Original Article

Prevalence of Empty Follicle Syndrome in King Chulalongkorn Memorial Hospital

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Objective: To investigate the prevalence of empty follicle syndrome (EFS), a condition in which no oocytes were retrieved after ovarian stimulation, categorized into genuine EFS (g-EFS) and false EFS (f-EFS), at the King Chulalongkorn Memorial Hospital (KCMH), Thailand.

Materials and Methods: A retrospective study was conducted at the infertility clinic of the KCMH. Medical records of the assisted reproductive technology (ART) patients between January 2001 and October 2019 (5,523 patients) were reviewed. Exclusion criteria were the cases where ovulation occurred before oocyte retrieval or the cases with less than four follicles larger than 14 mm diameter on the day of triggering ovulation to minimize the absence of oocyte from the poor response. The patients with EFS, g-EFS, which are EFS with detectable urinary human chorionic gonadotropin (hCG), and f-EFS, which are EFS with undetectable urinary hCG, were identified. Prevalence of EFS was calculated.

Results: There were three cases with EFS in the present study, which g-EFS was identified in one case and f-EFS in two cases. The prevalence of EFS was 0.054%, which g-EFS was 0.018% and f-EFS was 0.036%.

Conclusion: EFS is a rare condition, particularly the g-EFS. Although EFS is rare, it causes tremendous stress and anxiety to both patients and physicians. Further study in the etiopathogenesis of EFS is required.

Keywords: Empty follicle syndrome; Infertility; In vitro fertilization

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Empty follicle syndrome (EFS) is a complete failure to retrieve oocytes after ovarian stimulation, despite normal follicle development and appropriate estradiol production by granulosa cells (GC)[1]. The term EFS was first described by Coulam et al in 1986[2]. However, Ben-Shlomo et al suggested that this condition was a sporadic event and should not be referred to as a syndrome[3]. The prevalence of EFS has been reported in various studies and ranged from 0.045% to 7.0%[1,4]. Currently, EFS is categorized into genuine EFS (g-EFS) and false EFS (f-EFS). The g-EFS is a complete failure to collect oocytes despite the presence of adequate human chorionic gonadotropin (hCG) or luteinizing hormone (LH) level at the time of oocyte retrieval. The f-EFS occurs when the hCG or LH level is below the critical threshold due to errors during ovulation triggering[5].

EFS is a rare but frustrating condition for both the couples and the physicians in assisted reproductive technology (ART). EFS can cause physical and psychological stress, including economic consequences[4]. Although, several previous studies regarding the causes of EFS have been reported, the etiology and mechanism of this syndrome is still obscure[1]. Therefore, it is essential to understand its prevalence, risk factors, pathophysiology, and chance of recurrence.

In Thailand, there is only one case report regarding the mechanism of g-EFS[6]. However, the prevalence of EFS has not been published. The King Chulalongkorn Memorial Hospital (KCMH) is one of the largest public general and tertiary referral hospitals in Thailand. The objective of the present study was to investigate the prevalence of EFS at the KCMH.

Materials and Methods

This retrospective study was conducted at the Infertility Clinic, KCMH, Thailand. The present study was approved by the Institutional Review Board, Faculty of Medicine, Chulalongkorn

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University, Bangkok, Thailand (IRB No. 363/62) and was registered at the Thai Clinical Trials Registry (TCTR20200917003). The procedures used in the present study adhere to the tenets of the Declaration of Helsinki.

Inclusion and exclusion criteria

Medical records of all ART cases between January 2001 and October 2019 were reviewed. The cases where no oocytes were retrieved during oocyte retrieval after follicles aspiration and multiple times of follicle flushing were recruited. Exclusion criteria were patients with a) premature ovulation before oocyte retrieval or, b) less than four follicles larger than 14 mm diameter on the day of hCG trigger, to minimize the chance of absent oocyte from poor responders or technical errors of oocyte retrieval[7-9].

Urinary hCG was examined when no oocytes were identified in the aspirated follicular fluid. EFS with detectable urinary hCG was defined as g-EFS and EFS with undetectable urinary hCG was defined as f-EFS[10].

Ovarian stimulation protocols

The patients in the present study were prescribed either recombinant follicle stimulating hormone (rFSH: Gonal-F®, Merck-Serono, Geneva, Switzerland; or Puregon®, Organon, Oss, The Netherlands) or human menopausal gonadotropin (hMG: Menopur®, Ferring, Saint-Prex, Switzerland) for ovarian stimulation. For pituitary suppression, either GnRH antagonist or GnRH agonist protocol were prescribed[7].

In the GnRH antagonist protocol, daily injections of rFSH or hMG (150 to 375 IU/day) were started on the third day of the menstrual cycle and continued for 8 to 12 days. Follicular growth was monitored by transvaginal sonography (TVS). GnRH antagonist 0.25 mg/day (Ganirelix: Orgalutran®, Organon, Oss, The Netherlands or cetrorelix: Cetrotide®, Merck-Serono, Geneva, Switzerland) was administered once when the leading follicles reached 14 mm diameter or on day 6 of ovarian stimulation.

In the agonist protocol (long protocol), the patients received 600 μg/day Buserelin acetate (Suprefact® Nasal Spray, Sanofi, Frankfurt, Germany) from the 21st day of the previous menstrual cycle. Then, at the beginning of gonadotropin administration, the dose was reduced to 400 μg/day and continued during ovarian stimulation. Gonadotropin was administered in the same manner as the antagonist protocol.

After individualized ovarian stimulation, either 250 μg recombinant human chorionic gonadotropin (rhCG: Ovidrel®, Merck-Serono, Geneva, Switzerland), 10,000 IU hCG (Pregnyl®, Organon Oss, The Netherlands), or 0.2 mg GnRH agonist (Triptorelin: Decapeptyl®, Ferring Saint-Prex, Switzerland or Diphereline®, Ipsen, Paris, France) was administered when at least three follicles reached the size of 18 mm as a mean diameter on transvaginal ultrasound. Oocytes were retrieved 36 hours after administration of hCG injection.

Statistical analysis

The prevalence of EFS, g-EFS, and f-EFS was calculated as the number of patients with EFS (g-EFS + f-EFS), g-EFS, and f-EFS divided by the total number of ART patients, respectively. The sample size was determined by the number of ART cycles between January 2001 and October 2019 and based on a power analysis from the previous studies[1,11].

The patient's characteristics and data were collected and analyzed. The data included age, body mass index (BMI), infertility diagnosis, cycle characteristics such as ovarian stimulation protocol, the total dose of gonadotropin, and duration of stimulation, laboratory results including basal FSH level, LH level and estradiol (E_2) level on the hCG triggering day, and transvaginal ultrasound data.

Results

Medical records of 5,523 ART patients were retrospectively reviewed. GnRH antagonist protocol was used in 70% of the patients to prevent premature LH surge. One or more oocytes were retrieved from 5,482 patients, subsequently, these patients were excluded from the study. No oocytes were retrieved in 41 patients. Of the 41 patients, 20 patients were excluded due to premature ovulation before the oocyte retrieval procedure and 18 patients were excluded because less than four follicles were larger than 14 mm by TVS.

Three patients were included in the analysis. The three patients underwent the GnRH antagonist ovarian stimulation protocol (Figure 1). Urinary hCG on the day of oocyte retrieval was not detected in two patients. These two cases were diagnosed with f-EFS. Only one patient had detectable urinary hCG and was diagnosed with g-EFS.

The overall prevalence of EFS in the KCMH was 0.054%. The prevalence of f-EFS was 0.036% and g-EFS was 0.018%. Baseline characteristics of the patients with EFS are described in Table 1.

The patient with g-EFS was a 45-year-old woman diagnosed with female factor infertility or reduced

Table 1. Baseline characteristic of patients with empty follicle syndrome

	False-EFS		Genuine-EFS
	Case1	Case2	
Age (years)	44	45	45
BMI (kg/m^2)	24.2	21.2	19.8
Infertility etiology	Advanced age	Tubal factor	Advanced age
Total days of stimulation	8	8	9
Total dose gonadotropin (IU)	2,000	2,400	2,950
No. of follicles on hCG triggering day	4	9	4
Size of follicles (mm)	19, 17, 15, 15	20, 19, 18, 16, 16, 15, 15, 15, 15	20, 18, 16, 15
Peak estradiol level (pg/mL)	816	1,462	1,416
LH level on hCG triggering day (IU/L)	9.9	4.2	9.0
Day 2 FSH level (IU/L)	5.5	6.2	5.0

EFS=empty follicle syndrome; BMI=body mass index; FSH=follicle stimulating hormone; LH=luteinizing hormone; hCG=human chorionic gonadotropin

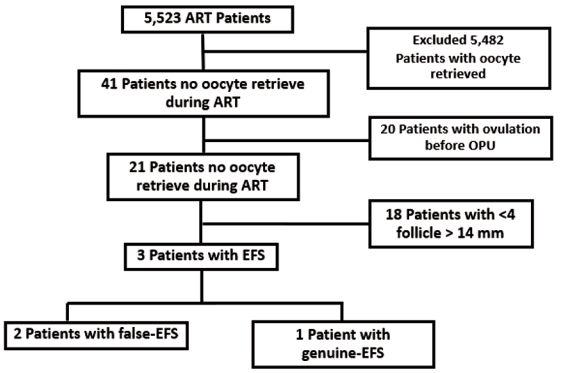

Figure 1. Diagram of the study.

EFS=empty follicle syndrome; ART=assisted reproductive technology

ovarian reserve, with a BMI of 19.8 kg/m². The GnRH antagonist stimulation protocol was started with rFSH (Puregon, Organon) 300 IU daily on Day 1 to Day 4 of stimulation and increased to 350 IU daily from Day 5 to Day 9 (total 2,950 IU). Pituitary suppression was achieved using a GnRH antagonist (Orgalutran, Organon) 0.25 mg daily started on day 6 of ovarian stimulation. After nine days of stimulation with gonadotropin, there were four follicles larger than 14 mm on the hCG triggering day with a peak E2 level of 1,416 pg/mL. At 36 hours after 250 mg rhCG (Ovidrel®, Merck-Serono) administration, no oocytes were recovered despite vigorous flushing and aspiration of follicles. The patient confirmed that she injected rhCG at the correct time, which was approximately 36 hours before oocyte retrieval. The urinary hCG test was positive in this patient.

There were hCG administration errors in the two patients with f-EFS. One patient injected the rhCG at an incorrect time. The other did not administer the rhCG before oocyte retrieval. The urinary hCG test was negative in both patients. These three patients did not continue their next ovarian stimulation cycles.

Discussion

EFS is a frustrating condition for both physicians and patients. Although, EFS is an infrequent event in ART, the economic and emotional consequences are enormous. The overall prevalence of EFS at the KCMH was 0.054%. The prevalence of f-EFS (0.036%) and the prevalence of g-EFS (0.018%) were slightly lower than in previous studies[1,8,12-14]. A systematic review by Stevenson et al[5] found that only one third of the patients were g-EFS. The results of the present study showed a similar proportion and suggested that g-EFS may be a rare condition.

The f-EFS is defined when a failure to retrieve oocytes occurs with a below critical threshold of hCG- or LH-circulating level, suggesting that the ovulation trigger has not functioned properly[1]. It is most likely associated with errors during the time of hCG administration, which is similar to the two patients in the present study. The definition of g-EFS has not been standardized because the threshold of hCG level to define an accurate pharmacologic trigger level has not yet been agreed on, which ranges from 5 to 160 hCG mIU/mL at 36 hours after hCG administration[1]. Due to the simplicity and non-invasive technique, urinary hCG was used in the present study instead of serum hCG level[10] to differentiate the types of EFS. In the present study, the patient with g-EFS was diagnosed when urinary hCG test was positive, which roughly corresponded to a serum hCG level of 10 to

20 mIU/mL[15].

Both the prevalence of g-EFS and the prevalence of f-EFS in the present study were less than the previous studies[8,14]. This was possibly due to the present study strict inclusion and exclusion criteria. Patients with fewer than four follicles larger than 14 mm diameter were excluded to avoid the patients with poor ovarian response[9] and technical issues of oocyte retrieval. Furthermore, recent studies indicated that follicular size larger than 12 mm or follicular volume larger than 0.6 mL are adequate for oocyte retrieval[16,17]. Thus, the present study chose the follicle size larger than 14 mm to reduce possible false-positive diagnosis of EFS from inadequate follicle size and oocyte immaturity.

The patient with g-EFS in the present study was prescribed a GnRH antagonist stimulation protocol. Similar to the present study, Baum et al[13,18] found that the incidence of EFS was highest at 3.8% in GnRH antagonist cycles. In contrast, Madani et al[14] reported that a high percentage of empty follicles was seen in the micro dose flare protocol, approximately 12.1%. However, the finding of the present study that all the patients with EFS were prescribed a GnRH antagonist stimulation protocol may be caused by the unequal distribution of the stimulation protocols in which the antagonist protocol comprised 70% of the cases.

The previous studies[1,18,19] proposed that EFS was associated with the advanced age of the female, prolonged infertility, decreased ovarian reserve, and poor respond to gonadotropins. Ovarian aging altered folliculogenesis has been postulated to be the cause of EFS[13,18]. Revelli et al[1] found a relationship between female age and EFS. They found the prevalence of EFS among the patients over 40 years was about five times higher than younger women (6.3% versus 1.8%, respectively). This is similar to the present study that all patients with EFS were older than 40 years old.

Other mechanisms such as genetic predisposition and molecular mechanism underlying the pathophysiology of EFS should be considered. Vutyavanich et al[6] reported a case of g-EFS that might result from a delayed maturation of oocyte cumulus complex in response to hCG that required a longer time to complete follicle maturation. Another possible mechanism, ovarian resistance to hCG, may explain why a repeated hCG administration, which increases the power and length of luteinizing hormone/choriogonadotropin receptor (LHCGR) stimulation, allowed retrieval of oocytes in some cases of EFS[20]. In addition, Inan et al also suggested that early atresia of the oocyte with the continuing growth of follicles was a possible mechanism of EFS[19]. They analyzed whole gene expression of GC from a patient with recurrent EFS. They identified 160 genes that were differentially expressed when compared to the control, which were the GC from a patient that underwent the same protocol and yielded oocytes[19]. Several growth factors such as amphiregulin, epiregulin, and betacellulin are essential stimulators of oocyte maturation and cumulus expansion in an animal study[21]. However, the altered expression of the genes that regulate cumulus expansion remains under investigated in human studies.

The present study included a large cohort of more than 5,000 patients in nineteen-year period. The authors did not investigate some other confounding factors such as genetic polymorphism, including some new hormonal assay such as Anti-Müllerian Hormone (AMH) level in all patients in the study. In addition, most of the patients used a GnRH antagonist protocol. Further studies are required to increase understanding of other confounding factors and the pathogenesis of EFS to avoid EFS in the future.

In conclusion, EFS is a rare condition, particularly the g-EFS. Only one patient with g-EFS in the review of 5,523 patients was identified, supporting the conclusion that EFS is a rare phenomenon.

What is already known on this topic?

EFS is an uncommon but frustrating condition in ART, which leads to physical and psychological stress, including economic consequences to the patients. However, the prevalence of EFS has not been published.

What this study adds?

Having a similar trend to global prevalence, EFS is a rare condition in Thailand, particularly the g-EFS. Further studies are required to increase understanding of other dimensions of empty follicle syndrome, to avoid EFS in the future.

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Authors' contributions

All authors contributed to the study conception

and design. Material preparation, data collection, and analysis were performed by Chanakarn Suebthawinkul. The first draft of the manuscript was written by Chanakarn Suebthawinkul and all authors commented on the previous versions of the manuscript. All authors read and approved the final manuscript.

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Conflicts of interest

The authors declare that they have no conflict of interest.

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