Original Article

Empty Follicle Syndrome: A Challenge to Physician

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Abstract

Background: Empty follicle syndrome (EFS) is a condition in which no oocytes are retrieved from normally growing ovarian follicles after ovarian stimulation. It is a rare and frustrating condition of obscure etiology. **Objective:** The objective of this study was to estimate the incidence of EFS and study factors related to it. **Design:** This was a retrospective study. **Setting:** This study was conducted in hospital-based research center. **Methods:** In 1968 *in vitro* fertilization cycles from January 2010 to August 2016 were studied. Agonist, antagonist, and miniflare protocols were used for the stimulation. **Results:** The incidence of EFS is 2.38% (47/1968 cycles). Antagonist protocol group (76.59%, n = 36) had highest incidence of EFS (6.69%). Literature on EFS depicts decreased ovarian reserve (DOR) as the main cause, but only 4.25% of patients had DOR in our study. Interestingly, polycystic ovary syndrome and unexplained infertility were found in 31.9% of the cases. Serum anti-Müllerian hormone (AMH) levels (mean ± standard deviation [SD]) were 4.47 ± 3.54 ng/ml, and antral follicle count (AFC) was 15.30 ± 8.07 (mean ± SD) emphasizing that diminished ovarian reserve is not the main factor for EFS. All patients (n = 95) who underwent ovum pickup on day when any patient had EFS were taken as control. Patients with EFS were compared with controls. A statistically significant difference was not observed in serum AMH (P = 0.38) and AFC (P = 0.52). **Conclusion:** EFS is an uncommon event. Antagonist cycles have higher chances of empty follicle at ovum pickup. Looking at the profile of patients in this study, we conclude that EFS is not a manifestation of DOR.

Keywords: *Decreased ovarian reserve, empty follicle syndrome, infertility*

Introduction

Coulam *et al*. gave the term "empty follicle syndrome (EFS)" in 1986.[1] It is a condition in which no oocytes are retrieved after ovarian stimulation and tedious follicular aspiration from normally growing follicles.[1]

EFS is a rare and extremely frustrating event. It causes stress and anxiety to both patients and physicians. Therefore, it is important to understand EFS. The mechanism responsible for this syndrome is unknown. However, early atresia of oocyte due to dysfunctional folliculogenesis is thought to be one of the causes of this syndrome.[2] Some believe that longer exposure to human chorionic gonadotropin (HCG) is necessary for causing detachment of oocyte-cumulus complexes from the follicular wall.[3] According to some studies, EFS results from the abnormal biological activity of some batches of HCG;[4] however, it does not explain recurrence in the next cycle. Another theory is about ovarian aging in older women presenting with varying function and growth of granulose cells resulting in altered oocyte growth and maturation of follicle.[5] The aim of this study was to estimate the incidence of EFS and to understand the factors associated with it.

Methods

All *in vitro* fertilization (IVF) cycles done from January 2010 to August 2016 were retrospectively

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studied from IVF database. Long agonist, antagonist, and miniflare protocols were the stimulation protocols used in the patients. In the long agonist protocol, injection leuprolide 1 mg s.c. (GnRH analog) was started from the 21st day of previous menstrual cycles. Recombinant follicle-stimulating hormone (Gonal-F) or human menopausal gonadotropin was used for ovarian stimulation, and the follicular growth was monitored. In the antagonist protocol, Gonal-F was started on the 2nd day of the menstrual cycle, and the follicular growth was monitored. Antagonist cetrorelix 0.25 mg/day was administered from day 6 of the cycle. In the microdose flare protocol, the patient received 0.5 μg leuprolide from the 2nd day of menstrual cycle. Gonal-F was started from day 3 of the cycle. The follicular growth was monitored using transvaginal sonography. Recombinant human chorionic gonadotropin (rhCG), 250 μg was administered when at least three follicle reaches the size of 18 mm, and ovum pickup was performed after 34–36 h.

Of 1968 IVF cycles, 47 patients had EFS. The information, including age, body mass index (BMI), follicle-stimulating hormone (FSH) level, anti-Müllerian hormone (AMH) level, and antral follicle count (AFC), type of protocol used for stimulation, number of days of stimulation, number of follicle on the day of trigger, amount of gonadotropin use, and estradiol levels (E2) on the day of trigger, were collected.

On the day, when any patient had EFS, other patients in whom oocytes were retrieved during ovum pickup were taken as control. These patients had HCG trigger from the same batch ruling out ineffective HCG as the cause of EFS. Patients were compared with respect to age, BMI, AFC, serum AMH, serum FSH, and protocols used for the stimulation.

Statistical analysis

Data collected was analyzed using the statistical software Statistical Product and Service Solutions (SPSS) IBM version 19.0, Armonk, New York, USA. Descriptive statistics, such as mean, median, standard deviation, and interquartile range, were calculated for various study parameters. Continuous variables data were tested for normality assumption using the Kolmogorov–Smirnov test**.** Two sample mean values of approximate to normally distributed data were compared using the Student's *t*-independent test. For data not normally distributed, median values were compared using the nonparametric Mann–Whitney U-test. Frequency data were expressed as number and percentage and compared using the Chi-square or Fisher's exact test as appropriate.

To find significant influencing variables for EFS, multivariate logistic regression analysis was carried out by taking EFS status as the dependent variable (Yes-1, No-0) and all other the study variables as independent variables. Backward elimination procedure with variable removal *P* of 0.06 was adopted. Overall fit was assessed by likelihood ratio, Chi-square, and individual logistics regression coefficient were tested by Wald statistics. Adjusted odds ratio (OR) and its 95% confidence limits were calculated for the final model with statistically significant variables. $P < 0.05$ was considered statistically significant.

Results

In this retrospective study, 47 cases of EFS were seen in 1968 IVF cycles accounting an incidence rate of 2.38% (95% confidence interval: 1.8%–3.2%). A wide variation in incidence was observed between different protocols. According to our data, the agonist protocol was used for 19.14% (n = 9) of women, the antagonist for 76.59% (n = 36) of women, and the microdose flare for 4.25% (n = 2) of women. The incidence of EFS was higher in antagonist cycle 6.69% (36/538), followed by 2.04% (2/98) in case of microdose flare protocol, and agonist 0.67% (9/1332) [Table 1].

The mean (mean ± standard deviation [SD]) age of the patients was 30.85 ± 4.51 years, and their mean BMI was 24.47 ± 3.54 kg/m^2. Indications of IVF were tubal 44.68% (n = 21), polycystic ovary syndrome (PCOS) 14.89% (n = 7), diminished ovarian reserve 4.25% (n = 2), male infertility factor 19.14% (n = 9), unexplained 17.02% (n = 8), and endometriosis 4.25% (n = 2).

About 60% (28/47) of the patients had ovarian reserve AMH value between 1and 5, and 30% of patients had AMH >5. Moreover, 65.26% had AFC value 5–15, and 31.9% (15/47) had AFC >15. Serum AMH levels were 4.47 ± 3.54 ng/ml (mean ± SD), and AFC was 15.30 ± 8.07 (mean ± SD) emphasizing that diminished ovarian reserve is not the main factor for EFS.

Looking at the stimulation sheets of the patients, total dose of gonadotropin use, number of follicle,

Table 1: Incidence of EFS in various IVF protocols

Protocols	Incidence	*P* fisher exact test
Agonist	0.67% (9/1332)	0.001
Antagonist	6.69% (36/538)	
Micro dose flare	2.04% (2/98)	
Total	2.38% (47/1968)	

and estradiol level on the day of trigger were studied. More than 40% (20/47) of patients achieved proper follicle development at <3000 of total gonadotropin dose. Moreover, on the day of trigger, five or more follicles of adequate size were seen in 63.85% (30/47) of patients showing that response to the gonadotropins was adequate. Median estradiol level (E2) was 939.0 pg/ml (interquartile range: 688–2274 pg/ml) which was low; however, there were few patients with higher estradiol (around 5000 pg/ml) levels had EFS.

All patients (n = 95) who underwent ovum pickup on day when any of the scheduled patients had EFS were taken as control. Baseline characteristics of EFS cases (47 patients) were compared with controls (95 patients) undergoing IVF in whom oocytes were retrieved on ovum pickup. Patients were comparable with respect to age, BMI, AFC, serum AMH, serum FSH, and protocols used for the stimulation [Tables 2 and 3]. When the cause of infertility was compared in both the groups, it was observed that PCOS was more common in patients with EFS (P = 0.041) and endometriosis in control group (P = 0.001) [Table 3]. Total follicles on the day of HCG trigger were significantly less in EFS patients ($P \leq 0.001$). Significantly, lower serum estradiol levels were observed on the day of trigger in EFS group ($P \leq 0.01$) [Table 4]. No significant difference was found in total days of stimulation (P = 0.48) and total dose of gonadotropin required in IVF cycle (P = 0.99) [Tables 2 and 4]. Distribution of EFS patients and controls by various study variables is summarized in Table 4. It was observed that the percentage of patients with < four follicles on the trigger day or the patients with serum estradiol value <1000 pg/ml was significantly ($P < 0.01$) more compared to controls [Table 5].

Results of multivariate logistic regression analysis

Statistically significant variables with adjusted OR and 95% confidence limits are presented in Table 6. Overall fit of the model was statistically significant (Likelihood ratio χ^2 (4) =50.8; $P < 0.001$; Pseudo R^2 = 0.42).

On reviewing the records, it was observed that out of these 47 patients, only three patients underwent repeat IVF. Oocytes were retrieved in all three patients by changing the protocol from antagonist to agonist type, but none of the cycle resulted in pregnancy. Interestingly, one PCOS patient who had EFS in her

Table 2: Baseline characteristics of EFS patients and controls

Baseline characteristics	Cases (n=47) Mean±SD	Control (n=95) Mean±SD	P (t-test)
Age (years)	30.85±4.51	31.09±3.52	o. 72
BMI (kg/m2)	24.47±3.54	24.48±3.332	0.99
Total AFC	15.30±8.06	14.39±6.522	0.47
S. AMH	4.40±3.456	4.47±3.076	0.90
S. FSH	6.43±2.019	6.45±2.221	0.94
Total days of stimulation	11.15±1.945	11.37±1.670	0.48
Total follicles on day of trigger	6.02±3.247	9.96±4.250	<0.001

Table 3: Distribution comparison between EFS patients and controls by base-line characteristics

Baseline characteristics	Case (n=47)	Control (n=95)	P by Chi square test
Cause of infertility			
Tubal	21 (44.68%)	46 (48.42%)	0.674
PCOS	7 (14.89%)	4 (4.21%)	0.041
DOR	2 (4.25%)	4 (4.21%)	0.99
Endometriosis	2 (4.25%)	30 (31.57%)	0.001
Male	9 (19.14%)	22 (23.15%)	0.586
Unexplained	8 (17.02%)	17 (17.89%)	0.0.898
Protocol			
Agonist	9 (19.14%)	24 (25.26%)	0.417
Antagonist	36 (76.59%)	66 (69.47%)	0.375
MDF	2 (4.25%)	5 (5.26%)	0.99
AFC			
<5	1 (2.12%)	1 (1.05%)	0.10
5-15	31 (65.95%)	62 (65.26%)	
16-29	9 (19.14%)	29 (30.52%)	
≥30	6 (12.76%)	3 (3.15%)	

Table 4: Comparison of serum estradiol and total dose of gonadotropin used in EFS patients and controls

Baseline characteristics	Cases (*n*=47) Median (range)	Control (*n*=95) Median (range)	*P* (Mann Whitney U test)
S. Estradiol on day of trigger (pg/ml)	939.00 (IQR: 688-2274)	4745.00 (IQR: 2848-5180)	<0.01
Total dose of gonadotropin	3200.00 (IQR: 2275-4050)	3150.00 (IQR: 2550-4125)	0.99

Table 5: Distribution comparison of EFS patients and controls by various study variables

Parameter	Case	Control	*P*
Age			
<35	37 (78.7%)	76 (80.0%)	0.85
≥35	10 (21.3%)	19 (20.0%)	
BMI			
<25	26 (55.3%)	49 (51.6%)	0.67
≥25	21 (44.7%)	46 (48.4%)	
S. FSH			
<9	41 (87.2%)	79 (83.2%)	0.52
≥9	6 (12.8%)	16 (16.8%)	
S. AMH			
<1.01	5 (10.6%)	5 (5.3%)	0.38
1.01-4.99	28 (59.6%)	54 (56.8)	
>4.99	14 (29.8)	36 (37.9%)	
Total dose of gonadotropins			
<3000	20 (42.6%)	34 (35.8%)	0.43
≥3000	27 (57.4%)	61 (64.2%)	
Follicles on day of trigger			
≤4	17 (36.2%)	6 (6.3%)	<0.01
>4	30 (63.85)	89 (93.7%)	
S. estradiol			
<1000 pg/ml	25 (53.2%)	1 (1.1%)	<0.01
>1000 pg/ml	22 (46.8%)	94 (98.4%)	

Table 6: Significant influencing variables for Empty follicle syndrome by multivariate logistic regression analysis

Variable	Adjusted odds ratio	*P*	95% confidence limits	
FSH on D2	0.78	0.037	0.62	0.98
LH on D2	1.28	0.005	1.07	1.51
Simulation Days	0.75	0.024	0.58	0.96
Follicles on day of HCG	0.65	0.000	0.55	0.77

first IVF cycle conceived spontaneously later on and delivered a healthy male child. In two patients of EFS, oocytes were retrieved in their previous IVF cycle but none had successful conception.

Discussion

EFS is a rare but extremely frustrating event in the field of *in vitro* reproduction. The existence of EFS has always been considered controversial due to successful oocyte retrieval in the next cycle.[6] There have been many studies in the past to prove its existence. Inan *et al*. have suggested early atresia of oocyte with continued growth of follicle as mechanism of EFS.[7] In his study, a 22-year-old patient with recurrent EFS was studied, and 160 genes were found different from those patients who yielded oocyte normally.

The estimated incidence of EFS is 0.045%–3.5% of IVF cycles.[5,8,9] In this study, the incidence was 2.38%. The results were slightly higher than the study by Madani *et al*. (1.7% in 3356 patients)[10] and a study by Zreik *et al*. (1.8%).[11]

The mean age of the patients was 30.85 ± 4.51 years in this study. Most previous studies have shown increased age (37.7 ± 6.0 years in Baum *et al*. study) as a risk factor for EFS.[12] According to Zreik *et al*.,[11] chances of recurrence of EFS increase with age. Ovarian aging through altered folliculogenesis has been postulated to be the cause of EFS. Revelli *et al*.[13] also found the link between age and EFS. They found the prevalence of EFS was about five times higher among patients over 40 years than in younger women (6.3% versus 1.8%).[13]

While studying the distribution of EFS in the study, it was seen that the majority of patients underwent antagonist protocol. Similar to the study by Baum *et al*.,[12] the incidence of EFS was also highest in antagonist cycle (3.8%). Contrary to this, high percentage (12.1%) of empty follicles in the microdose flare protocol was seen in a study by Madani *et al*.[10]

Low estradiol levels compared to controls were observed in a study by Baum *et al*.[12] (499.9 ± 480.9 pg/mL vs. 1516.3 ± 887.5 pg/mL, $P < 0.001$). Similar results were observed in this study ($P < 0.01$); however, some patients had estradiol levels reaching up to 5000 pg/ml.

Most of the studies suggest that genuine EFS is a variant of low ovarian reserve.[12,14,15] However, it was not so in our study. In fact, PCOS patients were significantly higher in EFS group compared to controls. In addition, 29.8% of patients with EFS had AMH >5 ng/ml and 31.9% had AFC >15.

In study by Stevenson *et al*.[16] and Revelli *et al*.,[13] EFS was classified into false and genuine type of EFS. In genuine type of EFS, no oocyte is retrieved despite optimal HCG levels. Failure to retrieve oocytes in patients with HCG <40 IU/L on the day of ovum pickup is the false type of EFS. Cause of latter can

be due to low bioavailability of HCG or problems in HCG administration. According to Stevenson *et al*. study, 67% of EFS are false type, and only 33% are of genuine type. As rhCG was used in all 47 patients in our study and ovum pickup was done 34–36 h after HCG trigger, false type of EFS is less likely. Moreover, in eight patients, EFS urine pregnancy test was done on the day of ovum pickup which was positive in all patients which show adequate HCG was present in serum. In rest of the 34 patients, records related to urine pregnancy test or HCG levels were not available. However, other patients who underwent ovum pickup on the same day and had HCG trigger from the same batch of drug retrieved oocytes which rule out ineffectivity of HCG injection.

Literature explaining the cause of genuine EFS is less. A study by Yuan *et al*.[17] described homozygous mutation in luteinizing hormone/choriogonadotropin receptor (LHCGR) gene, c.1345G>A (p.Ala449Thr) as a cause of genuine EFS. Screening for mutations in the LHCGR gene may assist in detecting genuine EFS. In another study, Chen *et al*.,[18] a paternally transmitted heterozygous missense mutation of c.400 G>A (p.Ala134Thr) in zona pellucida glycoprotein 3 (ZP3), was described as the genetic basis of EFS.

As the cause of false EFS is an ineffective HCG trigger, giving another HCG dose and scheduling oocyte retrieval later may yield some oocytes from another ovary. This was first described by Ndukwe *et al*. in 1996. According to the records available, in none of the patients in the present study, repeat trigger was given and pickup attempted after no oocytes from one ovary.

Important aspect to keep in mind is the significance of this event to patients' future fertility. However, prognosis after EFS varies from sporadic events[5] to being a predictor of poor outcome.[19] According to a study by Baum *et al*., recurrence is found in around 15% of cases;[12] however, the exact percentage is difficult to estimate due to less number of studies. As very few patients underwent repeat IVF cycle, the recurrence rate of EFS cannot be commented on. In the present study, one PCOS patient who had EFS in her first IVF cycle conceived spontaneously later on and delivered a healthy male child.

Conclusion

EFS is a rare condition. The use of antagonist protocol is associated with more number of EFS. In addition, EFS may not be a manifestation of the poor ovarian reserve.

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Conflicts of interest

There are no conflicts of interest.

References

1. Coulam CB, Bustillo M, Schulman JD. Empty follicle syndrome. Fertil Steril 1986;46:1153-5.
2. Tsuiki A, Rose BI, Hung TT. Steroid profiles of follicular fluids from a patient with the empty follicle syndrome. Fertil Steril 1988;49:104-7.
3. Meniru GI, Craft IL. Evidence from a salvaged treatment cycle supports an aetiology for the empty follicle syndrome that is related to terminal follicular developmental events. Hum Reprod 1997;12:2385-7.
4. Zegers-Hochschild F, Fernández E, Mackenna A, Fabres C, Altieri E, Lopez T, *et al.* The empty follicle syndrome: A pharmaceutical industry syndrome. Hum Reprod 1995;10:2262-5.
5. Aktas M, Beckers NG, van Inzen WG, Verhoeff A, de Jong D. Oocytes in the empty follicle: A controversial syndrome. Fertil Steril 2005;84:1643-8.
6. van Heusden AM, van Santbrink EJ, Schipper I, de Jong D. The empty follicle syndrome is dead! Fertil Steril 2008;89:746.
7. Inan MS, Al-Hassan S, Ozand P, Coskun S. Transcriptional profiling of granulosa cells from a patient with recurrent empty follicle syndrome. Reprod Biomed Online 2006;13:481-91.
8. Castillo JC, Garcia-Velasco J, Humaidan P. Empty follicle syndrome after gnRHa triggering versus hCG triggering in COS. J Assist Reprod Genet 2012;29:249-53.
9. Driscoll GL, Tyler JP, Knight DC, Cooke S, Kime L, Clark L, *et al.* Failure to collect oocytes in assisted reproductive technology: A retrospective. Hum Reprod 1998;13:84-7.
10. Madani T, Jahangiri N. Empty follicle syndrome: The possible cause of occurrence. Oman Med J 2015;30:417-20.
11. Zreik TG, Garcia-Velasco JA, Vergara TM, Arici A, Olive D, Jones EE, *et al.* Empty follicle syndrome: Evidence for recurrence. Hum Reprod 2000;15:999-1002.
12. Baum M, Machtinger R, Yerushalmi GM, Maman E, Seidman DS, Dor J, *et al.* Recurrence of empty follicle syndrome with stimulated IVF cycles. Gynecol Endocrinol 2012;28:293-5.
13. Revelli A, Carosso A, Grassi G, Gennarelli G, Canosa S, Benedetto C, *et al.* Empty follicle syndrome revisited: Definition, incidence, aetiology, early diagnosis and treatment. Reprod Biomed Online 2017;35:132-8.
14. Stevenson TL, Lashen H. Empty follicle syndrome: The reality of a controversial syndrome, a systematic review. Fertil Steril 2008;90:691-8.
15. Kim JH, Jee BC. Empty follicle syndrome. Clin Exp Reprod Med 2012;39:132-7.
16. Younis JS, Skournik A, Radin O, Haddad S, Bar-Ami S, Ben-Ami M, *et al.* Poor oocyte retrieval is a manifestation of low ovarian reserve. Fertil Steril 2005;83:504-7.
17. Yuan P, He Z, Zheng L, Wang W, Li Y, Zhao H, *et al.* Genetic evidence of "genuine" empty follicle syndrome: A novel effective mutation in the LHCGR gene and review of the literature. Hum Reprod 2017;32:944-53.
18. Chen T, Bian Y, Liu X, Zhao S, Wu K, Yan L, *et al.* A recurrent missense mutation in ZP3 causes empty follicle syndrome and female infertility. Am J Hum Genet 2017;101:459-65.
19. Lorusso F, Depalo R, Tsadilas S, Caradonna F, Di Gilio A, Capotorto MT, *et al.* Is the occurrence of the empty follicle syndrome a predictor that a subsequent stimulated cycle will be an unfavourable one? Reprod Biomed Online 2005;10:571-4.