Pituitary down-regulation in IVF/ICSI: consequences for treatment regimens
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Chapter 6

Progesterone alone versus progesterone combined with HCG as luteal support in GnRHa/HMG induced IVF cycles: a randomized clinical trial

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Abstract

Two different regimens of luteal support in gonadotrophin hormone-releasing hormone (GnRH) analogue/human menopausal gonadotrophin (GnRHa/HMG)-induced in-vitro fertilization cycle (IVF) were compared in a randomized clinical trial. After embryo transfer, either vaginal progesterone alone was administered (n = 89, P group), or a combination of vaginal progesterone and human chorionic gonadotrophin (n = 87, P/HCG group).

The primary aim of this study was to assess the effect of the different regimens of luteal support on the pregnancy rate. The secondary aim was to compare oestradiol and progesterone concentrations in the luteal phase between the two groups, and assess their effect on the pregnancy rate. A clinical pregnancy rate of 15% was found in the P/HCG group in comparison with 26% in the P group (odds ratio 0.49; 99% confidence interval: 0.18-1.3).

The luteal serum oestradiol and progesterone values in the P/HCG group were significantly higher when compared with the P group on the 6th, 9th and 12th day after oocyte retrieval (Wilcoxon P < 0.001). In accordance with the high oestradiol concentrations, more cases of ovarian hyperstimulation syndrome (OHSS) were found in the P/HCG group. Oestradiol values on the 9th day after oocyte retrieval, presumably the day of implantation, appeared to be higher in women who did not become clinically pregnant.

We conclude that vaginal progesterone alone provides sufficient luteal support in GnRHa/HMG induced IVF cycles. The combination of vaginal progesterone and HCG as luteal support leads to significant high luteal oestradiol and progesterone concentrations. But a high concentration of oestradiol seems to have a deleterious effect on the implantation process, resulting in a low pregnancy rate.
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Introduction

The first report of the combined use of pituitary suppression and ovarian hyperstimulation in in-vitro fertilization (IVF) and embryo transfer programmes was published in 1984 (Porter et al, 1984). Since then, the advantages of gonadotrophin hormone-releasing hormone analogues (GnRHa) have been well documented. The cancellation rate has been decreased through the prevention of premature luteinizing hormone (LH) surges, follicular recruitment has improved, and the ovarian response to hyperstimulation has been better synchronized, thus facilitating the scheduling of oocyte retrieval (OR).

This pituitary suppression however, results in an impaired gonadotrophin production later on, and the output of LH remains blocked for at least 10 days after cessation of GnRHa administration (Smitz et al, 1988; Broekmans et al, 1992). As gonadotrophins are necessary to maintain progesterone output by the corpus luteum, exogenous luteal support is mandatory. Without luteal support, a mid-luteal decline in sex steroids is seen, which adversely affects implantation (Hutchinson-Williams et al, 1989). Several randomized studies have indeed shown significantly higher pregnancy rates in GnRHa/human menopausal gonadotrophin (HMG)-stimulated IVF cycles with luteal support, when compared with a similar group who received no such support (Smith et al, 1989; Belaisch-Allart et al, 1990; Herman et al, 1990). In addition, various forms of luteal support have been tested, including human chorionic gonadotrophin (HCG), administered i.m., or progesterone, administered i.m., orally, or vaginally (Buvat et al, 1990; Claman et al, 1992; Smitz et al, 1992; Golan et al, 1993).

Although vaginal progesterone seems the optimal form of luteal support (Devroey et al, 1992), a meta-analysis performed by Soliman established a beneficial effect of HCG in particular (Soliman et al, 1994).

Both regimens of luteal support, i.e. progesterone and HCG, have different modes of action. Progesterone is a direct form of luteal support, i.e. the end-product of the corpus luteum. The advantage of a direct form of luteal support is its independence of corpus luteum function. As the amount of granulosa cells may be reduced by oocyte retrieval, the corpus luteum can fail to produce sufficient progesterone, despite adequate stimulation either by endogenous GnRH from the pituitary gland or by exogenous HCG administration (Garcia et al, 1981). HCG is an indirect form of luteal support, by stimulating the corpus luteum. It is known to generate an increase in both
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Oestradiol and progesterone concentrations, thus rescuing the failing corpus luteum in GnRHa/HMG-stimulated IVF cycles (Hutchinson-Williams et al, 1990).

As HCG stimulates all corpus luteum functions, we hypothesized that other, still unknown, products of importance for the maturation and sustaining of the endometrium would be stimulated as well. These products might have a beneficial effect on the hormonal milieu and, as a consequence, might lead to a higher pregnancy rate. We therefore performed a randomized clinical trial, comparing GnRHa/HMG-induced IVF treatment cycles supported with vaginal progesterone only, with an experimental group who received additional HCG i.m., in addition to the standard vaginal progesterone regime.

Our primary aim was to assess the effect of both regimens of luteal support on pregnancy rate, our secondary aim was to compare the concentrations of oestradiol and progesterone in the luteal phase between the two groups. In addition we assessed the effect of the concentration of sex steroids in the luteal phase on the pregnancy rate, by comparing the women who became pregnant with those who did not.

Materials and methods

From September 1991 until February 1992, all patients scheduled for IVF treatment in our infertility centre of the Academic Medical Center, Amsterdam, The Netherlands, were asked to participate in this study.

Multiple follicular development was induced with 225 IU i.m. HMG (Humegon, Organon, Oss, The Netherlands) from the fifth day of the cycle onward, preceded by either 200 mg buserelin intranasally (Suprefact; Hoechst Pharma, Amsterdam, The Netherlands) daily for 5 days starting on the first day of the menstruation, or 0.2 ml Leuprolide s.c. (Lucrin; Abbott, St Remi Sur Avre, France) starting on the third day of the cycle. HMG dosages were individually adjusted if necessary. When the leading follicle was >18 mm in diameter, ovulation was induced by administering 10 000 IU i.m HCG (Pregnyl, Organon)

Luteal support was started on the day of ovulation induction with 300 mg micronized progesterone orally (Progestan, Organon) for 6 days, until the day of embryo transfer. The patients who proceeded to embryo transfer were, after obtained informed consent, randomly allocated into two groups by opening an envelope containing the medication schedule. Patients with a history of ovarian hyperstimulation...
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syndrome (OHSS) or with a follicular oestradiol concentration >10.0 nM were excluded. One group received 400 mg vaginal progesterone daily until the onset of the menstruation, or until OR + 18 days (P group). The other group received, besides the same amount of 400 mg vaginal progesterone daily for the same period as the first group, an additional 1500 IU HCG i.m. on OR +3, +6, +9, and +12 days (P/HCG group). HCG administration was ceased if moderate OHSS occurred. The grades of OHSS were defined in accordance with the classification of Golan et al. (1989).

After the first, the second, and third treatment cycle, eligible non pregnant patients were again asked to participate in the trial. Blood samples for oestradiol (nM) and progesterone (nM) analysis were obtained at OR, embryo transfer and on OR +6, +9, +12, and +18 days thereafter. Oestradiol was determined by radioimmunoassay, (DPC, Los Angeles, CA, USA) with an intra-assay variation of 5-6%, and an inter-assay variation of 8-9% in the range of 1-5 nM. Progesterone was determined by radioimmunoassay (Orion Diagnostica, Espoo, Finland) with an intra-assay variation of 5-6% and an inter-assay variation of 7-8%.

Clinical pregnancies were defined as a positive urine pregnancy test, Tandem ICON Test, (Hybntech, SA, CA, USA) on OR +18 days. Ongoing pregnancies were defined as positive fetal heart beat at OR +10 weeks during transvaginal sonography.

In the period preceding the trial, a pregnancy rate of 18% per embryo transfer was found in our infertility centre An increase in pregnancy rate from 18 to 25%, i.e an odds ratio of 1.5, was considered clinically relevant. Power calculations showed that 566 patients would be required in order to prove, or refute, this 7% increase in pregnancy rate at the conventional 5% probability level with 80% power for both groups.

The clinical pregnancy rate was expressed as an odds ratio comparing the experimental P/HCG group with the standard P group. In order to correct for any difference that may occur in baseline characteristics, we adjusted this crude odds ratio for possible confounding factors by performing a logistic regression analysis. The confounding factors addressed in the analyses were age, indication for IVF, total number of embryos transferred, and the rank number of treatment cycle.

Interim analysis was performed after 176 cycles, with an alpha of 0.01. The crude odds ratio and the adjusted odds ratio were calculated for
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the occurrence of clinical pregnancy rate in the experimental P/HCG group compared with the P group. In order to decide whether to continue or discontinue the trial, we compared the adjusted odds ratio with the odds ratio of the above mentioned 1.5, that was required for clinical relevance. Where the upper limit of the 99% confidence interval (CI) of the adjusted odds ratio was under 1.5, the trial would be discontinued.

Serum oestradiol and progesterone concentrations on OR, OR + 3, + 6, +9, +12, +18 in the P/HCG group and the P group were compared with a Wilcoxon test. Furthermore serum oestradiol concentrations on OR +9 were compared with a Wilcoxon test, between women who became pregnant and those who did not.

Results

From September 1991 until February 1992, 176 consecutive cycles were included. The P/HCG group consisted of 87 cycles, and the P group of 89. Table I summarizes patient and cycle characteristics for both treatment groups. The mean age of the P/HCG group was lower and there were more patients with tubal disease and male infertility and less patients with unexplained infertility in the P group.

Table 1. Patients and cycle characteristics of the progesterone only (P) group and of the progesterone + human chorionic gonadotrophin (P+HCG) group.

<table>
<thead>
<tr>
<th></th>
<th>P-group</th>
<th>P/HCG-group</th>
</tr>
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<tbody>
<tr>
<td>age (± SD)</td>
<td>34.2 (± 3.5)</td>
<td>32.7 (± 3.8)</td>
</tr>
<tr>
<td>indication IVF:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>tubal disease</td>
<td>57 (67%)</td>
<td>46 (53%)</td>
</tr>
<tr>
<td>male factor</td>
<td>10 (11%)</td>
<td>6 (7%)</td>
</tr>
<tr>
<td>unexpl. infertility</td>
<td>20 (22%)</td>
<td>33 (37%)</td>
</tr>
<tr>
<td>other</td>
<td>2 (2%)</td>
<td>2 (2%)</td>
</tr>
<tr>
<td>Number of treatment cycles</td>
<td>89</td>
<td>87</td>
</tr>
<tr>
<td>rank number of treatment cycle:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>first treatment cycle</td>
<td>40 (45%)</td>
<td>58 (55%)</td>
</tr>
<tr>
<td>second treatment cycle</td>
<td>37 (42%)</td>
<td>25 (29%)</td>
</tr>
<tr>
<td>third treatment cycle</td>
<td>9 (10%)</td>
<td>9 (10%)</td>
</tr>
<tr>
<td>forth and more</td>
<td>3 (3%)</td>
<td>5 (6%)</td>
</tr>
<tr>
<td>number of retrieved oocytes (± SD)</td>
<td>7.55 (±4.58)</td>
<td>7.97 (±5.32)</td>
</tr>
<tr>
<td>number of transferred embryo's (± SD)</td>
<td>3.08 (±1.00)</td>
<td>3.01 (±1.00)</td>
</tr>
</tbody>
</table>
More first treatment cycles were found in the P/HCG group. No differences were found in the number of oocytes retrieved or in the number of embryos transferred.

In complete contrast to our hypothesis, a lower clinical pregnancy rate was found in the P/HCG group. In 13 of the 87 (15%) cycles in the P/HCG group a pregnancy occurred, compared to 23 of the 89 (26%) of the 89 cycles in the P group [odds ratio 0.49, 99% confidence interval (CI) 0.18-1.3].

Logistic regression analysis was performed in order to correct the above mentioned difference in the base-line characteristics and resulted in even lower odds ratio of 0.46 (99% CI 0.16-1.4) for the variable HCG. Since a higher abortion rate was found in the P group, a smaller difference for the ongoing pregnancy rate was seen. An odds ratio of 0.68 (99% CI 0.20-2.2) for an ongoing pregnancy in the P/HCG group was found; nine of the 87 cycles (10%) in the P/HCG group and 13 of the 89 cycles (15%) in the P group resulted in an ongoing pregnancy. In the P/HCG group, eight patients had mild OHSS, two patients had moderate OHSS and one patient had severe OHSS. In these 11 cases, HCG was withheld after the second injection in order to avoid aggravating the OHSS. In the P group, three cases of mild OHSS were seen.

Statistically significant higher oestradiol and progesterone concentrations were found in the P/HCG group when analysed with a Wilcoxon test in the luteal phase, especially on OR + 6, +9 and +12 days, as shown in Figures 1 and 2.

**Figure 1.**
Serum Oestradiol in nM.
Open circles = progesterone/human chorionic gonadotrophin group.
Closed squares = progesterone group.
Wilcoxon test on day of oocyte retrieval (OR) +6,+9,+ 12: P < 0.001.
OR = day of oocyte retrieval

**Figure 2.**
Serum progesterone in nM.
Open circles = progesterone/human chorionic gonadotrophin group.
Closed squares = progesterone group.
Wilcoxon test on day of oocyte retrieval (OR) +6,+9,+ 12: P < 0.001.
OR = day of oocyte retrieval
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In Figure 3, the serum oestradiol concentration on OR +9 (representing the implantation day) is shown. Both the P/HCG group and the P group are divided into subgroups for pregnant and non-pregnant women. In the P/HCG group serum oestradiol concentrations appeared to be lower in women who became pregnant, compared with the women who did not become pregnant. Although this difference was not statistically significant when analysed with a Wilcoxon test, we suspect that very high serum oestradiol concentrations have a negative effect during the implantation period on the pregnancy rate.

Fig 3. Serum oestradiol (E2) (median, range, middle two quartiles) on day of oocyte retrieval (OR) + 9, divided in progesterone (P) group/pregnant, P group/not pregnant, P/human chorionic gonadotrophin (HCG) group/pregnant, P/HCG group/not pregnant

Discussion

Contrary to our expectations, the interim analysis of our randomized clinical trial showed a lower pregnancy rate in the P/HCG group in comparison with the P group. An odds ratio of 0.49 with a 99% CI of 0.18-1.3 was calculated, meaning there was a 40% reduction in probability of becoming pregnant for the patients in the P/HCG group. As mentioned in the method section, an upper limit of the 99% CI >1.5 would justify us to continue the trial. Since we found, after regression analysis, an odds ratio of 0.46 with an upper limit of the 99% CI of 1.4, we decided to end the study. We can now refute, with 99% certainty, the hypothesis that the combination of HCG and progesterone does improve the pregnancy rate from 18 to 25%.

Significantly higher oestradiol and progesterone concentrations were found in the P/HCG group. As we expected, no mid-luteal decline in
die concentrations of sex steroids was seen. In order to show the potential beneficial effect of high luteal sex steroid on pregnancy rate, we divided die cycles of both groups into those which resulted in a pregnancy and those which did not. Again, contrary to our expectation, high concentrations of oestradiol and progesterone had no favourable effect on the pregnancy rate. In our study we found that women with oestradiol concentrations $>4.5$ mM on OR +9, have an odds ratio of becoming pregnant of 0.06 (99% CI 0.004-0.93) compared with the women with an oestradiol concentration $<4.5$ mM. We therefore suspect that an excessive oestradiol secretion on day OR + 9, presumably the implantation day, may interfere with implantation. The well-known contraceptive effect of exogenous oestrogen administration together with several studies on implantation rate in IVF cycles supports this assumption. (Gidley-Baird et al., 1986; Forman et al., 1988; Machn et al., 1990).

Finally an important point to be stressed is that a null hypothesis has been tested. Often null hypotheses and pre-test power calculations are disregarded in randomized clinical trials, and conclusions are drawn, based on false-positive or false-negative results. In fact all randomized clinical trials, cited in the introduction section and in the meta-analysis of Soliman et al. (1994) lack power calculations and null hypotheses. Therefore, the possibility remains that the results of these trials are false-positive. A meta-analysis, however, can overcome the problem of the insufficient power of studies, although judgement of the meta-analysis has to be done with reservations. Publication bias can have a distorting effect on meta-analysis. (Egger and Smith, 1995).

In conclusion, vaginal progesterone alone provides sufficient luteal support, despite a mid-luteal drop in sex steroids. However the duration and dosages of vaginal progesterone still need to be studied, in order to achieve the highest, ongoing pregnancy rate.
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