Outcome of in-vitro fertilization through natural cycles in poor responders

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This prospective study examines the benefits of using natural cycles instead of stimulated cycles in poor responders to in-vitro fertilization (IVF) treatment. Eleven patients in whom puncture was cancelled or who failed to conceive because of a poor response were included in the analysis. The data for natural cycles (n = 16) were compared with data obtained during previous stimulated cycles (n = 25) in the same women. Out of 16 natural cycles, 13 (81.3%) were scheduled for oocyte retrieval compared to 13 out of 25 stimulated cycles (52%). Eighteen metaphase II oocytes were obtained during stimulated cycles, giving a 66% fertilization rate. In natural cycles, 11 metaphase II oocytes were available giving a fertilization rate of 78.6%. A mean number of 51.5 ± 25 ampoules of gonadotrophins per cycle were used during ovarian stimulation. Three clinical pregnancies were obtained after embryo transfer in natural cycles (18.8%/started cycle) compared to none in stimulated cycles. Our findings demonstrate that an encouraging number of pregnancies can be achieved by IVF during natural cycles in poor responders to ovarian stimulation. This may not be the first approach to consider in IVF but it should be offered as an alternative after two ovarian response failures using classical protocols of stimulation.

Key words: IVF/natural cycles/poor responders

Introduction

The first successful birth after in-vitro fertilization (IVF) was achieved in a natural, unstimulated cycle (Steptoe and Edwards, 1978). However, pregnancy rates improved with the use of ovulation induction techniques, mainly as a result of transferring several embryos (Lopata et al., 1978; Testart et al., 1986; Wood et al., 1995). Such treatments are known to provoke several types of ovarian response. There are two extremes: the high responders with a risk of ovarian hyperstimulation syndrome and the poor responders with a poor prognosis for IVF outcome. The various treatments that have been proposed for the latter group of women cannot be easily compared because a uniform definition of a poor response is lacking (Serafini et al., 1988; Van Hoof et al., 1993; Ferldberg et al., 1994). This group of patients is heterogeneous, consisting either of young patients with early follicular depletion, or older patients with a declining follicular pool, or patients with ‘resistant ovary syndrome’ in whom follicles are still present in the ovaries. Nevertheless, this group shares one common characteristic as ‘poor responders’. Whatever stimulation protocol is used, it is often associated with a large consumption of gonadotrophins with a partial or complete lack of ovarian response. In our study, we investigated the benefits of using spontaneous cycles with timed triggering of ovulation using human chorionic gonadotrophin (HCG) in such patients.

Materials and methods

Patients

The study group consisted of 11 patients (mean age ± SD: 36.6 ± 6 years) whose treatment was cancelled or who failed to conceive during two previous IVF attempts because of poor response to regular protocols of ovulation induction. These patients failed to achieve oestradiol concentrations >200 pg/ml on the day of HCG, and failed to develop, or developed a maximum of one follicle during previous attempts. Oestradiol was routinely measured using an oestradiol kit (Costria 2°, BioMerieux, Brussels, Belgium) in serum on cycle days 7, 9, 10 and 11. Entry criteria included any cause of infertility amenable to IVF treatment, spontaneous ovulatory cycles (24–36 days long) and a willingness to undergo daily ultrasound controls and blood measurements of oestradiol during the reference cycle. Indications for IVF included mechanical infertility, endometriosis and male factors.

Ethical permission for this prospective study was obtained from the Board of the Ethical Committee of the Catholic University of Louvain. All the patients were fully informed about the prospective nature of the procedure, the chances of premature and untimely luteinizing hormone (LH) surges and the possibility of cancelling the procedure just before oocyte retrieval. Signed consent was obtained from all patients.

Cycle monitoring and oocyte retrieval

All the patients had a vaginal ultrasound scan of the pelvis on day 2 of the cycle. Patients with an ovarian cystic structure >14 mm in diameter were dropped for a subsequent cycle.

All the patients underwent vaginal ultrasound monitoring beginning on day 9 of the cycle, followed by daily scans once the leading follicle reached 15 mm in diameter. Serum oestradiol and LH concentrations were measured after each ultrasound examination. Once the follicle reached 17 mm in diameter, each patient was given a series of five test bands for urinary detection of the LH surge (RapiTest® LH; Norwell Diagnostics, Zurich, Switzerland). The patient was then asked to perform a test every 6 h until HCG administration. HCG (10 000 IU) was administered to all patients when the largest diameter of the follicles reached 18 mm and in the presence of oestradiol concentrations ≥100 pg/ml with an absence of any LH surge. Transvaginal ultrasound-directed oocyte retrieval was performed 35 h later. In the case of a spontaneous LH surge, oocyte
retrieval was scheduled 33 h after the observation of a positive coloration with urinary testing.

**The assay procedure for the urine test**

The Norwell Diagnostics RapiTest® LH is designed to determine the presence of human LH in urine samples. This test is capable of identifying LH concentrations of 45 mIU/ml as an LH surge. The test band contains polyclonal anti-LH-coated membrane and a pad with mouse monoclonal IgG anti-LH-dye conjugate in a protein matrix containing 0.1% sodium uzide. Three or four droplets of urine should be applied into the well of the test device. The results are read after 3–4 min but not later than 10 min.

Positive results: if there are two colour bands of equal intensity in each of the test windows (C and T) and if the test band (T) is darker than the control band (C).

Negative results: if no colour band appears in the test window (T) and if the colour band in the test window is lighter than the band in the control window.

**Embryo transfer and luteal phase support**

Embryos were transferred 48–72 h after oocyte retrieval using a Frydman catheter (CCD, Brussels, Belgium). The embryo was deposited ±0.5 cm below the uterine fundus in 10–20 µl of culture medium. Luteal phase support was given to all patients in the form of 30 mg of oral dyhydrogesterone (Duphaston®; Duphar, Brussels, Belgium) daily. Serological testing for pregnancy was performed 12 days after embryo transfer. Pregnancy was confirmed 21 days after serological testing. Only clinical pregnancies were included in the analysis.

**Cancellation criteria**

Treatment was abandoned if inadequate follicular growth was observed (defined as the absence of increase in follicular diameter after two ultrasound scans), if the serum oestradiol concentration was falling or <100 pg/ml or if there was evidence of a premature LH surge (defined as an LH value three times the basal LH value determined on day 2 and with a follicle <17 mm in diameter).

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### Table I. Cycle characteristics and in-vitro fertilization (IVF) outcome

<table>
<thead>
<tr>
<th>Cycle no. by patient code</th>
<th>Age (years)</th>
<th>FSH on cycle day 3 (mIU/ml)</th>
<th>Day of HCG administration</th>
<th>Oestradiol on the day of HCG (pg/ml)</th>
<th>LH on the day of HCG (mIU/ml)</th>
<th>No. follicles &gt;15 mm diameter</th>
<th>No. of oocytes</th>
<th>Mode of insemination</th>
<th>No. 2PN</th>
<th>No. 3PN</th>
<th>No. ET</th>
<th>Result</th>
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<td>2190</td>
<td>31.4</td>
<td>8</td>
<td>11</td>
<td>121</td>
<td>2.2</td>
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<td>1</td>
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<td>0</td>
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<td>-</td>
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<tr>
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<td>30.1</td>
<td>13.4</td>
<td>12</td>
<td>125</td>
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<td>-</td>
</tr>
<tr>
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<td>9.8</td>
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<tr>
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<tr>
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<td>0</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td><strong>Total (n = 13)</strong></td>
<td><strong>36.6 ± 6</strong></td>
<td><strong>12.9 ± 6.4</strong></td>
<td><strong>13.1 ± 2.3</strong></td>
<td><strong>187 ± 116</strong></td>
<td><strong>21.9 ± 13.6</strong></td>
<td><strong>14</strong></td>
<td><strong>11</strong></td>
<td><strong>0</strong></td>
<td><strong>6</strong></td>
<td><strong>2</strong></td>
<td><strong>6</strong></td>
<td><strong>3</strong></td>
</tr>
</tbody>
</table>

*nd: not determined.

As urinary LH testing indicates a timed LH surge, oocyte retrieval was scheduled 33 hours later (ultrasound performed before the retrieval showed the presence of the follicle).

ICSI was indicated in cases of severe male factors.

ICSI = intracytoplasmic sperm injection; LH = luteinizing hormone; FSH = follicle-stimulating hormone; HCG = human chorionic gonadotrophin; nl = normal; 2PN = pronuclear; ET = embryo transfer.

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**Statistical analysis**

The number of follicles observed on the day of oocyte recovery, the number of oocytes collected, the fertilization rate and the clinical pregnancy rate were recorded. These data were collected in natural cycles and compared with the data obtained after stimulated cycles.

**Results**

A total of 16 cycles was monitored for IVF during natural cycles. Cancellation occurred in three cycles (18.7%) and an LH surge was observed in five cycles (31.2%). In three of those cycles, the timing of the LH rise allowed oocyte retrieval. On the contrary, the other two cycles with an untimely LH surge had to be cancelled. The third cycle was cancelled because of the drop in the serum oestradiol concentration on the 11th day of the cycle.

Thirteen cycles were scheduled for oocyte puncture (Table I). From the 14 follicles >15 mm in diameter observed during ultrasound, 11 oocytes were obtained. In two cycles (15.4%), no oocytes were recovered. The oocyte recovery rate was 78.6%. The mean day of HCG administration was 13.1 ± 2.3 with mean oestradiol and LH serum concentrations of 187 ± 116 pg/ml and 21.9 ± 13.6 mIU/ml respectively. The mean day 3 FSH serum concentration was 12.9 ± 6.4 mIU/ml.

Intracytoplasmic sperm injection (ICSI) was performed in five cases (45.5% of inseminated cycles) for male factors, and the other six cases (54.5%) were normally inseminated. In the five ICSI cycles, there was no evidence of oocyte fertilization in two cases, and triploidy was observed in another two cases (Table I). Embryos suitable for transfer were available in six cycles (6/11 = 54.5%), one after ICSI and five after normal insemination. The mean number of blastomeres in the embryos replaced was 2.83 ± 0.9 cells.

Three clinical pregnancies were obtained, giving a pregnancy
rate of 23.1% per oocyte retrieval and 18.8% per started cycle. None of the women miscarried, one delivered a healthy baby of 2.350 g at 37 weeks gestation, and the other two pregnancies are still ongoing (25 and 34 weeks of gestation).

In patients who accomplished their IVF treatment during spontaneous cycles, a total of 25 previous attempts had been made using ovarian stimulation. During those cycles, the cancellation rate, the oocyte recovery rate, the fertilization rate and the percentage of cycles that ended in embryo transfer were respectively 48, 66.6, 22.2 and 20%. No pregnancy was obtained after those treatments (Table II). Apart from a mean consumption of gonadotrophin ampoules of 51.5 (SD ± 25) per started cycle, none of the IVF parameters indicated that ovarian stimulation was favourable (Table II).

### Discussion

The results of this prospective study, while including only a small number of cycles, tend to confirm the view that the natural cycle is a suitable alternative to stimulated cycles for IVF in such a group of patients. The pregnancy rate obtained was 18.8% per started cycle compared to none at all in stimulated cycles for the same group of patients. Considering the poor IVF prognosis (Wilcox et al., 1988; Toner et al., 1991; Medrum, 1993) in poor responders, this may be quite an acceptable rate. In their debate on ‘friendly IVF’, Olivennes and Frydman (1998) found a similar pregnancy rate using spontaneous cycles but with the administration of a gonadotrophin-releasing hormone (GnRH) antagonist to delay the LH surge (Olivennes and Frydman, 1998). Also, their oocyte recovery rate was 81%, which is similar to that observed in our group of patients. In these circumstances, there are several advantages to using a natural cycle for IVF. The cycle requires less monitoring than a stimulated one, and it also reduces anxiety and stress in the patient as she does not have to worry about the response of her ovaries to the daily injections of gonadotrophins. The costs to the patient and the fertility clinic are lower because fewer visits are required to monitor the cycle, and drugs for ovarian stimulation are not used (except for HCG). Furthermore, as discussed by Edwards et al. (1996), ovarian stimulation for IVF may not be good for women’s health and may not be the optimal treatment to achieve the best implantation rates (Edwards et al., 1996). One of the disadvantages of such an approach is the high risk of failure at each step of the process.

Cycles may be cancelled because of inadequate follicular development or LH surges. Cancellation rates varying from 17–47% have been reported during IVF in unstimulated cycles (Paulson et al., 1992; Claman et al., 1993; Fahy et al., 1995). In our study, oocyte retrieval was attempted in 13 out of 16 cycles (81.3%). The cancellation rate (18.8% in natural cycles) was significantly lower than the 48% cancellation rate during previous stimulated cycles in the same women. The reasons for cancellation are different during unstimulated and stimulated cycles. In spontaneous cycles, cancellation occurs mainly as a result of an untimely LH surge. However, in stimulated cycles, cancellation is mainly the result of an absence of ovarian response, an inadequate oestradiol rise or cystic follicular development. In the future, use of GnRH antagonist may prove helpful in reducing spontaneous LH surges during natural cycles, thus limiting cancellation to inadequate follicular development. However, it is of utmost importance to determine the exact timing of GnRH antagonist administration. Such administration in the late follicular phase or during the early phase of the LH surge may not prevent this surge, and its administration early in the follicular phase may decrease the oestradiol secretion and suppress the function of the corpus luteum, as demonstrated in animal models (Fraser et al., 1997).

If a cycle reaches the retrieval stage, there is a chance that the oocyte may not be recovered. We were able to collect one oocyte each from 11 cycles out of the 13 scheduled for retrieval. Previous accounts of IVF in natural cycles reported oocyte recovery rates varying from 85–97% (Edwards et al., 1984; Foulot et al., 1989; Muasher et al., 1990; Paulson et al., 1992; MacDougall et al., 1994; Daya et al., 1995). All these studies compared IVF outcome in unstimulated and stimulated cycles in supposed normal responders. Our study is the first prospective study evaluating the IVF outcome in natural cycles in poor responders. Our lower recovery rate is related to the characteristics of our group of patients, with an elevated FSH concentration on cycle day 3. This may explain the poor stimulation outcome while patients are still menstruating regularly.

Once the oocyte has been obtained and inseminated, it may not fertilize or it may become fertilized by more than one spermatozoon. Intracytoplasmic sperm injection (ICSI) is helpful in cases of male infertility and it may also be helpful to avoid polyspermic fertilization. Out of the 11 cycles with successful oocyte collection in this study, five utilized ICSI and six utilized classical insemination.

In these cases, indications for ICSI were severe oligozoospermia (n = 2), severe teratozoospermia (2% normal forms; n = 2) and a testicular sperm extraction procedure (n = 1). Surprisingly, two failures of fertilization and two triploidies occurred in the ICSI cycles and only one failure of fertilization was recorded in the other cases. We cannot draw any conclusions, with such a small number of cases, on the impact of ICSI in these conditions. However, in the case of normal sperm parameters, ICSI does not appear to be necessary as one might expect a good fertilization rate (5/6; 83%) with classical insemination. The occurrence of two triploidies after
ICSI is related to the quality of the oocytes injected. Indeed, one occurred in a patient of 47 years of age and the other in a younger patient (31 years) with a history of unexplained infertility for 12 years. The overall fertilization rate was 54.5% which was also higher than the rate observed after stimulated cycles in the same women. Fertilization rates varying from 52–96% have been reported (Edwards et al., 1984; Foulot et al., 1989; Paulson et al., 1992; Claman et al., 1993; MacDougall et al., 1994; Daya et al., 1995). Compared to stimulated cycles, a higher fertilization rate was reported during natural cycles (Foulot et al., 1989; Ramsewak et al., 1990; Paulson et al., 1992; Taymor et al., 1992). The high fertilization rate observed might be attributed to the physiological hormonal environment present during the natural cycle. In our population, high concentrations of exogenous FSH and/or LH were not administered; thus, the single developing follicle might have matured better as it was not exposed to high LH stimulus, as it would be during stimulation for IVF. Some authors (Foulot et al., 1989; Scarduelli et al., 1994) suggest that higher fertilization rates could be attributed to the timed triggering of ovulation with HCG but this was not confirmed in other studies (Edwards et al., 1984; Taymor et al., 1992).

In this series, the number of cycles with embryo transfer was slightly lower than expected as reported by Daya et al. (1995): 37.5% of natural cycles reached the embryo transfer stage, but only 20% of stimulated cycles reached transfer in the same women. Though not statistically significant, this difference is clinically relevant, as in the absence of a daily injection, and with lower stress concentrations due to the lack of ovarian monitoring, we achieved more transfers than during ovarian stimulation. The pregnancy rates observed during natural cycles were 18.8% per started cycle and 23.1% per oocyte retrieval. Such results were observed by others (Foulot et al., 1989; Paulson et al., 1992; Scarduelli et al., 1994) using spontaneous cycles in normally responding women. Comparing our results with the expected IVF outcome in poor responders, it seems that higher pregnancy rates can be achieved through natural cycles with HCG administration than with ovarian stimulation. On the other hand, one should view our results with caution, as we are dealing with a small series, and they need to be corroborated by other studies.

To the best of our knowledge, this is the first report on such management of patients with a previous poor ovarian response to stimulating agents. Our findings demonstrate that pregnancy can be achieved by IVF during natural cycles in such a group of patients at an encouraging rate. The results may not be so favourable in comparison with global IVF outcome but such management should be seriously considered after failure of IVF treatment because of poor ovarian response. It may not be the first choice of approach, and a classical protocol of stimulation may first be offered to the patient, but such a procedure should be kept in mind as an alternative. This approach has been proposed as the ‘gold standard’ of so-called ‘friendly IVF’, a concept developed by Olivennes and Frydman (1998). It is a low-cost alternative that may be more accessible, offering many advantages to patients. More studies are required to confirm our results and to identify the factors that predict a successful IVF outcome in unstimulated cycles in poor responders.

References


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