oocyte retrieval (OR), 3, 6, 9, 12 and 18 days thereafter (OR + 3, OR + etc) (closed circles = HCG-group, triangles = OR-group, closed squares = ET-group)
Timing luteal phase support

Lastly, there were no significant differences between the three groups in biochemical, clinical and ongoing pregnancies or live birth (Table III).

### Table III Pregnancy results*

<table>
<thead>
<tr>
<th>ITT</th>
<th>n (%)</th>
<th>RR**</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Biochemical</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OR-group</td>
<td>39 (30.5)</td>
<td>0.83</td>
<td>0.56-1.23</td>
</tr>
<tr>
<td>hCG-group</td>
<td>33 (25.4)</td>
<td>1.06</td>
<td>0.73-1.52</td>
</tr>
<tr>
<td>ET-group</td>
<td>41 (32.3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Clinical</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OR-group</td>
<td>36 (28.1)</td>
<td>0.82</td>
<td>0.54-1.24</td>
</tr>
<tr>
<td>hCG-group</td>
<td>30 (23.1)</td>
<td>1.04</td>
<td>0.70-1.53</td>
</tr>
<tr>
<td>ET-group</td>
<td>37 (29.1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Ongoing</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OR-group</td>
<td>29 (22.7)</td>
<td>0.92</td>
<td>0.58-1.45</td>
</tr>
<tr>
<td>hCG-group</td>
<td>27 (20.8)</td>
<td>1.04</td>
<td>0.66-1.62</td>
</tr>
<tr>
<td>ET-group</td>
<td>30 (23.6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Live birth</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OR-group</td>
<td>27 (21.1)</td>
<td>0.94</td>
<td>0.58-1.52</td>
</tr>
<tr>
<td>hCG-group</td>
<td>26 (20.0)</td>
<td>0.97</td>
<td>0.60-1.56</td>
</tr>
<tr>
<td>ET-group</td>
<td>26 (20.5)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* excl. 2 spontaneous pregnancies  
**RR and 95% CI were calculated for the hCG and ET groups compared to the OR group

### Discussion

Synchronization of the fertilized egg and the receptivity of the endometrium, i.e. the implantation window, are regarded as essential in the success rate of IVF/ET (Bourgain and Devroey, 2003). In vivo, a small, but distinct, increase of endogenous progesterone is already seen concurrently with the LH surge. After the LH surge, it takes 36–48 h for progesterone to transform the proliferative endometrium into secretory-phase endometrium. The in vivo fertilized embryo usually arrives at the uterine cavity 72–96 h after ovulation, leaving sufficient time for the completion of the transformation of the endometrium (Croxatto et al., 1978). Compared to in vivo fertilized embryos, the in vitro fertilized embryos arrive earlier in the uterine cavity, i.e. 72 h after OR. Starting luteal phase support early in order to achieve a more advanced endometrium seems, therefore, preferable (Garcia et al., 1984). This was also observed in an oocyte donor programme, where the highest pregnancy rates were found in patients who were pretreated with progesterone for at least 3 days or more (Prapas et al., 1998).
Pituitary down-regulation in IVF/ICSI

Another method for achieving synchronization between endometrial receptivity and the embryo is varying the day of ET. A Cochrane review showed significantly higher clinical pregnancy rates of day three transferred embryos versus day two transferred embryos. (Oatway et al., 2004). Unfortunately, in this review, data concerning the start of luteal phase support is lacking.

This randomized study aimed to create three different implantation windows by adjusting the luteal phase of an IVF treatment cycle, while the day of ET was fixed on day 3. As luteal support, we used progesterone administered vaginally and not HCG, since HCG significantly increases the risk of ovarian hyperstimulation syndrome (Odds Ratio 3.06) (95% CI 1.59–5.86) (Daya and Gunby, 2004) with similar ongoing pregnancy rates.

We could not identify, within the chosen time points and within the sample size, a specific implantation window period in which an optimal endometrial receptivity yields the highest pregnancy rate. This trial, however, only had sufficient power to detect a difference in ongoing pregnancy rates between the OR group and one of the two other groups of at least 18%. It is possible that smaller, also clinically meaningful, differences may be present depending on the time of onset of administration of luteal phase support.

In view of our findings, it is worthwhile to consider recent insights learned from GnRH antagonist studies. Instead of adjusting the luteal phase, adjusting the length of the follicular phase may be of importance. Two recent studies have suggested that prolonging exposure of the endometrium to high levels of $E_2$ results in a lower ongoing pregnancy rate of 10%, which can be explained by the stage of advancement of the endometrium (Kolibianakis et al., 2003; Mochtar et al., 2004). By varying the moment of HCG administration for final oocyte maturation, it is possible to fine-tune the duration of the follicular phase. Further studies are needed to explore whether timing of HCG according to predetermined criteria of follicular size, opposed to the until now rather loose criteria, leads to higher ongoing pregnancy rates in GnRH agonists down-regulated controlled ovarian hyperstimulation IVF/ET cycles.
Timing luteal phase support

References


