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Continuing the debate on empty follicle syndrome: can it be associated with normal bioavailability of β-human chorionic gonadotrophin on the day of oocyte recovery?

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This paper describes our experience with four ovarian stimulation in-vitro fertilization (IVF) cycles in which we failed to retrieve oocytes despite normal bioavailability of β-human chorionic gonadotrophin (β-HCG) in patients' blood 35 h after HCG administration. In three cases, the oocyte recovery procedure was interrupted, a second dose of HCG was administered and 24 h later mature oocytes were collected from two of the patients. In the first case, the three metaphase II oocytes collected fertilized after intracytoplasmic sperm injection (ICSI) and two cleaved grade three embryos were transferred but pregnancy did not ensue. In the second case, six out of eight metaphase II oocytes fertilized and cleaved following ICSI, leading to transfer of one grade two and two grade three embryos. This resulted in a clinical pregnancy which at the time of this report is ongoing. A similar rescue protocol was used for the third case who had empty follicle syndrome (EFS) in her previous treatment cycle but only cumulus–corona complexes were aspirated. Five additional patients who had EFS before instituting pregnancy diagnostic test screening have had further treatment cycles in which oocytes were collected but pregnancy did not ensue. We conclude that normal bioavailability of β-HCG on the day of oocyte recovery does not exclude the diagnosis of EFS. EFS does not predict a reduced fertility potential in future cycles, although it may recur due to a biological abnormality in the availability of mature oocytes that are retrievable. In such patients, oocyte donation may offer the chance of achieving a pregnancy.

Key words: empty follicle syndrome/human chorionic gonadotrophin/IVF/oocyte collection/ovarian stimulation

Introduction

Failure to retrieve oocytes from mature follicles—referred to as empty follicle syndrome (EFS)—was first reported by Coulam *et al.* (1986). Since then, there has been considerable interest in the literature on the subject regarding its aetiology (Ashkenazi *et al.*, 1987; Asch *et al.*, 1992; Zegers-Hochschild *et al.*, 1995; Khalaf and Braude, 1997), prediction (Ben-Shlomo *et al.*, 1991), diagnosis (Ndukwe *et al.*, 1996) and management (Ndukwe *et al.*, 1997; Shulman *et al.*, 1997). In their study, Zegers-Hochschild *et al.* (1995) found no detectable human chorionic gonadotrophin (HCG) in patients with EFS compared with a mean of 207.5 mIU/ml in the control while Ndukwe *et al.* (1996) found that a cut-off point of 10 mIU/ml was 100% predictive. The cause of the low bioavailability after HCG administration remains unclear but could be due to: (i) abnormality in the in-vivo biological activity of some batches of commercially available HCG (Zegers-Hochschild *et al.*, 1995); (ii) inappropriate timing (Khalaf and Braude, 1997) or lack of (Shulman *et al.*, 1997) HCG administration; or (iii) rapid clearance of HCG by the liver (Zegers-Hochschild *et al.*, 1995). That EFS can be associated with normal bioavailability of β-HCG on the day of oocyte recovery has hitherto not been reported. In this study, we report on four treatment cycles in three patients with EFS where the β-HCG concentrations were within the normal range on the day of oocyte recovery.

Materials and methods

Between 21 March 1994 and 15 August 1997, 2059 in-vitro fertilization (IVF) treatment [1078 standard and 981 intracytoplasmic sperm injection (ICSI)] cycles were performed at Midland Fertility Services using long-protocol buserelin acetate (Suprefact; Hoechst, Hounslow, UK) and gonadotrophin [human menopausal gonadotrophin (HMG)/urofollitrophin (uFSH)/urofollitrophin high purity (uFSH HP)/follitrophin alpha (rFSH)] (Pergonal; Metrodin; Metrodin HP; Gonal-F; Serono Laboratories, Welwyn Garden City, UK, or Humegon; Organon, Cambridge, UK). Monitoring was by transvaginal scan and serum oestradiol estimation performed on days 8–11 of HMG/uFSH/uFSH HP/rFSH stimulation. Buserelin acetate and gonadotrophin were stopped, and i.m. β-HCG 10 000 IU (Profasi; Serono, or Pregynl; Organon) was given when it was estimated that patients would have two or more follicles >17 mm diameter. Transvaginal ultrasound-directed follicle aspiration was performed 35 h after HCG injection. All oocyte collections in the unit were performed by experienced operators.

There were 19 cases in 18 patients in which no oocyte was recovered despite performing multiple flushes of the follicles present. In all these cases, enquiries confirmed that the HCG used had not expired, was properly stored, and was administered at the right time. Excluding cycles in which the patient responded poorly to ovulation induction (defined in our unit as patients who developed less than two follicles >10 mm on day 9 of ovarian stimulation; these patients fell below the 10th percentile for response), there were 12 cases in 11 patients in whom we would expect to have collected oocytes. This represents 0.6% of the total cycles during the study period.

Four cases in three patients occurred after the publication by Ndukwe *et al.* (1996) concerning the prediction of EFS. In each of

Table I. Demographic variables of patients with empty follicle syndrome

Patient	Age (years)	Partner's age (years)	Fertility status	Aetiology of infertility	Previous successful oocyte recovery	Duration of infertility (years)	Baseline FSH[a] (U/l) (day 3)	Baseline LH[b] (U/l) (day 3)
A	27	32	Primary	Male factor	No	3	8.4	5.4
B	24	27	Primary	Male factor	No	6	7.3	8.9
C	28	26	Primary	Male factor	No	3.5	5.1	6.9

[a]Normal range = 2.5–10.2.
[b]Normal range = 1.9–12.5.

Table II. Response to ovarian stimulation in patients with empty follicle syndrome

Patient	Type of HMG	Total dose of HMG (ampoules)	Serum oestradiol[a] on day of HMG (pmol/l)	No. of days on HMG[b]	Follicles ⩾12 mm on day of HMG	Follicles aspirated,[c] first OC	Follicles aspirated,[d] second OC
A, cycle 1	Metrodin	52	2260 (day 11)	13	13 (day 11)	21 (day 16)	–
B	Metrodin HP	20	3101 (day 8)	10	5 (day 8)	13 (day 13)	18
C	Gonal-F	20	4372 (day 8)	10	12 (day 8)	28 (day 13)	10
A, cycle 2	Gonal-F	60	350 (day 11)	15	7 (day11)	8 (day 18)	8

HMG = human menopausal gonadotrophin; OC = oocyte collection.
[a]Serum oestradiol was measured on the day the patients had their first and only transvaginal scan while on HMG, i.e. between days 8 and 11 of ovarian stimulation depending on the patient's age, baseline follicle stimulating hormone (FSH), previous response (if any) and whether or not polycystic ovaries were present.
[b]Buserelin acetate and gonadotrophin were stopped and i.m. HCG 10 000 IU was given when it was estimated that patients would have two or more follicles >17 mm diameter, assuming a growth rate of 2 mm/day.
[c]Transvaginal ultrasound-directed follicle aspiration was performed 35 h after HCG injection.
[d]A second scan was performed 3–4 days later where insufficient follicles >10 mm diameter had been recruited, in order to estimate timing for HCG injection.

these, blood was taken for β-HCG estimation. Following the first case where a detectable concentration of HCG was found in patient A's blood (cycle 1), an immediate pregnancy diagnostic test was performed to detect HCG in urine when EFS was suspected during oocyte recovery. Pregnancy test was by an immunoenzymetric assay for the semi-quantitative determination of HCG in urine [ICON II HCG (Urine): Hybritech Inc., San Diego, CA, USA]. The sensitivity of this test for urinary HCG is 70 and 100% at 10 and 20 mIU/ml respectively, with a specificity of 100%. β-HCG evaluation was by an automated chemiluminescence immunoassay (Ciba Corning ACS Total HCG System; Ciba Corning Diagnostics Corp., Medfield, MA, USA). This immunoassay uses constant amounts of two antibodies specific for different epitopes that are present on both the free β subunit and the β subunit of intact HCG.

In all but the last three cases in which an immediate assessment of urinary HCG concentration was done, follicle aspirations were completed but no oocyte was retrieved. In the three cases that followed, the procedure was suspended despite positive pregnancy diagnostic test results leaving 10 follicles in one, eight in another, and all the 18 follicles in the right ovary in the third. A second injection of HCG 10 000 IU i.m. from the same batch was administered and aspiration of those follicles left intact was planned for the following morning, approximately 24 h following the second HCG injection.

Results

The demographic variables from our last three patients with EFS are summarized in Table I. In the first case (patient A, cycle 1) a pregnancy diagnostic test was not performed on the day of oocyte recovery (21 follicles were aspirated) but blood drawn at the time of oocyte recovery showed 154.9 mIU/ml of β-HCG.

In the last three cases (A cycle 2, B and C) no oocyte was retrieved from 8–28 follicles despite a positive urinary pregnancy diagnostic test (Table II). Blood specimens taken at the time the patients had their first oocyte collection showed β-HCG concentrations of 145.1–377 mIU/ml (Table III). After the second i.m. injection of HCG, serum concentrations rose to 277–322 mIU/ml and none of the patients had ovulated (Table III).

In patients B and C, three and 10 oocytes were collected from the 18 and 10 follicles respectively that were left intact after the first attempted oocyte recovery. In patient B, the three metaphase II oocytes fertilized normally (two pronuclei) after ICSI, two cleaved, and two grade 3 embryos were transferred; however pregnancy did not ensue. In patient C, eight of the 10 oocytes collected were in metaphase II. Of these, six fertilized normally after ICSI and all cleaved, leading to transfer of one grade 2 and two grade 3 embryos. This resulted in a clinical pregnancy which at the time of this report was ongoing.

Patient A returned 3 months later for another cycle (A, cycle 2, Tables II and III). Before the start of the second cycle, chromosomal analysis revealed a normal female (46 XX) karyotype. A total of 16 follicles was aspirated in this patient, with an oestradiol concentration of 350 pmol/l on day 11 and 2239.5 pmol/l on the day of first oocyte collection (day 18). Follicle aspiration was abandoned when no oocyte was retrieved halfway into the procedure, despite a positive pregnancy diagnostic test. A repeat i.m. HCG 10 000 IU was given but despite repeated flushing during the second oocyte recovery 24 h later, no oocyte was retrieved. In this case, unlike the others, cumulus–corona complexes were seen in the aspirate.

Table III. Treatment outcome in patients with empty follicle syndrome

Patient	Pregnancy test screen on day of OC	HCG (mIU/ml) day of first OC	No. of oocytes, first OC	HCG (mIU/ml), day of second OC	No. of oocytes, second OC	No. of oocytes with normal fertilization	No. of oocytes undergoing cleavage and grade	No. of embryos transferred	Treatment outcome
A cycle 1	Not done	154.9	0	–	–	–	–	0	Not pregnant
B	Positive	377	0	Not done	3	3 (ICSI)	2	2 (2 × 4-cell, grade 3)	Not pregnant
C	Positive	236	0	322	10 (8 M II)	6 (ICSI)	6	3 (4-cell grade 2, 4-cell grade 3, 6-cell grade 3)	Clinical pregnancy
A, cycle 2	Positive	145.1	0	277	0	–	–	0	Not pregnant

HCG = human chorionic gonadotrophin; ICSI = intracytoplasmic sperm injection; M II = metaphase II; OC = oocyte collection.

The β-HCG concentration almost doubled between the first and second dose of HCG with both values within the normal range (106–360 mIU/ml) expected (Zegers-Hochschild *et al.*, 1995; Ndukwe *et al.*, 1996).

Discussion

Empty follicle syndrome, defined as the failure to aspirate oocytes from 'mature' follicles following ovulation induction for assisted reproductive treatment, occurs infrequently, with an incidence that varies between 1 and 7% in some series (Bartfai *et al.*, 1987; Ben-Shlomo *et al.*, 1991; Zegers-Hochschild *et al.*, 1995). The quoted incidence is higher than the 0.6% reported in this study. Nevertheless, this condition can be devastating when it occurs, the patients and their physician having invested time, effort and money to reach oocyte recovery.

The aetiology of EFS is conjectural. It was initially suggested that EFS may stem from the same cause leading to the patient's infertility (Coulam *et al.*, 1986; Ashkenazi *et al.*, 1987). Recently, attention has been focused on the fact that EFS may be a result of an abnormality in the in-vivo biological activity of some batches of commercially available HCG (Zegers-Hochschild *et al.*, 1995; Ndukwe *et al.*, 1996; Ubaldi *et al.*, 1997). Zegers-Hochschild *et al*. (1995) reported that immunoreactive HCG used in EFS cases was undetectable in plasma of male volunteers 10 min after i.v. injection of 5000 IU from the same batch. They and others (Ndukwe *et al.*, 1996), concluded that EFS is not an ovarian problem but the result of a lack of exposure to biologically active HCG. This was attributed to rapid clearance of the drug due to the high affinity of desialylated HCG to liver cells. While there may be no doubt that in some cases EFS results from a problem with the drug, this by no means explains the pathophysiology in all cases of this condition. In addition, a possible relationship may exist between poor ovarian response and failure to retrieve oocytes. Ben-Shlomo *et al*. (1991) reported poor follicular growth in 31% of their patients with EFS. They suggested that in some cases EFS may represent an advanced stage of ovarian ageing characterized by residual responsiveness of granulosa cells while oocytes can no longer develop adequately. One patient (A) with baseline FSH near the upper end of the normal range showed such a response in our series. Her first cycle using long-protocol buserelin/HMG was cancelled before oocyte recovery, after 11 days of ovarian stimulation with two ampoules daily of Metrodin. In her two subsequent cycles (those reported here) no oocyte was collected despite stimulation with a higher dose of gonadotrophins.

Highly coordinated and intricate mechanisms are involved in the processes leading to the release of the cumulus–corona–oocyte complex to the follicular fluid. This complex process requires the luteinizing hormone surge (or HCG as surrogate) which is also responsible for the resumption of meiosis, extrusion of the first polar body and subsequent ovulation. Understandably, EFS could be associated with low bioavailability of HCG as reported by Zegers-Hochschild *et al.* (1995) and Ndukwe *et al.* (1996). In their study, Ndukwe *et al.* (1996) found that a cut-off point of 10 mIU/I of β-HCG in serum 36 h after giving an ovulatory dose of HCG gave 100% sensitivity, specificity and predictive value in EFS. The result of the β-HCG estimation in the first cycle of patient A made us wonder whether the findings of Ndukwe *et al*. (1996) were universal. In the next three cycles that followed, despite a positive pregnancy diagnostic test, we decided to leave some follicles unemptied after aspirating a reasonable number of follicles with no oocyte retrieved. We did not feel it necessary to inject HCG from a different batch on the second occasion as there was evidence from the patients' history and the positive pregnancy diagnostic test that they had their HCG at the correct time. In addition, there were other patients who were injected with HCG from the same batch at the time and who had oocytes recovered. In the three patients described here, spontaneous ovulation had not occurred despite normal bioavailability of β-HCG 24 h earlier and in patients B

and C, three and 10 oocytes were retrieved respectively. Fertilization occurred normally in both cases and patient C became pregnant with a singleton which is ongoing. In these three cases, we were reluctant to wait 35 h to perform the second oocyte recovery for fear that the patients might ovulate spontaneously. It is possible that EFS in patients B and C represented lack of ovarian response to the administered dose of HCG, which would also explain why they had not ovulated 59 h after the first HCG injections were given.

It is generally believed that EFS does not represent a permanent pathophysiological condition, with most cases occurring sporadically. Cycles in which oocytes were not retrieved have been reported to be preceded (Zegers-Hochschild *et al.*, 1995; Shulman *et al.*, 1997; Ubaldi *et al.*, 1997) or followed (Tsuiki *et al.*, 1988) by cycles with successful oocyte retrieval. Similarly, pregnancy can occur before and after cycles with EFS (Ben-Shlomo *et al.*, 1991). Three other patients treated in our department had one previous oocyte recovery each when oocytes were collected. In one patient, nine oocytes were collected, four were fertilized and the three that cleaved were transferred; this resulted in a singleton pregnancy and birth. One patient had three children and another had a miscarriage before having treatment during which no oocytes were collected. Five additional patients who had EFS before instituting screening had further treatment cycles in which oocytes were collected but pregnancy did not ensue. In patient A, follicular fluid obtained from the follicles contained cumulus–corona cell complexes. In this patient, it is possible that oocytes were not present at all or had undergone an early process of atresia and degeneration (Ashkenazi *et al.*, 1987; Ben-Shlomo *et al.*, 1991). Recurrence of EFS has been reported by various authors (Coulam *et al.*, 1986; Khalaf and Braude, 1997) and may represent a manifestation of clinical dysfunction as suggested by Zegers-Hochschild *et al.* (1995) and Khalaf and Braude (1997).

EFS is a complex phenomenon with varying aetiology. It cannot be explained by low bioavailability of HCG alone, neither can it be reliably diagnosed by measurement of serum β-HCG on the day of oocyte recovery, except possibly where the serum β-HCG concentration is very low. In this case perhaps egg retrieval should not be attempted. It is important that physicians are aware that this condition can occur in the presence of normal bioavailability of HCG. Due consideration should be given to interrupting the oocyte collection procedure when an experienced operator recovers no oocyte, despite repeated aspiration and flushing of mature follicles irrespective of the HCG concentration in the patient's blood on the day. Another dose of HCG should be given and the second oocyte recovery rescheduled for the following day. Implantation does not appear to be hampered in cycles rescued by a second ovulatory dose of HCG, despite normal bioavailability of this hormone. EFS does not predict a reduced fertility potential in future cycles in the majority of cases. However, there is a small cohort of patients in whom this phenomenon tends to recur. Such cases may be due to a biological abnormality in the supply of mature oocytes that are retrievable. In these cases only oocyte donation offers the chance of achieving a pregnancy.

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