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Research Article

Empty Follicle Syndrome-Oocyte Could be Retrieved in Consecutive Cycle

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Abstract

Empty follicle syndrome (EFS), reported in patients undergoing IVF treatment with no oocytes is obtained after ovarian stimulation. The publications regarding EFS are limited and there is no consensus of its etiology. The aim of our study was to characterize the cycles with EFS and evaluate the possible reasons for its occurrence. The incidence of EFS in our cohort was 4.9% per cycle or 6.2% per patient. On the day of hCG administration the mean E2 and P4 levels were 1856 ± 1154 pg/ml and 0.98 ± 0.87 ng/ml, respectively, in our patients (control group) as compared to 610 ± 178 pg/ml ($p<0.05$) and 0.7 ± 0.4 ng/ml, respectively, for cycles with EFS. The mean follicle number per patient in the control group was 6.4 ± 4.1; however, the mean follicle number in patients with no oocytes in the follicular fluid (FF) was 2.5 ± 2.2. In the EFS group the measured level of AMH was 0.5 ± 0.3. Double dose of rec-hCG in consecutive cycles of the EFS group, using a similar COH protocol or changed COH regimen with regular dose of rec-hCG, resulted in oocyte aspiration in most cases with previous EFS cycles. From 150 oocytes retrieved in the 34 successful consecutive cycles of the EFS group, 37% of oocytes were immature (MI stage) and 63% mature (M II) oocytes, the same percentage as was found in our control sub-group (women over 35 years old). We suggest that EFS may be the result of a delayed maturation of oocyte-cumulus complexes. The cases of EFS are sporadic and, in our opinion, can't be defined as a syndrome. We suggest the use of a different name for EFS, such as "Failure of Oocyte Retrieval" (FOR).

Keywords: Empty follicle syndrome (EFS); Ovarian failure; Oocyte maturation; Control ovarian hyperstimulation (COH)

Introduction

Empty follicle syndrome (EFS) is defined as a condition in which no oocytes are obtained after successful ovarian stimulation [1,2]. It could be frustrating, either for the couple and/or for the clinical staff involved. The incidence of EFS has been estimated in a range of 0.6%-7% [3,4]. Some authors suggested that EFS is occurs due to a technical failure during oocyte aspiration, or inappropriate injection of hCG. Others suggested that EFS occurs due to dysfunctional folliculogenesis [5].

Empty follicle syndrome was first reported by Coulam et al. [6] in 4 patients with unexplained infertility. The authors suggested that EFS might represent an unknown, as yet, explanation of infertility. The EFS cases were reported [7-10] and described in reviews [2,11]. In a systematic review, Stevenson and Lashen [2] classified EFS into true and false EFS. False EFS was defined as a failure to retrieve oocytes in the presence of low hCG level.

Possible etiologies include inappropriate administration of hCG, defects in the biological preparations of hCG [12,13] or individual variation in the bioavailability and metabolism of hCG [1]. True EFS was defined as unsuccessful oocyte retrieval, after apparently normal follicular development, with optimal hCG levels on the day of oocyte retrieval [2], an extremely rare phenomenon [14]. Some authors suggest that genuine EFS do not exist and "Empty Follicle" is obtained due to some technical difficulties during oocyte aspiration [15,16]. Therefore, the existence of follicles without oocytes is an obscure phenomenon and under debate. The publications about EFS are limited and there is no consensus of the EFS etiology. The aim of our study was to characterize the cycles with EFS in our unit and evaluate the possible reasons for its occurrence.

Methods

Patients

This retrospective study, during two year period (2014-2015), included 856 ART cycles performed in 556 patients (mean age of 34.4 ± 6.2 years old; number of patients is 245<35 and 311>35 years old) in our IVF unit. Among the 42 cycles, done in 35 patients (mean age of 38.8 ± 5.0 years old, number of patients is 8<35 and 27>35 years old), the oocyte pick-up (OPU) procedure ended with EFS. According to this, all patients were divided into two groups: control group - patients with oocytes retrieved after (OPU) (n=556) and EFS group – patients with no oocytes retrieved after OPU (n=35). Patient's demographic data, method of control ovarian hyperstimulation (COH) protocols, parameters of ovarian response, dosage of ovulation triggering, as well as the outcome of previous and consecutive cycles were analyzed. Patients with an inappropriate use of hCG were excluded.

Ovarian stimulation and OPU

Controlled ovarian stimulation was performed by using routine GnRH agonist or antagonist individualized COH protocol. To trigger the final oocyte maturation, rec-hCG was injected in a dose of 250 IU. The administration of this dose of rec-hCG was performed when at least two follicles reached a diameter of 18 mm. In some cases, due to EFS incidents occurrence, a double dose of rec-hCG was administered. Ultrasound-guided oocyte retrieval was performed at 34-36 h after hCG administration. The exact time interval between hCG administration and OPU was agreed upon with the patients before performing the OPU. After general anesthesia (fentanyl 50-100 μg/I.V; propofol 100-300 mg/I.V.), under ultrasound guidance, a needle was inserted through the vaginal wall into an ovarian follicle. The other end of the needle was attached to a suction device. Once the follicle was entered, suction was gently applied to aspirate follicular fluid. Immediately after follicular

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puncture, the follicular fluid was transferred to the IVF laboratory for microscopic examination. The retrieved oocytes were subsequently fertilized by ICSI. Endometrial thickness was measured by vaginal ultrasound (ClearVue 350, Philips) on day of hCG administration.

Statistics

Data are presented as mean ± SD and frequencies, presented as percentage. Statistical analysis was performed using the χ^2 test for the comparison of the groups' outcome variables and other categorical variables, and Student's two-sided *t* test, for continuous variables. ANOVA was used to estimate differences of means between the groups. A p-value of <0.05 was considered statistically significant. The data were analyzed using SPSS (version 21, SPSS, Inc.).

Results

The incidence of EFS in our cohort was 4.9% per cycle (42/856) or 6.2% per patient (35/556 cases), Table 1. In most of these cycles (65%) antagonist protocol was used. The baseline (day 3 of the cycle) of the hormonal profile in the EFS group was 7.2 ± 2.7 IU/ml and 5.3 ± 3.7 IU/ml for FSH and LH, respectively, and 38.3 ± 29.3 pg/ml and 0.8±1.4 ng/ml for E2 and P4, respectively, in the profile of control patients (Table 1).

In the EFS group, 35 out of 42 cycles were the first treatment cycle, and 7 out of 42 were repetitive cycles. On the day of hCG administration, mean estradiol levels were 1856 ± 1154 pg/ml in the control group in contrast to 610 ± 178 pg/ml (p<0.05) in the EFS group. Mean progesterone levels were 0.98 ± 0.87 ng/ml in the control group, as compared to 0.7 ± 0.4 ng/ml in the EFS group (Table 1). Eight patients in the EFS group underwent at least one cycle of IVF previously, resulting in the recovery of oocytes with 2 ± 1 oocytes [range 1-4 oocytes] from which mature oocytes were 1 ± 1 (Table 2).

Endometrial thickness in the control group was 10.6 ± 2.5 mm, as compared to 9.2 ± 2.9 mm in the EFS group (Table 1). The mean follicle number in the control group was 6.4 ± 4.1, with 7.6 ± 6.1 aspirated oocytes and 5.0 ± 4.3 mature oocytes. The mean follicle number in the EFS group was 2.5 ± 2.2 (Table 1). Cumulus cells were observed in aspirated follicular fluids (FF) from the EFS group, however no oocytes were found under microscopic examination. In the EFS group the level of AMH, as a marker of ovarian reserve, was lower than 1 (0.5 ± 0.3; Table 1).

In 41 repeated cycles EFS was recurred only in 6 cycles (14%, 3 cases out of 34). Use of a double dose of rec-hCG in 20 consecutive cycles with the same COH protocol resulted in oocyte aspiration in most cases, while EFS was recurred in only 5 out of 20 cycles (2 patients). In 14 consecutive cycles with aspirated oocytes (EFS group), the COH regimen was changed (Table 3). From 150 oocytes retrieved in the 34 successful consecutive cycles, 55 oocytes (37%) were immature (stage M I), (Table 3 and Figure 1) and the maturity (stage M II) was 63%. In contrast to the EFS group, mature (M II) oocytes in the control cohort was 83.2%; however, in aged women of the control group (subgroup over 35 years old) the maturity of oocytes was 65.5%, which was statistically different from the younger women in the control group (subgroup <35 years old) patients (p<0.008, Table 3).

	Control group	EFS group	Statistical significance
n	556	35 (6.2%)	
Age	34.4 ± 6.2	38.8 ± 5.0	n.s.
FSH (IU/ml) [baseline]	7.0 ± 3.1	7.2 ± 2.7	n.s.
LH (IU/ml) [baseline]	4.9 ± 3.6	5.3 ± 3.7	n.s.
E2 (pg/ml) [baseline]	32.1 ± 26.2	38.3 ± 29.3	n.s.
P4 (ng/ml) [baseline]	0.6 ± 0.5	0.8 ± 1.6	n.s.
E2 (pg/ml) [day of ovulation trigger]	1856 ± 1154	610 ± 178	p<0.05
P4 (ng/ml) [day of ovulation trigger]	0.98 ± 0.87	0.7 ± 0.4	n.s.
AMH	-	0.5 ± 0.3	
Follicle number	6.4 ± 4.1	2.5 ± 2.2	p<0.05
Aspirated oocytes	7.6 ± 6.1	0	
Mature oocytes	5.0 ± 4.3	-	
Endometrium (mm)	10.6 ± 2.5	9.2 ± 2.9	n.s.

Table 1: Association between patient characteristics in Control and EFS groups.

EFS group (n)	EFS patients with oocytes in previous cycle (n)	Number of oocytes in previous cycle	Number of mature oocytes in previous cycle
35	8	2 ± 1	1 ± 1

Table 2: Ovarian reserve characteristics of women in cycle prior to EFS.

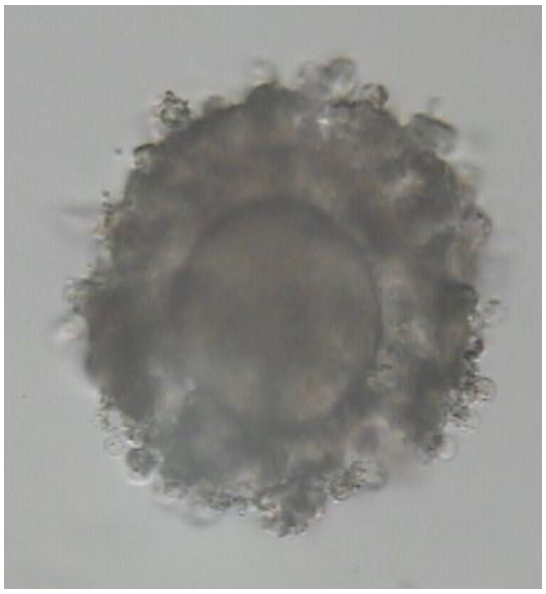

Figure 1: The immature oocyte with tightly compact cumulus cells, accepted at 3rd treatment cycle after 2 previous cycles with follicles (by US) and EFS.

	EFS group (age>25)	Consecutive cycle (age>25)	Control subgroup (age<35)	Control subgroup (age>35)
Cycles	42	41	479	377
Oocytes	0	150	4850	2187
M II oocytes	-	63%	83.2%	65.5% (p<0.008)
Immature oocytes	-	37%	17.8%	34.5%
Double dose hCG		20 cycles		
Change of COH regiment		14 cycles		

Table 3: Oocytes in consecutive cycle of EFS group compared to control.

Discussion

EFS has been reported in patients undergoing COH for ART. It was noted that some patients with EFS had a history of poor response to ovarian stimulation, implying that ovarian dysfunction could play a role [17]. Our findings suggest that most cases of EFS are observed in patients with a diminished ovarian reserve. Low levels of AMH in our EFS cohort indicate decreased ovarian function and/or low ovarian reserve. In our study a high incidence of EFS could be explained by the patient's cohort, 56% of which consists in women, whose age was older than 35 years. This observation has been supported by previous reports associating EFS to female age [18,19]. A high incidence of immature oocytes retrieved in the consecutive cycle suggests that EFS may result from a delayed maturation of oocyte-cumulus complexes. One of the possible solutions to avoid EFS is applying a different COH regimen and/or triggering of ovulation, by an enhanced dose of hCG in the subsequent cycle, as proposed previously by Reichman et al. [20].

Can a follicle be empty of an oocyte? In the growing follicle, the presence of an oocyte is obligatory and a situation of genuine EFS can't exist. It has been shown by a tight support between different cell types in the follicle. Cooperation between the oocyte and follicular cells in the growing follicle is extremely important. The oocyte regulates cumulus cell functions by agents such as GDF9, BMP15 and others [21]. However, cumulus cells coordinate oocyte development and maturation, provide energy substrate for oocyte meiosis resumption, regulate oocyte transcription, and promote nuclear and cytoplasmic maturation of the oocyte [22-26].

Different ovarian factors originating from oocyte and/or follicular cells are involved in firm oocyte-follicle interaction and development. For example, deletion of Pdk1 in mouse oocytes damages the survival of primordial follicles [27]. Deletion of *Pten* in oocytes leads to enhanced activation of primordial follicles, indicating that an intra-oocyte PI3K pathway is needed for follicular activation [28]. Communication between oocyte and surrounding follicular cells could be reached via gap junctions of connexin (C×43), which is under the control of LH. Interruption in this communication could prevent maturation of the oocyte. It has been shown that preovulatory LH surge changes the status of these junctions by a phosphorylation state of the C×43 protein (gap close/open), as well as by a later response which is manifested by a reduction of C×43 protein concentration [29]. This action of C×43 could play an important role in female fertility, as recurrent EFS was demonstrated in 3 stimulated cycles in 2 sisters with an abnormality of C×43 protein. They suffered from a congenital hearing loss, which also resulted from an abnormal function of connexin [30]. An additional possible factor for EFS could be mutation in LH receptor. It was observed that a substitution of aspargine by serine in the LH receptor impaired follicular function and explains the lack of response of the two sisters with EFS to the repeated administration of β-hCG [31].

Oocyte-cumulus cells cross-talk is extremely important for the follicle, as well as oocyte development and competence. For this reason, unsuccessful oocyte aspiration is not evidence for the real empty follicle. Our results revealed that successful oocyte aspiration in such cases may be achieved by recruiting a different COH regimen and augmentation of the rec-LH dosage. In conclusion, our belief is that the definition of this entity "Empty Follicle Syndrome" (EFS) is not correct. In our opinion, the more appropriate definition is a sporadic lack of luck in the oocyte's maturational process, resulting in "Failure of Oocyte Retrieval" (FOR).

Conclusion

In conclusion, our results revealed that successful oocyte aspiration in such cases may be achieved by recruiting a different COH regimen and augmentation of the rec-LH dosage.

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