Original Article

Characteristics of Empty Follicular Syndrome during *In vitro* Fertilization Embryo Transfer and its Association with Various Etiologies in Comparatively Young Patients

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Abstract

Background: Nearly 0.6%–7% of patients undergoing *in vitro* fertilization embryo transfer (IVF ET) will not be able to yield any oocyte despite successful ovarian stimulation and this condition is called as empty follicular syndrome (EFS). EFS is a dreadful situation for clinicians as well as patients, seems to be an unavoidable clinical condition despite a proper ovarian stimulation. **Materials and Methods:** This was a retrospective observational study conducted at a tertiary hospital; 1103 patients who underwent IVF ET between January 2016 and May 2017 were included in the study. **Study Outcome:** To estimate the incidence of empty follicle syndrome (EFS) and to study the associated factors. **Results:** There were 53 (4.8%) cases of EFS out of 1103 cycles of IVF ET; 43 (3.9%) cases were false EFS and 10 (0.9%) cases were genuine EFS. Mean age of EFS group and oocyte retrieved group was 30.17 years and 29.12 years respectively. Recurrence rate of EFS during the next IVF cycle was 36.8%. Decreased ovarian reserve was associated with an increased chance of EFS (54.7%) with a recurrence rate as high as 57%. **Conclusion:** The incidence of EFS is not an uncommon clinical scenario; it depends upon ovarian reserve to a great extent. Young age is not immune for the occurrence of EFS as there is a similar incidence in comparatively younger age group in our study. EFS is seen in all etiological groups of infertility, but only respite is that there is a chance of about 63.2% oocyte retrieval during repeat IVF cycle.

Keywords: *Decreased ovarian reserve, empty follicular syndrome, in vitro fertilization embryo transfer, oocyte retrieval*

Introduction

The success of *in vitro* fertilization embryo transfer (IVF ET) is not 100%, and mere completion of IVF ET cycle is associated with hurdles at many stages. Various stages at which the treatment cycle gets stuck starting from getting an opportunity to undergo this costly treatment, response to stimulation protocol, retrieval of gametes, fertilization, embryo transfer with good-quality embryos, implantation and clinical pregnancy, continuation of pregnancy, and finally a live birth.

Retrieval of oocytes during IVF ET is the most important milestone for the completion of IVF ET cycle. Empty follicular syndrome (EFS) is a stressful clinical situation where there are no oocytes retrieved despite successful stimulation with apparently normal follicular development and E2 levels, even after repeated aspiration and flushing. EFS is generally seen in 0.6%–7% of the patients undergoing IVF ET. There are two types of EFS: genuine EFS and false EFS. Genuine EFS means the occurrence of EFS in the presence of optimal beta-human chorionic gonadotropin (βhCG) levels on the day of oocyte retrieval, and in false EFS, there are

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no adequate βhCG levels.[1] βhCG level >40 IU/L is essential for successful oocyte retrieval.[2]

Ovulation trigger is very essential for final maturation of oocytes and loosening the cumulus oocyte complex from the follicular wall. In a normal cycle, this role is played by luteinizing hormone (LH) surge. During controlled ovarian hyperstimulation (COH) for IVF, there is a requirement of supraphysiological level of LH for ovulation trigger, which cannot be met with the administration of external LH. Human chorionic gonadotropin (hCG), due to its similarities with LH, has been used to induce final oocyte maturation in case of long-agonist cycle. However, in antagonist cycle, agonist can be used for ovulation trigger as an alternative to hCG for prevention of ovarian hyperstimulation syndrome (OHSS) in polycystic ovarian syndrome (PCOS).[3]

The probable etiologies assumed for false EFS are hCG-related faults such as improper hCG administration, rapid metabolic clearance, manufacturer defects, and low bioavailability.[1,4] Genuine EFS is presumably related to intrinsic ovarian dysfunction, dysfunctional folliculogenesis or premature apoptosis of the growing oocytes, defective function of granulosa cells,[5-7] strong attachment of cumulus cell complexes to the follicular wall, and improper timing of ovulation trigger. In rare instances, follicles may need longer exposure to βhCG to undergo cumulus expansion and to separate from the follicular wall.[8,9] Genetic causes of EFS have also been suggested such as LH/hCG receptor mutations.[10] Altered expression of genes regulating cumulus expansion and cellular apoptosis may result in increased loss of oocytes during late folliculogenesis. Pericentric inversion of chromosome 2 has been reported by Vujisic *et al*. in a patient who had multiple EFS.[11] Fault in the maintenance of correct suction is also a known technical cause for EFS which may be ignored due to fatigue or complacency.

EFS is a tremendously stressful situation to a patient as well as a clinician to face a nil oocyte retrieval situation. It is very important to understand about this situation and to formulate precautionary measures at every IVF center; hence, this study was initiated to study the characteristics of empty follicular syndrome during IVF ET and its association with various etiologies.

Materials and Methods

This is a retrospective observational study. Patients who underwent IVF cycle at our Reproductive Medicine Centre from January 2016 to May 2017 (n = 1103), meeting inclusion and exclusion criteria, were included in the study. Approval was taken from the institutional ethical committee.

Inclusion criteria

1. All willing patients undergoing IVF ET with age ranging between 22 and 40 years during the study period
2. Presence of two functioning ovaries.

Exclusion criteria

1. Patients who underwent ovum pick up (OPU) for fertility preservation.
2. Presence of single ovary.

Methodology

Controlled ovarian stimulation for IVF ET was done by two IVF protocols such as long-agonist protocol (LP) and short-antagonist protocol (AP). In LP, oral contraceptive was started on the 5th day of menstruation and short-acting gonadotropin-releasing hormone agonist (GnRH). Injection leuprolide acetate 1 mg subcutaneously (sc) daily (Luprorin®-4 mg/4 ml vial, Intas Pharmaceuticals, India) was started from day 21 of the cycle for downregulation.

Ovarian stimulation was started from the 2nd day of menstruation in a downregulated cycle with injection recombinant follitropin alpha (Gonal F, MerkSereno, Italy), and injection recombinant LH (Luveris, MerkSereno, Switzerland) was added toward the end for follicular maturation in PCOS cases. In cases of normal ovarian reserve and in decreased ovarian reserve (DOR) cases, highly purified human menopausal gonadotropin (Menodac 150 IU, Bayer Zydus Pharma, India) was added after six doses of injection recombinant follitropin alpha. Dosages were titrated as per ovarian response with ultrasound follicular monitoring. In AP, stimulation was started from the 2nd day of menstruation, and GnRH antagonist injection cetroreli × 0.25 mg SC (Cetrolix, Intas Pharmaceuticals, India) was added when the leading follicle reached 15 mm or on day 5 of stimulation whichever was early. Ovulation trigger was given with injection recombinant hCG 250 µg prefilled syringe (Ovitrelle, Merk Sereno, Italy) when the follicles reached 18 mm or more in size. OPU was done around 35 h after ovulation trigger. If no oocytes were identified, a dip stick urine test was done at the earliest. Demographic profile, cycle characteristics including induction protocol, infertility diagnosis, oocyte retrieval, and IVF outcome were tabulated. Estimation of the incidence of empty follicle syndrome and its characteristics were done; subsequently, the association of empty follicle syndrome with various etiologies during IVF ET was studied.

Statistical analysis

Details of all cases were recorded on a structured format and analyzed with the help of Statistical Package for the Social Sciences (SPSS) version 22 (IBM Corp., Armonk, NY, USA). Group comparisons were made using independent *t*-test and paired *t*-test. Cross-tabulation and Chi-square test were done for protocol-wise analysis of empty follicular syndrome. One-way analysis of variance was done to assess empty follicular syndrome in different categories. Statistical significance was assessed at $P < 0.05$.

Results

Mean age of the patients who underwent IVF ET was 29.17 years and common etiological factors of the patients who underwent IVF ET are unexplained (22.9%), PCOS (21.9%), DOR (19.1%), tubal factor (9.9%), azoospermia (8.0%), oligoasthenoteratozoospermia (5.4%), and other causes as mentioned in Figure 1. 78.6% of the patients underwent stimulation by LP and remaining 21.4% by AP. EFS was noticed in 53 cases [Table 1] and one patient had the suspicion of premature ovulation despite down-regulation and OPU was attempted for the remaining two follicles. Mean anti-Müllerian hormone (AMH) is 3.13 and 4.97 in the EFS and oocyte retrieved group [Table 1]. Nineteen cases who had EFS during the first cycle of IVF underwent repeat cycle and EFS did not recur in 12 patients (63.2%) and recurred in 7 (36.8%); recurrence was more common in DOR group (57%). Thirty-four patients did not report for the repeat IVF during the study period. Overall pregnancy rate is 43.87% which included three patients who had EFS during first IVF cycle.

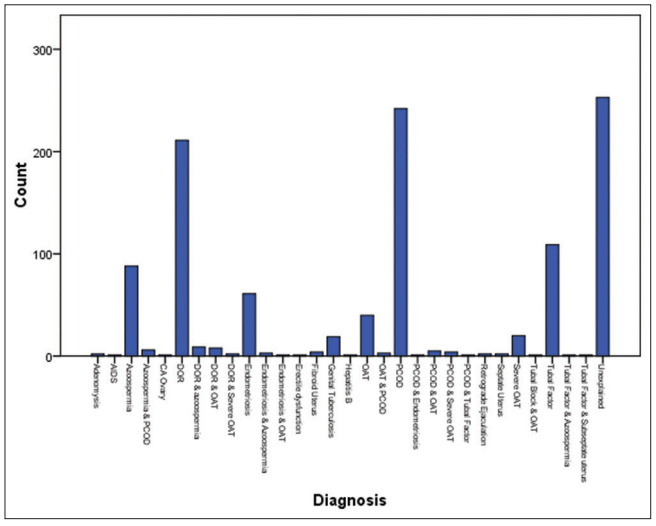

Figure 1: Etiological aspects of infertility

Discussion

Retrieval of oocytes during OPU is the most important stage of the IVF ET procedure, and the reported oocyte recovery rate in natural cycles is approximately 80%.[2] The yield of adequate gametes may be considered as the success for the role of the clinician as well as the patient.

The advanced age has also been attributed as the cause for EFS, study by Kim JH *et al* showed there was significant association between two age groups 37.7 ± 6.0 years and 34.2 ± 6.0 years with EFS, $P < 0.001$.[2] In our study, overall mean age was 29.17 years and 30.17 years in the EFS group and 29.12 in the oocyte retrieved group ($P = 0.05$); comparatively, our patients are in the young age group [Table 1]. The overall incidence of EFS was 4.8%; among these, 3.9% cases were false EFS and 0.9% of them were genuine EFS; the incidence of genuine EFS is a rare phenomenon. Similar overall incidence of about 4.9% was noticed in the study by Girsh *et al*.[12] and 2.4% in another study.[2]

In our study, 236 patients (21.4%) underwent AP of which 11.4% had EFS, and 867 patients (78.6%) underwent LP of which 2.9% had EFS [Table 2]. This major difference in the incidence of EFS between the two protocols is because most of the DOR patients underwent AP. A study by Castillo *et al*. showed the incidence of EFS between two protocols to be same, i.e., 3.5% versus 3.1%, respectively, in agonist and antagonist protocol; in this study, antagonist was primarily used in PCOS patients to avoid OHSS.[13] In a study by Kim and Jee, the incidence of EFS between antagonist and agonist cycle was 2.5% versus 1.4%,

Table 1: Average age and anti-Müllerian hormone levels in empty follicular syndrome and oocyte retrieved

Category	Number of cases	Age (years)	AMH level (mean±SD)
False EFS	43	29.84±4.73	3.16±4.77
Genuine EFS	10	31.6±4.45	2.99±3.37
Oocytes retrieved	1050	29.12±3.74	4.97±4.26
Total	1103	29.17±3.79	4.88±4.29

SD=Standard deviation, EFS=Empty follicular syndrome, AMH=Anti-Müllerian hormone

Table 2: Protocol and empty follicular syndrome cross tabulation

Protocol	Protocol distribution of the cases	Type of EFS		Total number of EFS
		False EFS	Genuine EFS	
AP	236	23	4	27
LP	867	20	6	26
Total	1103	43	10	53

EFS=Empty follicular syndrome, AP=Antagonist protocol, LP=Long agonist protocol

Table 3: Empty follicular syndrome and etiology cross tabulation

Category	Etiology of infertile couple					
	DOR	DOR and azoospermia	OAT	PCOS	Unexplained	Total
False EFS	27	1	1	4	10	43
Genuine EFS	2	1	0	2	5	10
Total	29	2	1	6	15	53

EFS=Empty follicular syndrome, DOR=Decreased ovarian reserve, OAT=Oligoasthenoteratozoospermia, PCOS=Polycystic ovarian syndrome

respectively, and the difference is not statistically significant (P = 1.000).[2]

In our study, when the incidence of EFS was studied by etiology-wise, the incidence of EFS was highest in DOR cases (58.49%) [Table 3]. Similar association was not seen when AMH was considered separately as it is supposed to be less in EFS group, maybe because of small number of EFS group. EFS group also contained PCOS and normal ovarian reserve cases which must have altered the mean AMH level in EFS group. A study by Madani and Jahangiri also showed that 51.7% of women had AMH levels ≤0.5 ng/mL.[14] A similar finding was noticed in a study by Girsh *et al*. in which the EFS group had very low AMH, i.e., 0.5 ± 0.3, which suggests DOR.[12]

In our study, EFS recurred in 36.8% of 19 cases who underwent repeat IVF following an episode of EFS, and the recurrence was very high in cases of DOR (57%). EFS did not recur in 12 cases (63.2%), and 3 of them had successful IVF ET with a positive pregnancy. Thirty-four patients did not report for the second attempt of IVF ET during the study period. The study by Zreik *et al.* estimated that women with one EFS cycle had a 20% risk of recurrence in subsequent IVF cycles,[5] and Baum *et al*. have reported a recurrence of about 15.8%.[7] The recurrence rate in our study is high because most of the cases were DOR who were at high risk for EFS.

Every clinician practicing IVF should know strategies to prevent an occurrence of EFS during subsequent IVF cycle. Some of the strategies elaborated in various studies are correct timing of ovulation trigger and administration of recombinant βhCG for ovulation trigger instead of urinary βhCG under supervision by a person trained in reproductive medicine unit. Checking for optimum level of βhCG before OPU, the optimum level ranges from 40 to 161 IU/L.[15] Following GnRH agonist trigger in an antagonist cycle, the measurements of LH value at 8–12 h probability of EFS were unlikely when it was ≥15 IU/ml, as elucidated by Kummer *et al*.[16] There is also a case study by Deepika *et al*., which mentions about dual trigger with recombinant βhCG and GnRH agonist in antagonist cycle which yielded good number of oocytes in three cycles after two cycles of EFS.[17] Ensuring proper suction pressure during OPU should be a drill of every technician and clinician during OPU. If EFS is detected soon after completion of one side ovary repeating a rescue dose of βhCG and re-aspiration after around 35 h later would yield oocytes, successful outcome of IVF cycles has also been reported. Recently, a review of literature has shown that 42.8% of cycles resulted in a healthy live born fetus when rescue protocol was used.[1]

Conclusion

The incidence of an empty follicular syndrome is not an uncommon clinical scenario; to a great extent, it depends on ovarian reserve. Our study confirms its association with DOR even in younger age group. EFS is seen in all etiological groups of infertility, but only respite is that there is a chance of about 63.2% oocyte retrieval during repeat IVF cycle with a positive pregnancy rate of 25%. Adequate counseling before OPU in high-risk cases and compassionate approach after OPU in EFS cases, during this stressful situation, is very essential as there is a chance of hope during the next IVF.

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Conflicts of interest

There are no conflicts of interest.

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