‘Curing’ empty follicle syndrome

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We report a novel method of rescuing empty follicle syndrome (EFS) and provide evidence that it is a drug-related problem rather than a clinical dysfunction. In a preliminary study we established that in EFS the serum β-human chorionic gonadotrophin (β-HCG) concentrations 36 h after HCG administration never exceeded 10 mIU/ml. β-HCG concentrations were thus used to confirm EFS when oocytes were not retrieved from one ovary after controlled ovarian hyperstimulation. The procedure was suspended leaving intact all follicles in the second ovary. After confirmation of EFS, a second HCG from a different batch was administered and 36 h later mature oocytes were retrieved from the intact ovary, suggesting a fault with the HCG previously administered. Three patients have been treated in this way. In the first case, four out of five mature eggs were fertilized after intracytoplasmic sperm injection (ICSI) resulting in the transfer of three top grade (grade 1) embryos. In the second case all seven mature oocytes fertilized after in-vitro fertilization (IVF) and three grade 1 embryos were transferred resulting in a twin pregnancy, now delivered. In the third case, five out of nine oocytes were fertilized after ICSI and one out of the three treated with high insemination concentration IVF fertilized, resulting in the transfer of three ICSI embryos.

Key words: controlled ovarian hyperstimulation/empty follicle syndrome/human chorionic gonadotrophin/IVF

Introduction

In assisted reproductive technologies, sometimes oocytes are not retrieved from follicles after controlled ovarian hyperstimulation, despite repeated aspiration and flushing. This is known as the empty follicle syndrome (EFS). It cannot be predicted by the pattern of ovarian response to stimulation either sonographically or hormonally (Ben Shlomo et al., 1991). It has been described as a clinical syndrome and a cause of infertility (Coulam et al., 1986; Ashkenazi et al., 1987; Galache Vega et al., 1989). It has also been suggested that it may reflect dysfunctional ovulation induction (Tsuki et al., 1988). Despite the recognized occurrence of EFS over the years, a full explanation for it has been slow in coming. A better understanding of this condition is necessary before a means of predicting and perhaps treating it can be devised. We have recently described a simple test for predicting EFS (Ndukwe et al., 1996) and in this report we describe a successful method of rescuing the EFS cycle and present evidence, using EFS patients as their own controls, which confirms that EFS is a drug-related problem rather than a clinical dysfunction (Zegers-Hochschild et al., 1995).

Materials and methods

This study involved three women undergoing assisted reproductive technology (ART) at Nottingham University Research and Treatment Unit in Reproduction (NURTURE). In all cases controlled ovarian hyperstimulation was with gonadotrophin-releasing hormone agonist (GnRHa), buserelin, 500 mg s.c., daily (Suprefact; Hoechst UK Ltd, Hounslow, Middlesex, UK), starting in the mid-luteal phase of the preceding cycle, and human menopausal gonadotrophin (HMG) 150–225 IU, i.m. daily (Pergonal; Serono Laboratories Ltd, Welwyn Garden City, UK or Humegon or Normegon; Organon Laboratories Ltd, Cambridge, UK). Ovarian stimulation was monitored by ultrasound and serum oestradiol measurements. When at least three follicles were ≥18 mm, i.m. HCG 10 000 IU was administered (Profasi; Serono or Pregnyl; Organon). Transvaginal ultrasound-guided oocyte retrieval was carried out 36 h post-HCG administration. In a previous study of EFS cycles (Ndukwe et al., 1996), we described a serum β-HCG value of <10 mIU/ml as predictive (100% predictive value) of EFS. Therefore, in all three cases when no oocytes were retrieved from any of the follicles in one ovary despite repeated aspiration and flushing, blood was taken for urgent serum β-HCG estimation. If the value was <10 mIU/ml, EFS was confirmed and the procedure was suspended without aspiration of the follicles in the second ovary. An enquiry was conducted on the appropriateness of the storage, timing, administration and expiry dates of the HCG administered. A second HCG, 10 000 IU i.m. from a totally different batch, was then administered and serum β-HCG evaluated 12 and 36 h later. Oocyte retrieval from the ovary whose follicles had been left intact was then carried out 36 h following the second HCG administration. Evaluation of β-HCG was by Microparticle Enzyme Immunoassay, MEIA (1 Mx Total β-HCG System; Abbot Diagnostics, Berkshire, UK).

Results

No suggestion of EFS was found during the monitoring of controlled ovarian stimulation (COH) in any of the three women studied, as ovarian response to stimulation was adequate sonographically and hormonally (Table I). Prior to the first HCG administration, β-HCG was not detectable in the serum. Thirty-six hours after HCG administration, when oocytes were not retrieved from any of the follicles on one ovary, serum β-
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Table I. Characteristics of assisted reproductive technology cycles up to the diagnosis of EFS

<table>
<thead>
<tr>
<th>Case</th>
<th>Day of first HCG administration</th>
<th>Day of first oocyte retrieval (36 h post-HCG)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Oestradiol (pmol/l)</td>
<td>Follicles &gt;14mm (n)</td>
</tr>
<tr>
<td>Case 1</td>
<td>7312</td>
<td>13</td>
</tr>
<tr>
<td>Case 2</td>
<td>14 900</td>
<td>19</td>
</tr>
<tr>
<td>Case 3</td>
<td>17 729</td>
<td>28</td>
</tr>
</tbody>
</table>

HCG = human chorionic gonadotrophin.

Table II. Outcome following rescue of EFS cycles by the administration of a second HCG injection from a different batch

<table>
<thead>
<tr>
<th>Case</th>
<th>Serum β-HCG (mIU/ml)</th>
<th>Follicles left</th>
<th>Oocytes retrieved</th>
<th>Mature oocytes (metaphase II)</th>
<th>Normal fertilization (2PN)</th>
<th>Cleavage</th>
<th>Embryo transfer</th>
<th>Pregnancy outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>12 h post-2nd HCG</td>
<td>36 h post-2nd HCG</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Case 1</td>
<td>327</td>
<td>291</td>
<td>6</td>
<td>6</td>
<td>5</td>
<td>4 (ICSI)</td>
<td>3 (grade 1)</td>
<td>Negative</td>
</tr>
<tr>
<td>Case 2</td>
<td>230</td>
<td>242</td>
<td>9</td>
<td>7</td>
<td>7</td>
<td>7 (IVF)</td>
<td>3 (grade 1)</td>
<td>Twins (delivered)</td>
</tr>
<tr>
<td>Case 3</td>
<td>204</td>
<td>260</td>
<td>15</td>
<td>12</td>
<td>12</td>
<td>5 (ICSI)</td>
<td>3 (grade 1)</td>
<td>Negative</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1 (IVF)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

2PN = 2 pronuclear; HCG = human chorionic gonadotrophin; ICSI = intracytoplasmic sperm injection; IVF = in-vitro fertilization.

HCG was below 10 mIU/ml in every case, confirming EFS (Table I). After the second administration of HCG serum, β-HCG concentrations in the three subjects rose accordingly to expected values (Table II). During the second oocyte retrieval, oocytes were recovered from all six follicles left intact in case 1, seven out of the nine follicles in case 2 and 12 out of the 15 follicles left intact in case 3. In case 1, four out of the five oocytes were mature (metaphase II) and all four achieved normal fertilization [two pronuclei (2PN)] after ICSI, leading to transfer of three grade I embryos; however, pregnancy was not achieved. In case 2, all seven oocytes were mature (metaphase II) and all fertilized normally (2PN) after in-vitro fertilization (IVF). Three grade I embryos were transferred, resulting in a twin pregnancy, now delivered. In case 3, all 12 oocytes retrieved were mature (metaphase II); nine oocytes underwent ICSI and three high insemination concentration IVF (IVF–HIC), resulting in five ICSI embryos (2PN) and one embryo (2PN).

Three grade I embryos were transferred but pregnancy did not occur. In all subjects studied, detailed enquiry confirmed appropriate storage, timing, administration and non-expiry dates of the HCG given.

Discussion

Our data are in agreement with the observations of Ben Shlomo et al. (1991) that EFS cannot be predicted by the pattern of ovarian response to stimulation. Previous work by ourselves (Ndukwe et al., 1996) and Zegers-Hochschild et al. (1995) showed that EFS is consistently characterized by very low serum β-HCG concentrations after HCG administration i.m. The concentrations of β-HCG in the serum were <10 mIU/ml in all cases of EFS encountered by our group. This was the basis of the test we proposed for the prediction of EFS.

Careful and detailed investigation in all three cases confirmed that the HCG doses administered had not expired and were obtained from reliable sources. Drug storage was strictly according to the manufacturer’s advice and appropriate i.m. administration was performed without any difficulty, by a trained nurse.

The problem of EFS is that of very low, inadequate bioavailability of HCG when used as a surrogate for the luteinizing hormone (LH) surge. Because of the inconsistency of the spontaneous LH surge in controlled ovarian stimulation, HCG has been adopted uniformly by all successful assisted reproductive technology programmes to effect the final triggering of ovulation. When pre-ovulatory follicles are present, administration of HCG is followed by granulosa cell luteinization, the switch from oestradiol to progesterone synthesis, resumption of meiosis and oocyte maturation and general preparation of the follicle for oocyte extrusion culminating in follicular rupture after 36–40 h. The threshold duration of LH (HCG) surge concentrations required to re-initiate meiosis seems to be 14–18 h (Seibel et al., 1982; Itskoitz-Eldor et al., 1993) whereas metaphase II oocytes are obtainable 28 h after the onset of the LH (HCG) surge.

The threshold amplitude of the LH (HCG) surge is not known for certain, but our data show that a serum β-HCG concentration <10 mIU/ml prevents pre-ovulatory changes within the follicle. In our previous work (Ndukwe et al., 1996) the lowest concentration of serum β-HCG when oocytes were successfully retrieved following controlled ovarian stimulation was 106 mIU/ml. These data are similar to the lowest concentration of 110 mIU/ml reported by Zegers-Hochschild et al. (1995).

Studies in rats have shown that only 5% of normal LH (HCG) surge amplitude is necessary for oocyte maturation, while >85% is needed to activate actual ovulation (Peluso...
et al., 1990). Similar data in humans are not available, but if these percentages are applied to our data, the minimum β-HCG concentrations necessary for oocyte maturation would be ~5 mIU/ml and that needed to activate actual ovulation ~90 mIU/ml. The projected HCG concentration for oocyte maturation of 5 mIU/ml is close to the 10 mIU/ml below which no oocytes were retrieved. It is therefore not surprising that the crucial factor in the pathogenesis of EFS is the inappropriately low bioavailability of HCG.

The cause of the low bioavailability after HCG administration is unclear. Postulates include: a problem with the drug itself, its administration or a very rapid metabolic clearance of the drug by the liver. The possibility of a problem with drug administration was excluded, because in all our cases, it was confirmed after careful investigation that the HCG was administered correctly and at the relevant time.

The appropriate rise in serum β-HCG following the administration of HCG from a different batch rules out an intrinsic liver problem as the cause of EFS, as the second dose of HCG administered would have been similarly cleared. The subjects have in essence acted as their own controls. It would be reasonable to surmise from these findings that the problem may be an intrinsic defect in the HCG administered initially in all three cases. This would support the findings of Zegers-Hochschild et al. (1995) that EFS may be the result of an abnormality in the in-vivo biological activity of some batches of commercially available HCG and not an ovarian problem. One possible explanation is the desialylation of the HCG administered which may make it subject to rapid metabolic clearance by the liver (Hoermann et al., 1993). The possible causes of desialylation merit further study.

In EFS, therefore, an intrinsic fault with the HCG administered seems to be the basic underlying pathology. Empty follicle syndrome is thus neither a reflection of dysfunctional ovulation as suggested by Tsuiki et al. (1988) nor a cause of infertility per se as suggested by Coulam et al. (1986), Ashkenazi et al. (1987) and Galache Vega et al. (1989). Rescue of EFS cycles is thus possible by administering HCG from a different batch and does not seem to compromise the assisted reproductive technology cycle even if the oocyte recovery had started before the diagnosis was made. The success of the assisted reproductive technology cycles apparently was not jeopardized by the long ‘coast’ of up to 120 h between the last HMG injection and the administration of a second HCG injection. This was clearly shown by the number of mature oocytes retrieved after such rescue, the fertilization and cleavage rates and the quality of embryos transferred which resulted in a twin pregnancy in one subject.

We have shown EFS to be the result of inadequate exposure of pre-ovulatory ovarian follicles to HCG due to the very low, inadequate bioavailability which seems to occur when HCG from certain batches of commercially available HCG is administered. Using the subjects essentially as their own controls, we have shown that in EFS the underlying problem seems to be a fault with the HCG administered rather than an intrinsic problem with the patient per se. Empty follicle syndrome can be diagnosed by a single evaluation of serum β-HCG after HCG administration and the cycle rescued by administering a second HCG from a different batch. As far as we know, this is the first description of a successful rescue of EFS cycles, and provides direct evidence, using EFS patients as their own controls, that EFS is a problem with the drug administered rather than a problem with the patient.

An attractive proposition would be to measure routinely serum concentrations of β-HCG 12 h post-HCG administration for the timely prediction and rescue of EFS cycles in assisted reproductive technology. Although this seems to be a simple way of predicting EFS before attempting oocyte retrieval, it is debatable whether this will be cost effective given the low incidence of this condition. The alternative would be to follow our protocol in this report, i.e. an urgent check of serum β-HCG if no oocytes are retrieved from the follicles in one ovary despite aspiration and several flushes, and then to administer HCG from a totally different batch if EFS is confirmed, followed by retrieval of the oocytes 36 h later.

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References


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