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CASE REPORT *Infertility*

Empty follicle syndrome: successful treatment in a recurrent case and review of the literature

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ABSTRACT: Empty follicle syndrome is a condition in which no oocytes are retrieved after an apparently adequate ovarian response to stimulation and meticulous follicular aspiration. It is a rare condition of obscure etiology. A patient with primary infertility who underwent seven assisted reproductive technique cycles is described. In spite of a satisfactory ovarian response, aspiration yielded no oocytes in four cycles and 1–4 low quality oocytes in three cycles. In the index treatment cycle, ovulation was triggered using GnRH agonist 40 h prior to ovum pickup and hCG was added 6 h after the first trigger. Eighteen oocytes were recovered, of which 16 were mature and were inseminated by ICSI. Two embryos were transferred 48 h after aspiration and nine embryos were cryopreserved. The patient conceived and delivered a healthy boy at 38 weeks of gestation. The literature is reviewed and possible etiologies and treatment options of this enigmatic syndrome are suggested.

Key words: empty follicle syndrome / *in vitro* fertilization / GnRH agonist / oocyte maturation failure / follicle development

Introduction

Empty follicle syndrome (EFS) was first described by Coulam *et al.* (1986). It is a condition in which no oocytes are retrieved after an apparently adequate ovarian response to stimulation and meticulous follicular aspiration. EFS in the presence of dominant follicles is an uncommon event, estimated to occur in 0.045–3.5% of patients undergoing ovum pickup (OPU) (Ben-Shlomo *et al.*, 1991; Awonuga *et al.*, 1998; Driscoll *et al.*, 1998; Zreik *et al.*, 2000; Aktas *et al.*, 2005; Coskun *et al.*, 2010; Mesen *et al.*, 2011; Castillo *et al.*, 2012).

EFS has been classified into 'genuine' (GEFS) and 'false' EFS (FEFS). The former was defined as a failure to retrieve oocytes from mature ovarian follicles after ovulation induction and apparently normal follicular development and steroidogenesis in the presence of optimal β-hCG levels on the day of oocyte retrieval. The latter was defined as a failure to retrieve oocytes in the presence of low β-hCG due to an error in the administration, or the reduced bioavailability, of choriogonadotrophin (Stevenson and Lashen, 2008).

The underlying mechanism of GEFS remains obscure. Some have even cast doubt regarding the existence of the syndrome (Ben-Shlomo *et al.*, 1991; Harrison and Fawzy, 1996).

In this report, we present a patient who underwent four OPUs in which no oocytes were aspirated. In three other cycles, the aspiration yielded between one and four low quality oocytes. Changing the treatment protocol brought about a successful outcome. We also review the literature about this debated syndrome.

Patients and Methods

Case report

A couple with primary infertility of 25 months presented to our fertility clinic in 2003. The 24-year-old patient had irregular menses since menarche at the age of 12, with maximal amenorrhea periods of 50 days. Her BMI was 26. Physical examination was unremarkable with no acne or hirsutism. Pelvic sonography revealed normal pelvic findings with 8 mm regular endometrium and a 20 mm ovarian follicle. The early follicular phase hormone profile was normal and FSH was 4.89 IU/l. Previously, prolactin was slightly elevated and a computerized tomography scan demonstrated a 6 mm structure suspected to be a microadenoma, although the patient never received anti-dopaminergic treatment; all her subsequent prolactin levels were normal. Hysterogalpingography was unremarkable.

Owing to a family history, coagulation tests were done and the patient was found to be heterozygote for the prothrombin mutation (FII G20210A) and homozygote for MTHFR (C677T mutation). Treatment with folic acid 5 mg/day was advised.

The husband was a 28-year-old truck driver who smoked 30 cigarettes/day. Physical examination revealed third degree varicocele. Sperm analysis demonstrated oligo-asthenozoospermia (13 M/ml, 40% motility). He subsequently underwent subinguinal ligation of the spermatic vein.

The couple returned to our clinic 9 months later, since no spontaneous pregnancy was achieved. Ovulation induction with clomiphene citrate (two cycles) or gonadotrophins (two cycles), combined with IUI, was carried out. Despite a normal ovarian response and a total count of 3–10 million motile spermatozoa per insemination, pregnancy was not achieved. In the years 2005–2011, the patient went through eight assisted reproductive technique (ART) cycles, described in Table I. Low molecular weight heparin (Enoxaparin 40 mg/day, Sanofi Winthrop Industrie) was added concomitant with ovarian stimulation because of the hypercoagulabile state. Ovarian response was adequate, according to estradiol (E_2) levels and sonographic appearance, but no oocytes were aspirated in four cycles. In one of the cycles, but not in others, high progesterone levels indicated premature lutealization as a possible cause for the failure to retrieve oocytes. Quantitative β-hCG levels were obtained on two occasions after the failed retrievals and found to be 44 and 70 IU/l. Three additional cycles yielded between one and four oocytes, only two of which were mature and were injected. No embryos were ever available for transfer. The patient underwent chromosomal analysis which showed a normal female karyotype.

In the last and finally successful cycle, we chose to administer a GnRH agonist (Triptorelin acetate, decapeptyl 0.1 mg*2, Ferring Pharmaceuticals Israel) 40 h prior to OPU. In order to avoid the unfavorable clinical pregnancy rate reported in similar protocols, recombinant hCG (Choriogonadotropin alfa, ovitrelle 250 μg, Serono) was given 34 h prior to OPU. Aspiration yielded 18 oocytes, 16 of them were mature oocytes which were injected. A total of 11 embryos developed; 2 were transferred 48 h after OPU and 9 were cryopreserved. Luteal support with progesterone (Endometrin 100*2/day, Ferring Pharmaceuticals Israel) and E_2 (Estrofem 2 mg*2/day, Novo Nordisk) was initiated on the day of embryo transfer. A singleton pregnancy was achieved and a healthy 2600 g boy was delivered at 38 weeks of gestation.

Literature search

MEDLINE was searched using the phrase 'EFS' no restrictions (language or other) were placed. The search yielded 60 citations. The reference lists from all citations were examined to identify cited articles not captured by electronic search, revealing 11 additional references. After this, nine studies were excluded for the following reasons: two were not in English, one described a case of a menopausal woman, one is a reply letter with the same data as described elsewhere, in two articles EFS was mentioned in a general discussion about infertility without specific consideration and three citations described sonographic assessment of EFS without follicle aspiration. All together, the 62 papers included were 43 case reports or case series, 10 observational studies, 3 reviews and 6 letters or comments.

In this study, 117 cases of EFS were described (Table II). Only in 29 cases, positive hCG levels at OPU confirmed the diagnosis of GEFS. In 15 articles, data are presented on the prevalence of the syndrome (Table III). These studies add 487 more cases of EFS, of which 28 were genuine.

Discussion

Definitions

EFS is an uncommon, complex and frustrating phenomenon in which no oocytes are retrieved after ovarian stimulation, despite apparently normal follicular development and E_2 levels. It is classified as 'genuine' in the presence of optimal hCG levels on the day of oocyte retrieval and 'false' when the levels are low (Stevenson and Lashen, 2008). Optimal hCG levels are not unequivocally defined.

Aktas *et al.* (2005) reported that hCG levels on the day of OPU for women who correctly administered hCG were 98–161 IU/l. Driscoll *et al.* (2000) reported a median serum hCG concentration of 117.1 IU/l (range 48–249) after s.c. administration of recombinant hCG 250 μg, and 83.6 IU/l (range 32–99) after i.m. administration of 5000 IU urinary hCG. Stevenson and Lashen (2008) in a comprehensive review, offered that hCG levels of 40 mIU/ml should be the cutoff between normal to low hCG levels on the day of OPU. Ndukwe *et al.* (1996), trying to predict EFS, stated that serum hCG levels were all <10 mIU/ml in cases of EFS.

A borderline form of EFS was also suggested in cases in which very few mature or immature oocytes are recovered from several mature follicles (Işik and Vicdan, 2000; Nikolettos *et al.*, 2004; Duru *et al.*, 2007; Desai *et al.*, 2009; Vutyavanich *et al.*, 2010).

Incidence

The incidence of EFS according to our literature review is 0.045–3.4% (Table III). These differences may result from different exclusion criteria. In some studies patients with poor response to ovulation induction or premature ovulation were included while in others not. GEFS prevalence is 0–1.1%. Recurrent GEFS cases are scarce. Being such a rare event, recurring EFS escapes even large series and thus might be erroneously regarded as non-existing.

Underlying mechanism

The etiology of GEFS is obscure. Since it was first described, various authors have been skeptical about its existence (Ben-Shlomo *et al.*, 1991; Harrison and Fawzy, 1996; van Heusden *et al.*, 2008). It was argued that the EFS phenomenon could be explained by premature ovulation, a poor ovarian response or hCG-related faults (Awadalla *et al.*, 1987; Ben-Shlomo *et al.*, 1991; Asch *et al.*, 1992; Greb *et al.*, 1993; Aktas *et al.*, 2005). Failure to retrieve oocytes, despite correct hCG administration, may be due to low bioavailability which results from variation in the absorption or clearance of hCG (Zegers-Hochschild *et al.*, 1995; Hirshfeld-Cytron and Kim, 2008), variation in the threshold for follicular response to hCG, variation in the time needed from hCG exposure to maturation of oocyte–cumulus complexes (Vutyavanich *et al.*, 2010) or intrinsic defects in the biological activity of hCG preparation (Zegers-Hochschild *et al.*, 1995). Supporting the hypothesis that ovarian low availability of hCG is a cause of EFS, was a case of unilateral EFS due to ovarian torsion occurring between the time of hCG administration and follicular aspiration. Oocytes were aspirated from the unaffected ovary (Stefunidis *et al.*, 2002). Other etiologies have been suggested, including ovarian aging (Ben-Shlomo *et al.*, 1991; Greb *et al.*, 1993; Lorusso *et al.*, 2005), dysfunctional folliculogenesis due to increased apoptosis and follicular atresia (Desai *et al.*, 2009), defective granulosa cell function (Zreik *et al.*, 2000), faulty oocyte development and maturation (La Sala *et al.*, 1991; Bustillo, 2004; Kourtis *et al.*, 2004; Inan *et al.*, 2006; Duru *et al.*, 2007), strong attachment of cumulus cell complexes to the follicular wall, dysfunctional ovulation induction

Table I ART cycles description.

Date	Gonadotrophin	Dose Amp/ units per day	Total	GnRH analog	Protocol	Ovulation triggering	Estradiol levels (pg/ ml)	Follicle no.	Progesterone levels at hCG day (ng/ml)	Oocyte no.	Oocyte description
1/2005	hMG	3	21	None	Gonadotrophin only	r-hCG*2	569	18	<0.5	0	
3/2005	hMG	4	36	GnRH agonist (0.1 mg/day)	LD	r-hCG*2		15		0	
7/2005	hMG	4	36	GnRH antagonist (cetrorelix acetate)	Antagonist, flexible	r-hCG	2027	12	8	0	
9/2005	hMG	4	36	GnRH agonist (3.75 mg)	LD	r-hCG	1810	30	<0.5	3	M1*1 M2*2[a]
3/2006	rFSH	300	2400	GnRH antagonist (Ganirelix)	Antagonist, flexible	r-hCG	2224	16	0.94	0	
7/2008	hMG	4	32	GnRH agonist (0.1 mg/day)	LD	r-hCG	934	13	<0.5	1	M1*1[a]
9/2008	HP-hMG	4	40	GnRH agonist (0.1 mg/day)	LD	hCG	4030	14	1.23	4	M1*2[a] GV*1 Atretic*1
2/2011	hMG	4	32	GnRH antagonist (Ganirelix)	Antagonist, flexible	GnRH agonist (0.1 mg) + r-hCG	1841	10	0.91	18	M2*16[b] M1*1 GV*1

hMG (Menogon , Ferring Farmeceuticals); rFSH (Puregon, MSD); HP-hMG (Menopur, Ferring Farmeceuticals);GnRH agonist, Triptorelin acetate (decapeptyl, Ferring Farmeceuticals); r-hCG, Choriogonadotropin alfa 250 μg, (Ovitrelle, MerckSerono); GnRH antagonist cetrorelix acetate (Cetrotide, MerckSerono); GnRH antagonist Ganirelix (Orgalutran, MSD); hCG (Prgnyl, MSD).
SD, short decapeptyl.
[a]Low quality. Perivitaline space filled with fragments.
[b]Fragmented polar body, light and gargled cytoplasm.

Table II Case reports and series.

Author (year)	No. of patient with EFS[a]	No. of patient with GEFS[b]	No. of patient with FEFS[c]	No. of cycles	Protocol (no. of cycles)	Final oocyte maturation	Summary
Coulam *et al.* (1986)	4			5	hMG (3); CC + hMG (2)	Spontaneous (4)/U hCG 10 000 IU (1)	Presented a new syndrome—EFS
Ashkenazi *et al.* (1987)	4			4	uFSH	U hCG 10 000 IU	A new entity in etiology of unexplained infertility versus unusual reaction to uFSH stimulation
Tsuiki *et al.* (1988)	1			1	CC + hMG	U hCG 10 000 IU	Follicular fluid from empty follicles demonstrated increased E_2/progesterone and increased androstenedione level in comparison to follicular fluid from two successful OPUs in the same patient
Rudak *et al.* (1990)	1			3	CC + hMG (2)	U hCG 10 000 IU	
La Sala *et al.*, (1991)	1			2	Agonist + hMG	U hCG 10 000 IU	
Asch *et al.* (1992)			5	5	Agonist + hMG	none	Absence of hCG injection resulted in empty follicle syndrome
Greb *et al.* (1993)		1	1	3	Short agonist + hMG (2)	None (1); hCG 10 000 IU (2)	One patient missing hCG injection; one patient with recurrent EFS albeit proper hCG injection suspected to have early perimenopause
Traina *et al.* (1993)		1		1	Long agonist + hMG	U hCG 10 000 IU	In the previous cycle, nine oocytes were obtained under the same ovarian stimulation protocol
Zegers-Hochschild *et al.* (1995)			6	6	Long agonist + hMG	U hCG 10 000 IU	Abnormalities in hCG batches
Ndukwe *et al.* (1996)			6	6	Long agonist + hMG	U hCG 10 000 IU	Serum hCG levels in EFS significantly lower than in cycles in which oocyte were retrieved. Low bioavailability of bhCG as the cause of EFS
Ndukwe *et al.* (1997)			3	3	Long agonist + hMG	U hCG 10 000 IU	Rescue of EFS cycles by re administration of hCG and aspiration of the intact ovary
Meniru and Craft (1997)	1			1	Long agonist + hMG	U hCG 10 000 IU	Failure to retrieve oocytes 12 h after hCG injection. Successful pickup 24 h later
Khalaf and Braude (1997)	2		1	3		U hCG 10 000 IU	One case in which hCG was injected only 12 h before OPU. Further aspiration 24 h later resulted in twin pregnancy. Second case in which despite confirming proper hCG administration, no oocyte were aspirated in two cycles

Ubaldi *et al.* (1997)		1		1	Long agonist + hMG	U hCG 10 000 IU	Second OPU, 24 h after readministration of hCG yielded 13 M1 oocytes. Twin pregnancy resulted from IVM and frozen-thawed embryo transfer
Awonuga *et al.* (1998)	8	3		12	Long agonist + hMG/uFSH/rFSH	U hCG 10 000 IU	The patients with GEFS had a second hCG dose and repeat OPU 24 h later, two oocytes were retrieved
Hassan *et al.* (1998)		1		1	Long agonist + hMG	U hCG 10 000 IU	Despite high hCG on OPU day, a second hCG dose was administered, repeated OPU 24 h later yielded 11 oocytes
Quintans *et al.* (1998)			5	5	Long agonist + rFSH	None (error in administration)	Administrating hCG and reaspiration resulted in three clinical pregnancies, four infants were born
Peñarrubia *et al.* (1999)		1		2	Long agonist + HP FSH(1); Long agonist + rFSH (1)	U hCG 5000 IU	In the third cycle, rhCG was used. five oocytes were aspirated
Evbuomwan *et al.* (1999)	1			1	Long agonist + rFSH	U hCG 10 000 IU	A second dose of hCG was injected and OPU was repeated 38 h later yielding nine oocytes. The patient had two additional cycles in which OPU was uneventful
Işik and Vicdan (2000)	1			1	Long agonist + rFSH	U hCG 1000 IU (error in administration)	Serum b-hCG level at the time of OPU was 21 mIU/ml
Khalaf *et al.* (2000)	1			1	Long agonist + rFSH	U hCG 5000 IU	Successful retrieval after sole administration of GnRH agonist in another cycle.
Esposito and Patrizio (2000)			1	1	Long agonist + rFSH	None (error in administration)	Successful retrieval 36 h after hCG administration
Papier *et al.* (2000)			1	1	Long agonist + rFSH + hMG	U hCG 1000 IU (error in administration)	A second dose of hCG was given, re-aspiration yielded 12 oocytes. Frozen-thawed embryo transfer resulted in delivery
Milki and Mooney (2001)			2	2	Long agonist + FSH	U hCG 10 000 IU	A second dose of hCG was given, re-aspiration yielded 0 oocytes in one case and 10 in the second. Frozen-thawed embryo transfer resulted in delivery
Stefunidis *et al.* (2002)	1			1	Long agonist + rFSH	U hCG 5000 IU	No oocytes were aspirated from one ovary. Ovarian torsion which probably occurred prior to OPU was diagnosed a few hours later
Lok *et al.* (2003)		1		2	rFSH + GnRH antagonist (1); Long agonist + rFSH (1)	U hCG 10 000 IU	On the third cycle GnRH agonist was used for final oocyte maturation, nine oocyte were aspirated

Continued

Table II *Continued*

Author (year)	No. of patient with EFS[a]	No. of patient with GEFS[b]	No. of patient with FEFS[c]	No. of cycles	Protocol (no. of cycles)	Final oocyte maturation	Summary
Onalan *et al.* (2003)		2		3	Long agonist + rFSH	U hCG 10 000 IU	EFS in two sisters
Uygur *et al.* (2003)		1		2	Long agonist + rFSH	U hCG 5000 IU	
Nikolettos *et al.* (2004)		1		1	Long agonist + rFSH		
Aktas *et al.* (2005)		14	11	25	GnRH analog + hMG/rFSH	U hCG 10 000 IU	Mistake in hCG administration in 11 cases. Twelve women with GEFS underwent another IVF cycle, in all oocytes were retrieved
Lorusso *et al.* (2005)	3			3	Long agonist + rFSH	U hCG 10 000 IU	A second cycle was carried in all cases, In one, nine oocytes were retrieved but number of mature oocytes was low
Vujisic *et al.* (2005)	1			4	Spontaneous (2); Long agonist + hMG(1);rFSH(1)	U hCG 5000–10 000 IU	Pericentric inversion of chromosome 2: 46, XX,inv(2)(p11q21)
Inan *et al.* (2006)	1			3	Long agonist + hMG	U hCG 10 000–30 000 IU	Gene expression in granulosa cells from EFS patient was altered. Genes involved in metabolism, cellular processes and apoptosis were affected
Ng *et al.* (2006)		1		1	FSH + GnRH antagonist	U hCG 10 000 IU	A second cycle using the same protocol 3 months later yielded 14 mature oocytes
Duru *et al.* (2007)	1			2	Long agonist + rFSH (1); Long agonist + rFSH + hMG(1)	rhCG 250 μg	Third cycle: antagonist protocol, final oocyte maturation with GnRH agonist and hCG (added later) yielded eight oocytes, four were inseminated, none fertilized
Krishna *et al.* (2008)		1		1	Long agonist + rFSH + hMG	U hCG 10 000 IU	Second cycle: antagonist protocol, final oocyte maturation with rhCG 500 μg yielded 13 oocytes and resulted in a single life birth
Hirshfeld-Cytron and Kim (2008)			1	1	Long agonist + rFSH	rhCG 250 μg	A patient with dramatic weight lost and abdominal skin redundancy. Levels of hCG after s.c. injection were 19 IU/ml. In the next cycle hCG was given i.m., its level was 45 IU/ml and 19 oocytes were retrieved
Snaifer *et al.* (2008)			1	1	rFSH + GnRH antagonist	None (error in administration)	Administration of rhCG and reaspiration yielded four oocytes and resulted in delivery
Qublan *et al.* (2008)	1			1	Long agonist + hMG	U hCG 10 000 IU	After the failed cycle, the patient had 6 weeks of amenorrhea, ovarian pregnancy was diagnosed

Hourvitz *et al.* (2010)	2		5	NM	NM	Successful IVM cycles in these patients are described
Reichman *et al.* (2010)		7	7	GnRH analog + hMG/rFSH	hCG 10 000 IU (error in administration in three cases)	Repeat administration of hCG in the setting of low serum hCG levels results in unsuccessful outcome
Honnma *et al.* (2011)	2		2	rFSH/hMG + GnRH antagonist	Nasal buserelin 300 μg	Second OPU 36 h after hCG administration yielded 28–35 oocytes
Yariz *et al.* (2011)[d]		2	3	Long agonist + rFSH	U hCG 10 000 IU	Mutation in the LH/choriogonadotrophin receptor

[a]EFS, hCG levels are not detailed.
[b]Genuine EFS, hCG levels are positive.
[c]FEFS, hCG levels are negative or low or mistake in administration was revealed.
[d]The same two sisters described by Onalan *et al.* (2003).

(Tsuiki *et al.*, 1988; Khalaf *et al.*, 2000) ora genetic defect (Papier *et al.*, 2000; Onalan *et al.*, 2003; Vujisic *et al.*, 2005; Yariz *et al.*, 2011).

Tsuiki *et al.* (1988) showed high E_2 and androstendione levels and low progesterone levels in follicular fluid from a patient with EFS. Low levels of progesterone and high levels of testosterone were also reported by Phocas *et al.* (1992) although they reported low levels of E_2 as well (Tsuiki *et al.*, 1988; Phocas *et al.*, 1992). Gene expression of granulosa cells from a patient with recurrent EFS was significantly altered compared with a control. The most prominent effect was demonstrated in genes involved in metabolism, cellular processes and apoptosis. The authors suggested that the increased apoptotic gene expression and reduction in transcripts whose products are responsible for proper follicular growth is the cause of EFS in that patient (Inan *et al.*, 2006). A possible mechanism is that the oocytes are actually lost during late folliculogenesis due to apoptosis. Recently a novel mutation in the LH/choriogonadotropon receptor was identified in two sisters with GEFS (Yariz *et al.*, 2011). Borderline cases of EFS led us to suspect a defect in the process that results in oocyte maturation and cumulus cell expansion.

Therapeutic approach

Patients with EFS present a challenge to the treating physician. No single treatment is universally effective. Some authors, relying on the low frequency of recurrence, recommend repeating the standard ART cycle, regardless of the treatment protocol (Ben-Shlomo *et al.*, 1991). Since in most EFS cases, down-regulation was achieved by GnRH agonist (possibly presenting the higher prevalence of agonist over antagonist in ART cycles), shifting from an agonist to antagonist protocol was suggested (Krishna *et al.*, 2008). In cases where no oocytes are aspirated from one ovary and hCG levels are low, some have suggested readministering hCG from a different batch and aspirating the second ovary (Khalaf and Braude, 1997; Ndukwe *et al.*, 1997; Awonuga *et al.*, 1998; Quintans *et al.*, 1998; Evbuomwan *et al.*, 1999; Esposito and Patrizio, 2000; Papier *et al.*, 2000; Reichman *et al.*, 2010) or even reaspirating the same follicles (Meniru and Craft, 1997; Hassan *et al.*, 1998; Snaifer *et al.*, 2008). Others suggested changing the hCG from a urinary to a recombinant preparation (Peñarrubia *et al.*, 1999). Hourvitz *et al.* (2010) presented two women with EFS who were successfully treated with *in vitro* maturation.

The two treatment remedies that were used in the case presented here, and thus discussed thoroughly, are using GnRH agonist for final oocyte maturation (Lok *et al.*, 2003) and prolonging the interval between ovulation triggering and OPU (Uygur *et al.*, 2003).

GnRH agonist for triggering final oocyte maturation

hCG has long been used as a surrogate for the LH surge. Later on, it was demonstrated that ovulation triggering may be achieved by GnRH agonist (Shalev *et al.*, 1994; Fauser *et al.*, 2002; Griesinger *et al.*, 2006). Among the possible advantages of GnRH agonist for final oocyte maturation is the simultaneous induction of an FSH surge (Gonen *et al.*, 1990; Fauser *et al.*, 2002). The role of the natural mid-cycle FSH surge is not fully clear. FSH was reported to induce LH receptor formation in luteinizing granulosa cells, promote oocyte nuclear maturation and cumulus expansion (Byskov *et al.*, 1997; Humaidan *et al.*, 2011). FSH also has a role in keeping the gap junctions open

Table III Cohort studies.

Author (year)	No. of OPU cycles	No. of EFS cycles (genuine)	EFS prevalence % (genuine %)	No. of patients with EFS	Recurrent EFS	Summary
Ben-Shlomo *et al.* (1991)	1321	26 (NM)	2 (NM)	26	1/21	
Harrison and Fawzy (1996)	1418	1 (NM)	0.07 (NM)	1		
Ndukwe *et al.* (1996)[a]	716	6 (0)	0.8 (0)	6		
Fiszbain *et al.* (1997)	376	9 (0)	2.4 (0)	9		In all cycles a mistake in hCG administration was discovered. In some cases(number not mentioned) immature oocytes were retrieved
Awonuga *et al.* (1998)[a]	2059	11 (3)	0.92 (0.58)	11	0/5	
Driscoll *et al.* (1998)	4236	43 (NM)	1.01 (NM)	42		30 cases due to poor response or age, six premature ovulation, one did not inject hCG, six unknown reason
Quintans *et al.* (1998)[a]	1118	5 (0)	0.44	5		
Zreik *et al.* (2000)	3004	57 (NM)	1.89	37	55/200	No recurrences under the age of 34. The recurrence rate is higher in older patients
Aktas *et al.* (2005)[a]	3060	25 (14)	0.81 (0.45)	25	0/12	
Coskun *et al.* (2010)	5238	NM (58)	NM (1.1)	26	4/13	The occurrence of EFS indicates poor IVF success in subsequent IVF cycles
Reichman *et al.* (2010)[a]	15 729	7 (0)	0.045 (0)	7		After administrating hCG and reaspiration, fewer oocyte were retrieved (in comparison to subsequent cycle), no pregnancy resulted
Griesinger *et al.* (2006)	51	1 (NM)	2 (NM)	1		GnRH agonist induces final oocyte maturation in all patients. LH levels were not measured thus it is unclear whether the injection was properly administered
Mesen *et al.* (2011)	12 359	11(2)	0.089 (0.016)	11		
Baum *et al.* (2011)	8292	163 (NM)	2 (NM)	136	16/101	
Castillo *et al.* (2012)	3467	118	3.4	118		No difference was found in EFS prevalence between ovulation triggering with GnRH agonist (2034 cycles) or hCG (1433 cycles)

NM, not mentioned.
[a]These articles were referenced in Table II as well.

between the oocyte and cumulus cells and thus may have an important role in signaling pathways (Godard *et al.*, 2009; Lamb *et al.*, 2011). FSH stimulates plasminogen activator activity within granulosa cells, which results in production of plasmin in the follicular fluid (Strickland and Beers, 1976). Plasmin, in turn, generates active collagenase which disrupts the follicular wall (D'Alessandris *et al.*, 2001; Lamb *et al.*, 2011). Expansion and dispersion of the cumulus cells allows the oocyte–cumulus cell mass to detach from the follicular wall before ovulation. This process involves synthesis of hyaluronic acid matrix, which is stimulated by FSH (Dell'Aquila *et al.*, 2004). Adding FSH at the time of the hCG trigger enhances oocyte recovery and improves fertilization (Lamb *et al.*, 2011). GnRH receptors have been identified in a wide variety of human tissues including pre-ovulatory granulosa cells. Mammalian oocytes remain at prophase of first meiosis from birth until the gondotropin surge at puberty. During this long period, intra oocyte cAMP and cGMP prevents oocyte meiosis resumption. The cGMP level decreases in response to LH and meiosis resumes (Sun *et al.*, 2009). It was demonstrated that peripheral GnRH receptor activation leads to a decrease in the intracellular cAMP level. GnRH induces transcription of several genes that are involved in follicular rupture and oocyte maturation (Yu *et al.*, 2011). The FSH surge and the direct action of the agonist on the ovarian GnRH receptor might explain the favorable results in the eighth cycle in our patient. One can speculate that, for an unknown reason, the LH path is blocked in the patient and the GnRH agonist activates a different path.

Prolonging the interval between ovulation triggering and OPU

In a natural cycle, the onset of LH surge occurs 34–36 h prior to follicular rupture (Hoff *et al.*, 1983). Similarly, administering exogenous hCG causes follicular rupture after ~37 h (Edwards and Steptoe, 1975). Resumption of meiosis begins 18 h after the onset of the LH surge (Seibel *et al.*, 1982). LH concentration must be maintained above a threshold for 14–27 h in order to maximize oocyte maturation (Zelinski-Wooten *et al.*, 1992). Follicular rupture and oocyte maturation are time-dependent processes, with different times needed in different patients. It might be hypothesized that cumulus expansion which allows the oocyte to detach from the follicular wall also requires longer time periods in certain patients. In these cases, EFS may result when aspiration takes place 34 h after hCG administration.

Summary and conclusions

The case presented here demonstrates the existence of a GEFS. The patient was obviously not a low responder since E_2 levels were consistently high after stimulation and many follicles could be visualized. She correctly administered hCG, as was demonstrated by positive hCG in her serum. We cannot exclude anomalous absorption or clearance as the cause of the slightly low serum hCG levels in our patient, but because the hCG levels were higher than those described in connection to EFS, we think another reason should be sought.

In the index cycle two treatment options were combined, using GnRH agonist for final oocyte maturation and prolonging the time between ovulation triggering and OPU. It is impossible to differentiate which of the two strategies yielded the desired outcome. Adding hCG 34 h prior to the OPU was most likely not the reason for the successful recovery, in light of the prior multiple failures with this regimen although one cannot reject that combining hCG with GnRH agonist caused the desired effect. It can also not be excluded that the 29 months, that passed between prior treatments and the successful treatment, influenced the outcome. In other words, EFS may be self-limiting phenomena.

We assert that GEFS does exist and submit this case as the proof. We argue, however, that EFS is a misnomer since the follicles are not actually empty, but rather that the oocytes are not aspirated or identified utilizing standard ART methods. EFS may represent a syndrome of impaired granulosa cell function, in which oocyte meiotic maturation is not resumed, cumulus expansion does not ensue and the immature oocyte–cumulus complexes are resistant to follicular aspiration. In the case presented here, prolonging the interval between ovulation triggering and OPU and inducing ovulation using GnRH agonist resulted in aspiration of mature oocytes, pregnancy and delivery. The concealed variation that caused our patient to be resistant to the common IVF regimen, yet sensitive to a novel one, remains a mystery.

Authors' roles

R.B.-F. conceived the concept and wrote the manuscript; A.W. wrote the manuscript; M.L., Y.G. and E.S. revised the manuscript. All authors read and approved the final manuscript.

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Conflict of interest

We declare that there are no conflicting interests between the authors and the completion or publication of the study.

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