

## OOCYTES DERIVED BY MILD IVF-IVM AFTER REPEATED EMPTY FOLLICLE SYNDROME: A CASE REPORT

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### ABSTRACT

Empty follicle syndrome (EFS) is one of the most disappointing events in assisted reproductive technology that cannot be predicted before retrieval of the oocytes. The case presented here is of a 27-year-old woman with polycystic ovary syndrome whose husband had azospermia. She had experienced EFS in previous in vitro fertilization attempts and came to our clinic for a new approach. Microsurgical testicular sperm extraction with sperm freezing was offered to her husband first, and then the patient underwent an antagonist cycle with good follicular growth. However, neither oocytes nor cumulus cells in the follicular fluid were collected. Repeated genuine EFS was diagnosed and in vitro maturation (IVM) with follicle-stimulating hormone priming was planned for the next cycle. Six germinal-vesicle oocytes were collected; 4 matured and were injected with thawed sperm. A single embryo was transferred, but the beta-human chorionic gonadotropin (beta-hCG) test was negative. To our knowledge, this is the first case to obtain oocytes by IVM, and this treatment could be a promising choice in repeated genuine EFS cases.

### INTRODUCTION

Empty follicle syndrome (EFS) is an uncommon complication of in vitro fertilization (IVF) treatment, with a prevalence ranging from 0.045% to 7%.<sup>1-4</sup> The existence of EFS is still under debate, and whether it is a cause or a result of infertility is as yet unclear. Most cases of EFS are due to insufficiency of human chorionic gonadotropin (hCG) for triggering, with fewer cases considered to be genuine EFS.

The case presented here is one of genuine EFS—the patient experienced recurrent EFS in stimulated IVF cycles. With the decision to attempt in vitro maturation (IVM) and the couple's consent, a mild IVF-IVM program was planned and 6 germinal-vesicle (GV) oocytes were collected, 4 of which were injected with thawed sperm by intracytoplasmic sperm injection (ICSI) performed 30 hours after oocyte pickup (OPU) following IVM. One 4-cell embryo with grade 1 morphology was transferred, but the pregnancy test was negative on the 12th day.

### CASE

A 27-year-old woman whose husband had male infertility due to nonobstructive azospermia (NOA) came to our clinic because she had previously experienced EFS. Microsurgical testicular sperm extraction (microTESE) with sperm freezing was offered to her husband before we attempted IVF. After sperm cryopreservation, an antagonist IVF cycle was administered but neither

oocytes nor cumulus cell mass was retrieved. The couple was informed about the EFS outcome. Mild IVF-IVM was recommended as an alternative to the stimulated cycle, and this treatment modality was accepted. The patient had no history of health problems, but her husband had a history of type 2 diabetes mellitus together with NOA. Laboratory and blood analyses of the couple were normal. It was unfortunate that her husband had azospermia and that the microTESE-derived frozen sperms used for ICSI were found to have poor morphology and motility, which may impact clinical outcome.

The patient was evaluated on day 3 by transvaginal ultrasound, and a follicle-stimulating hormone priming cycle was started with follitropin alfa for injection (Gonalf, EMD Serono, Geneva, Switzerland) 75 IU subcutaneously for 3 days together with estrogen on day 3 for endometrial thickening. On day 8, she was evaluated by ultrasound, which revealed endometrial thickness of 9.2 mm with follicles less than 12 mm in size. hCG priming with choriogonadotropin alfa (Ovitrelle, EMD Serono) 250 µg subcutaneously was given on day 8, and 36 hours later, OPU was performed. One oocyte from the left ovary and 5 oocytes from the right ovary were collected and placed into the IVM medium for 28 to 30 hours; 4 of 6 GV oocytes (66% maturation) were found to be mature. Thawed sperms were injected by ICSI at 30 hours, and only 1 fertilized oocyte and 2 pronuclear oocytes (25% fertilization rate) were observed

the next morning. On day 12, the 4-cell, grade 1 embryo was transferred. The IVM protocol is well accepted and used widely in indicated cases. Although the beta-hCG test was negative, the couple was hopeful and decided to repeat the treatment because they still had frozen sperm in the laboratory.

## DISCUSSION

EFS is a condition in which no oocytes can be obtained from the follicular fluid of properly stimulated IVF patients. It is quite rare, and the etiology is unknown. Two types have been defined according to hCG levels, genuine and false, and the existence of the genuine type is a matter of debate. No single treatment option is available, but the improper administration of hCG, which may cause EFS, can be corrected. EFS needs to be further researched in regard to oocyte maturation and ovarian biology.

EFS was first described by Coulam et al, as a condition of no oocytes in apparently normal growing follicles of stimulated ovaries with meticulous follicular aspiration.<sup>5</sup> The genuine type has been defined as failed retrieval in case of appropriate hCG levels, whereas the false type has been defined as a low level of hCG (<40 IU/L) due to misadministration or low bioavailability of medication.<sup>6</sup> EFS is a rare complication of IVF that cannot be anticipated before the OPU procedure. Occurrence has been estimated to be 0.0045% to 7% of patients undergoing OPU.<sup>3</sup> Aktas et al found 25 cases among 3060 cycles, with a prevalence of 0.81%.<sup>1</sup> Reichman et al estimated false group incidence at 0.045%.<sup>2</sup> Mesen et al evaluated the genuine and false types of EFS separately and found 0.016% to be genuine and 0.072% to be false among a total of 18,294 cycles.<sup>4</sup>

Castillo et al reported an incidence of EFS of 3.5% among 2034 oocyte donor cycles and 3.1% among 1433 IVF cycles performed between years 2009 and 2010 was retrospectively analyzed to identify cases of EFS in each group.<sup>7</sup> That study also reported that the triggering method does not significantly change the outcome.

In a case report by Vutyavanich et al, follicular fluids were filtrated at the stimulated cycle after EFS, and immature oocytes were collected and matured in vitro.<sup>8</sup>

The most common underlying mechanism in the false group has been shown to be inefficient hCG blood levels. Defects in manufacturing, rapid plasma clearance of hCG, and misuse by the patient also have been suggested as causes<sup>3,6</sup> as well as early oocyte atresia in continued follicular growth.<sup>4,7</sup>

Inan et al analyzed whole gene expression of granulosa cells from a 22-year-old patient with recurrent EFS and found a total of 160 differently expressed genes.<sup>9</sup> According to the investigators, the absence of oocytes may have been due to “the increased apoptotic gene expression and the reduction of transcripts whose products are responsible for healthy follicular growth.” In another case, the presence of oocytes and their apoptosis was proposed to be due to the presence of thin zona pellucida of 200 preantral follicles in the follicular aspirates.<sup>10</sup> Ovarian aging has also been suggested to have a significant role in genuine EFS.<sup>9</sup> Other investigators have considered low ovarian reserve as the cause.<sup>11</sup>

In addition, genetic causes of EFS have also been proposed. Onalan et al reported on a possible inherited condition of EFS with moderate sensorineural deafness affecting 2 sisters.<sup>12</sup> Any alteration that changes the transient and sequential expression of epidermal growth factor in family members might affect the oocyte growth in follicles, owing to impaired cumulus expansion and oocyte release.

Although some believe there is no recurrence of EFS in subsequent treatments, it has been shown that among patients with EFS, recurrent EFSs occurred in 15.8% of subsequent cycles.<sup>13</sup>

## CONCLUSIONS

In light of the literature, this case appears to be the first to attempt to manage EFS with a mild IVF-IVM modality. Selection of this mode of treatment is compatible with the etiopathogenesis of EFS. If the oocytes were present but failed to mature in the follicles during stimulation, it may be more effective to remove the immature oocytes and apply the maturation process in vitro. Although the beta-hCG was negative, this case could lead to an alternative approach to genuine EFS and may encourage investigation of the underlying reasons for this condition.

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