

## Empty follicle syndrome: Frequency and probable causes in Pakistani population

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### Abstract

**Objective:** To determine the frequency of empty follicle syndrome in in-vitro fertilization-stimulated cycles and to evaluate the causes associated with this phenomenon.

**Method:** The retrospective study was conducted at the Baqai Institute of Reproduction and Developmental Sciences, Karachi, and comprised data related to period from January 2012 to December 2016. Other than demographic information, laboratory investigations, ultrasound findings and treatment protocols were noted and evaluated. Data was statistically analysed using SPSS 21.

**Result:** Of the 1120 cycles studied, empty follicle syndrome was found in 72 (6.4%). The possible leading causes included advanced age in 64 (88.8%) patients, longer duration of infertility in 56 (77.7%), higher baseline follicle stimulating hormone in 56 (77.7%), and lower oestradiol level before human chorionic gonadotrophin injection in 58 (80%). Empty follicles were significantly correlated with age of the patient, years of infertility, serum follicle stimulating hormone level and stimulation length ( $p < 0.05$ ).

**Conclusion:** Empty follicle syndrome is a rare occurrence. Screening of patients for risk factors before considering IVF cycles is mandatory. The patients should be counseled for possible EFS even in first cycle.

**Keywords:** Empty follicle syndrome, Infertility, Ovarian stimulation, Oocyte retrieval.

(JPMA 70: 1538; 2020) DOI: <https://doi.org/10.5455/JPMA.18623>

### Introduction

In-vitro fertilisation (IVF) is an assisted reproduction technique (ART) during which ovarian stimulation is performed to retrieve mature oocytes at metaphase II stage that are capable of fertilisation. The process correlates with the number of developed mature follicles and the number of oocytes retrieved. Empty follicle syndrome (EFS) is a condition when there is a failure to retrieve oocyte in a controlled IVF cycle after repeated flushing even with normal oestradiol (E2) level and ultrasonic evidence of the presence of many potential follicles. The reported incidence is estimated to be 0.2-7%<sup>1</sup> and was first described in 1986.<sup>2</sup>

Literature describes two variants of EFS depending upon human chorionic gonadotrophin (hCG) level at the time of oocyte retrieval; genuine or true EFS and false EFS. Genuine EFS is defined as failed or altered folliculogenesis because of which early oocyte atresia occurs despite normal hCG level at the time of oocyte aspiration.<sup>3</sup> Other proposed mechanisms include either impaired granulosa cells function or metabolism in older females leading to altered oocyte growth or strong attachment of oocyte-cumulus complex resulting in EFS. The presence of cumulus cells during oocyte retrieval is highly suggestive of abnormal

folliculogenesis.<sup>4</sup> The incidence of false EFS is seen in cases with low hCG levels that might result from human error in hCG administration, such as wrong timing, inadequate dosage, variation in individual hCG threshold, increased plasma clearance leading to poor bioavailability or substandard pharmacological preparations.<sup>5</sup> Among all these causes, low hCG bioavailability remains the commonest cause of EFS.<sup>6</sup>

The exact mechanism responsible for this syndrome remains unclear. The associated risk factors include advanced age, longer duration of infertility, higher baseline follicle stimulating hormone (FSH) level and lower E2 levels before hCG injection.<sup>7</sup> It has been proposed that the reasons for EFS may be the same as those that have caused sterility in the patients previously. Moreover, it may be considered a side effect of super ovulatory treatment in which oocytes due to an inappropriate hormonal microenvironment are damaged, absorbed and failed to be released from the follicles.<sup>8</sup> Nonetheless, many other causes have been suggested up till now, including short-term exposure to hCG, ovarian aging in older women with low ovarian reserve, disorders of generation and maturation of follicle leading to early atresia and involvement of genetic factors etc.<sup>9,10</sup>

The IVF treatment cycles are good option for infertile couples, but, being very expensive, they have low affordability for lower and middle-class families, requiring extreme care and concern on behalf of the doctor. The

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current study was planned to estimate the frequency of EFS in females presenting for IVF treatment at an urban centre, and to assess the underlying causes associated with unsuccessful oocyte retrieval after apparently successful ovarian stimulation.

## Materials and Methods

The retrospective study was conducted at the Baqai Institute of Reproduction and Developmental Sciences, Karachi, and comprised data related to period from January 2012 to December 2016. After approval from the institutional review board, all ART cycles carried out between the study period were assessed. Participants included married women aged 24-45 years having regular menstrual cycles, normal hormonal profile and presenting for the first time for IVF treatment and whose record comprised detailed history and a baseline ultrasonography along with hormonal profile that ruled out common medical disorders, like endocrine diseases. Data of women aged >46 years and suffering from uterine tumours, uterine fibroids, ovarian tumours and endometrioma was excluded.

Data collection included information about patient's age, duration and type of infertility, gravidity and parity, cycle characteristics, including ovulation induction protocol and infertility diagnosis. Pertinent laboratory tests included early follicular-phase baseline FSH, and E2 levels on the day of hCG Injection (pg/ml) and transvaginal ultrasound (TVU) examination reports. Length of stimulation cycles was also recorded (Appendix).

For the induction of follicular growth, two types of IVF stimulation protocols were used according to the number of follicles, baseline FSH level and age of patient. Long protocol was used when the age was <35 years, FSH level <8 and number of follicles >10. Short protocol was used if FSH level was high with the number of follicles around 5 and age of patient between 35-40 years. Flareup protocol was used if the number of follicles was <5, age between 40-45 year and there was a previous history of failed stimulation from any other IVF centre.<sup>8</sup>

For the long protocol, the subjects were given gonadotrophin releasing hormone analogue (GnRH-a) in the form of nasal spray (Suprefact) one puff in each nostril, 2-5 times a day as daily dose at fixed timings in the mid-luteal phase (day-21) of previous menstrual cycle. Each puff contained a dose of 100 microgram of active ingredient; and the total dose depended on female age, FSH level and antral follicles. This was followed by recombinant FSH (rFSH) (Gonal-F, Puregon) in the dose of 200-450 IU depending on female age and ovarian response from day 2 of menstrual cycle when complete suppression of ovaries

was achieved by GnRH-a.

In the short Protocol, the subjects underwent a suppression protocol using GnRH antagonist (Cetrotide, Serono 0.25mg / day subcutaneous injection). Patients stimulated using the flareup protocol were started on day 2 of menstrual cycle with GnRH-a along with injection human menopausal gonadotrophins (HMG) or rFSH. Also, hCG 10,000 IU (Pregnyl 5000 IU injection Organon) was administered when at least 2 follicles of more than 18mm average diameter were observed on TVU.

TVU-guided follicular aspiration was done 36-37 hours after hCG injection. Flushing was conducted liberally to all patients during follicular aspiration.

EFS was defined as complete failure to retrieve oocytes despite average follicular size of 18mm on TVU on the day of hCG injection. Positive pregnancy was defined as serum hCG >10IU/l after 12 days of embryo transfer. Clinical pregnancy was defined as finding a gestational sac, foetal pole and foetal heart beat on ultrasound at 5-6 weeks of gestation.

Data was analysed using SPSS 21. The frequency of EFS was calculated as the number of patients with EFS divided by the total number of patients. The demographic information, cycle characteristics, including induction protocol, infertility diagnosis and relevant laboratory tests, were described as frequency and percentage. Pearson's correlation and regression coefficient were computed to analyse correlation between variables and empty follicles without eggs.

## Results

Of the 1120 IVF cycles, 72 (6.4%) had EFS. Length of stimulation was less than 9 days in 20 (27.7%) patients, while >9 days was in 52 (72.2%) (Table 1). Besides, 34 (47.2%) patients underwent long protocol, 16 (22.2%) short protocol, and 22 (30.5%) had flareup protocol. Mean thickness of endometrium on day 5 varied between 0.5 and 0.9 with a mean of  $0.623 \pm 0.123$ , whereas on day 8, it varied between 0.7 and 1.1 with a mean of  $0.89 \pm 0.101$ .

Cycles of empty follicles occurred in 60 (83.3%) patients who were aged >35 years while 12 (16.6%) were aged <35 years. The duration of infertility was >5 years in 56 (77.7%) patients, while it was <5 years in 16 (22.2%). Higher baseline FSH level >8 was found in 56 (77.7%) subjects; among them, the level was >10 in 40 (71.4%) cases, and 8-10 in 16 (28.5%) subjects. FSH level was <8 in 16 (22.2%) subjects. Primary infertility was found in 58 (80.5%) patients, while 14 (19.4%) subjects had conceived before. Lower E2 level was found in 64 (88.8%) patients, while 8 (11.2%) had borderline levels. Endometriosis was diagnosed in 16

Table-1: Demographic characteristics, baseline hormone level and stimulation protocol of patients.

Characteristics	n (%)
Age	
≥ 35	64 (88.8)
<35	8 (11.2)
BMI	
20-25	34 (47.2)
26-30	38 (52.7)
Type of infertility	
Primary	58 (80.5)
Secondary	14 (19.4)
Period of infertility	
≥5years	56 (77.7)
< 5 years	16 (22.2)
Baseline FSH level	
≥ 10	40 (55.5)
8 - 9	16 (22.2)
<8	16 (22.2)
E2 level	
lower	64 (88.8)
Borderline	8 (11.2)
Stimulation protocol	
Long	34 (47.2)
Short	16 (22.2)
Flare up	22 (30.5)
Length of stimulation	
≤ 9 days	20 (27.7)
> 9 days	52 (72.2)

BMI: Body mass index; FSH: Follicle stimulating hormone; E2: Oestradiol

Table-2: Causes of infertility associated with EFS in our population.

Characteristics	n (%)
Endometriosis	16 (22.2)
Decreased ovarian reserve	12 (16.7)
Premature ovarian failure & TB	2(2.8)
Tuberculosis	8 (11.1)
Azoospermia	6 (8.3)
Increased FSH decreased ovarian reserve	10 (13.9)
Increased Age and FSH	12 (16.7)
Hypothyroidism	4 (5.6)

EFS: Empty follicle syndrome; TB: Tuberculosis; FSH: Follicle stimulating hormone.

Table-3: Correlation analysis of variables with empty follicles.

Causes	r	Crude Regression Coefficient	p-value	r Adjusted	p-value
Age of Patients (yrs)	0.043	0.001	0.028*	0.029	0.563
Year of Infertility	0.741	0.042	0.034*	0.036	0.356
No of Ampoules per day	0.309	0.003	0.049*	0.032	0.048*
Stimulation length per day	0.285	0.065	0.011*	0.005	0.043*
Serum FSH level	0.309	0.054	0.007*	0.178	0.018*
BMI (kg/m <sup>2</sup> )	0.098	0.044	0.415	-	-
Endometrial thickness D5	0.022	0.249	0.856	-	-
Endometrial thickness Day 8 (cms)	-0.089	-1.235	0.457	-	-
Day of follicle aspiration	0.051	0.803	0.677	-	-
Follicles > 18mm	0.178	0.302	0.135	-	-

\*p-value ≤ 0.05 is considered as significant. FSH: Follicle stimulating hormone;

BMI: Body mass index

(22.2%) patients, tuberculosis (TB) in 10 (13.8%) and hypothyroidism in 4 (5.6%) (Table 2).

Plasma hCG level on the day of ovum pick-up was 45-55 IU in patients with BMI 20-25 and it was 30-40 IU in patients with BMI 26-30. BMI, endometrial thickness (at days 5, 8 and day of follicular aspiration in cm), and follicle size >18mm

had no significant relationship with empty follicles ( $p > 0.05$ ). Although, age, serum FSH level, number of ampoules (2 ampoules of Puregon 100 IU, amounting to a dosage of 1ml/day and/or Injection Gonal F, 75 IU, 1.5-2 ml/day) depending on the patient's ovarian response and stimulation length had significant positive relationship with follicles and strongest correlation with years of infertility ( $p < 0.05$ ) (Table 3).

Infertility had significant strongest correlation with number of follicles ( $r = 0.74$ ). Crude regression coefficient described the effect of variables on number of follicles. Coefficient of infertility revealed that with one-year increase of infertility and one-unit increase in stimulation length, the number of follicles increased, but they were empty without eggs. Multivariate analysis showed that one-unit increase in serum FSH level had significant strongest effect on follicles.

## Discussion

This study found EFS frequency to be 6.4%. Many studies have suggested age as a risk factor for the occurrence of EFS due to progressively diminishing ovarian reserve.<sup>9-11</sup> Our results showed similar findings where an overwhelming majority of patients (83.3%) were aged >35 years.

Baseline FSH level is used as a predictor of ovarian reserve and has important impact on oocyte maturation during menstrual as well as fertilization cycles. The FSH surge induces luteinising hormone (LH) receptor formation on luteinized granulosa cells and promote oocyte maturation and cumulus expansion.<sup>13</sup> A study reported that low levels of FSH and LH on day 2 and low LH on day of trigger, lead to suboptimal response and requires longer stimulation and more gonadotrophin with adequate response.<sup>14</sup> Another study documented that abnormally high levels of FSH and anti-Mullerian hormones (AMH) reflect highly beneficial outcomes in IVF, suggesting greater importance of FSH in early follicle maturation.<sup>15</sup> On the contrary, the baseline FSH level in the current study was higher in majority of cases (77.7%), whereas in 30.5% cases, both increased age along with increased FSH levels were found. Thus, both increased age and serum FSH levels showed significant positive correlation with empty follicles ( $p < 0.05$ ).

Our study reported endometriosis in 22.2% cases, TB in 13.8% cases and hypothyroidism in 7% subjects, which is line with literature.<sup>16</sup> It is estimated that 30-50% of women with endometriosis have infertility problems.<sup>17</sup> A meta-analysis suggested that the presence of endometriosis may be considered to reduce the ovarian reserve, but endometriosis-related infertility has similar cycle outcomes as other patients undergoing ART.<sup>18</sup> Another study

documented that advanced age and endometriosis are high-risk factors contributing to oocyte retrieval failure in infertile patients receiving IVF treatment.<sup>19</sup> The role of thyroid autoimmunity among women of reproductive age was stated to have no impact on IVF or intracytoplasmic sperm injection (ICSI) outcome in terms of number of oocytes retrieved and likelihood of fertilization, implantation and clinical pregnancy.<sup>20</sup>

In many patients, unsuccessful oocyte retrieval appears to be due to an underlying ovarian dysfunction and some may have a genuine empty follicle syndrome. A study reported low progesterone levels and low progesterone-E2 ratio in EFS cases, suggesting that the process of luteinisation was disturbed and LH effect was inappropriate. The E2 level provides a measure of ovarian response and correlates with the number of follicles and oocytes.<sup>9</sup> Previous studies have indicated that late follicular phase means E2 level <2800 nmol/L, and it represents reduced ovarian response associated with poor outcome and low implantation rate in a given stimulation protocol.<sup>9</sup> Our study also found E2 level to be lower in majority of cases (88%), while it was borderline in 8 (11.2%) cases.

The cause of false EFS has been clearly identified as an error in hCG administration at the time of ovulation by many studies.<sup>21</sup> A study documented failure to achieve mature oocytes in two subsequent IVF cycles despite the presence of normally growing follicles and rise in E2 level. One of the cycles was triggered with rhCG while other with GnRH agonist.<sup>22</sup> In many cases, technical problems, such as error in hCG administration or defects in hCG batches, have been identified but this is not sufficient to account for all reported cases.<sup>23</sup> Our study ruled out this false cause of EFS as injections were administered at proper timings by trained staff at the centre, which is associated with a fully equipped hospital working 24 hours. The hCG injections were given at a fixed time selected on the basis of egg collection through ultrasound-directed follicular aspiration.

Possibility of an underlying post-receptor cyclic adenosine monophosphate (cAMP) signalling system inducing early expression of apoptotic gene has been suggested previously.<sup>24</sup> Recently, a study reported a missense mutation of LH/chorio-gonadotrophin receptor as genetic evidence of EFS.<sup>25</sup> Another genome-wide linkage analysis study identified a paternally transmitted heterozygous missense mutation in zona pellucida glycoprotein-3 (ZP3).<sup>26</sup> These studies confirmed a genetic basis for genuine EFS and oocyte degeneration due to genetic mutations.

In line with literature, the current study found that EFS was a genuine disorder found more in older patients with longer infertility years, a higher baseline serum FSH level

as well as low E2 levels.

The fact that all patients underwent single cycle of IVF meant that subsequent cycle results were not available for comparison which is a limitation of the current study.

## Conclusion

EFS was not found to be a rare occurrence. It was associated with endometriosis, TB and decreased ovarian reserve. Healthcare professionals should educate and counsel infertile couples to consider IVF as early as possible.

**Disclaimer:** None.

**Conflict of Interest:** None.

**Funding disclosure:** This research was funded by the Deanship of Scientific Research at Princess Nourah bint Abdulrahman University through the Fast-track research funding program.

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