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The Prevalence of Genuine Empty Follicle Syndrome

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Abstract

Objective—To describe the prevalence of “genuine” empty follicle syndrome and “false” empty follicle syndrome at ART.

Design—Retrospective cohort.

Setting—Large private fertility center.

Patient(s)—12,359 patients who underwent ART between 2004 and 2009.

Intervention(s)—None.

Main Outcome Measure(s)—The failure to recover an oocyte during oocyte retrieval at ART, with and without a detectable serum β -hCG on day of retrieval.

Result(s)—Two cases of genuine empty follicle syndrome and 9 cases of false empty follicle syndrome were identified in the cohort examined. The prevalence of genuine empty follicle syndrome was 0.016% and the prevalence of false empty follicle syndrome was 0.072%. Only 2 out of 11 cases of EFS were considered genuine.

Conclusion—Genuine empty follicle syndrome is a rare occurrence. Because this syndrome tends to recur with dismal pregnancy rates at ART, continued identification and further investigation of the syndrome is needed.

Keywords

Empty follicle syndrome; oocyte maturation failure; follicle maturation; assisted reproduction; oocyte development; follicle development; HCG resistance; oocyte apoptosis

INTRODUCTION

The failure to recover any oocytes from mature-sized ovarian follicles during oocyte retrieval was first described 25 years ago by Coulam et al. (1) and is now known as “empty follicle syndrome” (EFS). The etiology remains enigmatic and the very existence of this

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syndrome has been questioned (2–4). The prevalence of EFS has been reported to vary between 0.6% and 7% (2,3,6,7). Cases of EFS can be subdivided into “false” (F-EFS) or “genuine” (G-EFS). False EFS is rigorously defined as instances where no oocytes are retrieved in the setting of undetectable serum or urine levels of β -hCG, suggesting either a human or pharmaceutical error. Genuine EFS is defined as cases where no oocytes are retrieved despite appropriate levels of β -hCG (7). In a review of EFS by Stevenson et al. (7), 33% of EFS cases were labeled as genuine and 67% as false. Regardless of the etiology, EFS is a condition that may cause substantial stress and anxiety for both the patient and physician during ART.

False EFS has been documented to result from errors of pharmaceutical preparation (8), provider error, or patient error (9). Zegers-Hochschild et al. (8) described a series of 6 consecutive EFS cases within a 48 hour period caused by accelerated metabolism and clearance of a pharmaceutical preparation of hCG. In addition, other reports have described patient and provider errors in administration of hCG during ART (4, 10–11). Common mistakes include failure to give the injection, improper timing of the injection, or improper re-suspension of hCG solute into solution. In such cases administration of hCG and retrieval 36 hours later has been suggested (12), but is often unsuccessful (13).

A subset of patients have appropriate serum β -hCG levels at time of oocyte retrieval and report receiving the medication at the appropriate time; but despite adequate follicle size and vigorous follicle aspiration, no oocytes are recovered. Furthermore, there are recurrent cases of EFS in the same patient (14–18), suggesting the existence of a problem other than hCG administration. Notably, patients with repeated EFS have a significantly reduced likelihood of pregnancy from 0% to 6.25% per cycle (15–18). Even more intriguing, instances of repeated GEFS have been reported in sisters (15), or in the setting of a chromosomal anomaly (19), suggesting a genetic basis for the syndrome. While a genetic etiology seems likely in some cases, the prevalence of the condition is reported to vary widely (3, 6, 7). The aim of this study was to investigate the prevalence of F-EFS and G-EFS in a large cohort.

MATERIALS AND METHODS

All ART cases (18,294 cycles in 12,359 patients) at a single fertility center from 2004 to 2009 comprised the study cohort for this retrospective review. The protocol was IRB-approved. In brief, patients underwent standardized stimulation protocols that used a combination of purified or recombinant FSH and hMG with either leuprolide acetate (Lupron) or a GnRH antagonist (Ganirelix Acetate), as described previously (20). HCG (either 250 mcg r-hCG, Ovidrel; or 10,000 IU hCG) was administered when at least one follicle was greater than 18mm in mean diameter on transvaginal ultrasound (TVUS). Thirty-six hours after hCG injection subjects underwent oocyte retrieval. Inclusion criteria consisted of cases where there was a failure to identify any oocytes after follicles were aspirated and flushed multiple times and the aspirate re-examined. Patients with fewer than 5 follicles with a diameter of at least 14mm on the day of hCG were excluded in order to minimize the likelihood that an absence of oocytes was caused by a poor response to ovarian stimulation, technical issues during retrieval, or the likelihood that chance alone could explain the absence of retrieved oocytes. If no oocytes were identified, a serum β -hCG level was obtained. Genuine EFS was defined as failure to retrieve oocytes in subjects with a serum β -HCG ≥ 5 mIU/ml at the time of attempted retrieval. False EFS was defined as failure to retrieve oocytes when a serum β -HCG level was < 5 mIU/ml. The prevalence of G-EFS was calculated as the number of patients with G-EFS divided by the total number of patients. Sample size was determined solely by the number of ART cycles between 2004 and 2009 and was not based on a power analysis. For the subjects that met inclusion criteria,

demographic information and cycle characteristics, including induction protocol, infertility diagnosis, and pertinent laboratory and TVUS data, were analyzed and described.

RESULTS

Within the cohort of 18,294 ART cycles, there was a failure to recover an oocyte in 46 subjects. Of these 46 patients, 13 were treated with Ovidrel and the remainder with purified hCG. Thirty-five of these subjects had fewer than 5 follicles measuring ≥ 14 mm visualized on TVUS, and therefore were excluded from further analysis. Of the remaining 11 subjects, there was no detectable serum β -HCG on the day of retrieval in 9 cycles (0.072%) and these cases were defined as F-EFS. Only 2 patients (0.016%) had a detectable serum β -HCG level and satisfied criteria for G-EFS (Figure 1).

The first patient with G-EFS was a 33-year-old woman with a body mass index (BMI) of 26 kg/m² diagnosed with male factor infertility and uterine fibroids (Table 1). The ART stimulation protocol consisted of a combination of rFSH (Follistim) and human menopausal gonadotropin (hMG, Menopur). Pituitary suppression was achieved using Ganirelix. The patient had 11 days of stimulation with a total dosage of 6225 IU of gonadotropin and produced 7 follicles ≥ 14 mm with a peak estradiol level of 1730 pg/ml. At retrieval thirty-six hours after 250 mcg hCG (Ovidrel) was administered, no oocytes were recovered despite vigorous flushing and re-aspiration of 5 follicles. A serum β -hCG level was 124 mIU/ml. Follicles in the opposite ovary were aspirated, but no oocytes were recovered.

The second patient with G-EFS was a 27-year-old woman with a BMI of 40 kg/m² and PCOS (Table 1). She underwent a luteal phase leuprolide pituitary suppression followed by a combination of follitropin alfa (Gonal-F) and hMG (Menopur) with 11 days of ovarian stimulation for a total dosage of 674 IU of gonadotropin. A peak estradiol level was 2340 pg/ml and the patient received 250 mcg of hCG (Ovidrel) 36 hours prior to retrieval. On the day of retrieval 9 follicles were larger than 14mm in average diameter. Ten large follicles were aspirated and no oocytes were recovered. A serum β -hCG level was 61 mIU/ml at retrieval. Neither patient has since reattempted ART.

DISCUSSION

This is one of the largest cohorts where the prevalence of both G-EFS and F-EFS has been rigorously examined. The prevalence of both categories of EFS were substantially lower than previously reported (2,3,6,7). A review of published reports suggested that approximately two thirds of the cases of F-EFS could be attributed to errors in hCG administration (7), however our study found a larger percentage of cases of EFS were attributed to F-EFS (82%) compared to G-EFS. Our findings suggest that G-EFS is an extremely rare phenomenon.

It is possible that the reduced prevalence of both G-EFS and F-EFS noted in this study resulted from our stringent inclusion and exclusion criteria. Patients with fewer than 5 follicles of 14mm were excluded to avoid incorrectly designating patients with a poor follicular response to ovarian stimulation as having EFS. Notably, technical issues or chance can certainly affect oocyte recovery, especially in cases with low follicle numbers, which is why we chose the more stringent minimum criteria of 5 follicles to be present on ultrasound. Studies suggest an oocyte may be expected to be recovered from a mature follicle in 80% of aspirations (21), overall. However, our experience is that the failure to retrieve an oocyte is a function of the number of oocytes present. In our cohort, ovum recovery occurred in 87.5%, 91.5%, 97.5% and 98.5% in patients with 1, 2, 3 and 4 follicles, respectively.

Based on the suggestion by van Heusden et al. (3) that chance may explain why no oocyte would be retrieved (likelihood of failed oocyte retrieval= $[0.2]^n$; where n=number of follicles), we calculated the likelihood of failing to retrieve an oocyte due to chance alone for the cases of G-EFS reported here to be 0.001% and 0.00005%, for 7 and 9 follicles respectively. The prevalence of G-EFS we detected was substantially greater (>16 fold) than might be expected due to chance alone; therefore, we interpret the findings to support the existence of genuine EFS. We have anecdotally observed, albeit not in the current cohort, that genuine EFS can be present when >20 follicles are present on ultrasound, strongly suggesting that chance alone is not a satisfactory explanation for the phenomenon (based on $\text{above}=1.04 \times 10^{-14}$).

Our criteria of follicle size >14 mm. was also chosen to avoid a possible false positive diagnosis of EFS. Follicles of 14 mm. in size correspond to a volume of 1.4 ml. A recent study of 58 patients indicated that follicular volumes > 0.6ml are highly associated with the number of oocytes retrieved (22). Another study determined that 12 mm was adequate for oocyte recovery (23). Thus, our criterion of 14 mm. is a conservative benchmark that should reduce the likelihood of a failure in oocyte retrieval due to inadequate follicle size and inadequate oocyte maturity.

Several possible causes of genuine EFS have been proposed. Some reports propose an error in folliculogenesis or premature apoptosis of the oocytes, yet continued follicular growth (24, 25). Other investigators suggest that in rare cases follicles may need longer exposure to hCG to undergo cumulus expansion and separate from the follicular wall (26, 27). It is worth noting that EFS has been reported with immature oocytes that were zona-free (28) or zona that were lacking in oocytes (25), suggesting abnormal oocytes may cause some cases.

Another possibility is a yet-to-be discovered genetic cause of the syndrome. Possible candidate genes include genes shown in studies of knock-out mice to be required for folliculogenesis and fertility (29). LH-dependent cumulus expansion requires communication between the mural granulosa cells and the cumulus complex involving amphiregulin, epiregulin, and beta-cellulin (30–33). These growth factors induce expression of prostaglandin synthase 2 (*Ptgs2*), tumor necrosis factor alpha-induced protein (*Tnfaip6*), and hyaluronan synthase 2 (*Has2*), which in turn are necessary for cumulus expansion, and subsequent oocyte release. It remains to be determined whether G-EFS is accompanied by altered expression of genes regulating cumulus expansion, but studies in mice support the possibility of such a mechanism (30,31).

In summary, we have identified two cases of G-EFS in a cohort of 12,359 patients, supporting the conclusion that G-EFS is in fact a rare disease. However, G-EFS is a syndrome that deserves additional study because such investigation could lead to a further understanding of ovarian biology and infertility.

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Figure 1.
Flow diagram of patients who underwent ART with no oocytes retrieved

Table 1

Clinical characteristics of patients with G-EFS

	Patient 1	Patient 2
Infertility Etiology	Male Factor	PCOS
Age	33	27
Days of Stimulation	11	11
Total Medication Usage (IU)	6225	674
Peak Estradiol (pg/ml)	1730	2340
β -hCG on Day of Retrieval (mIU/ml)	124	61
Repeat ART Cycle	No	No

Note: G-EFS = genuine empty follicle syndrome