Follicular activation in women previously diagnosed with poor ovarian response: a randomized, controlled trial

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Objective: To investigate whether ovarian fragmentation for follicular activation (OFFA) improves ovarian reserve markers and in vitro fertilization (IVF) outcomes in women with poor ovarian response (POR).
Design: Randomized, controlled trial, with parallel assignment.
Setting: University hospital.
Patient(s): Thirty-four women with POR according to the European Society of Human Reproduction and Embryology criteria.
Intervention(s): Women with POR were randomly allocated to receive ovarian fragmentation in 1 ovary or to no intervention (control group). Ovarian reserve markers were followed at 2-week intervals for 6 months. In vitro fertilization cycles were initiated when the antral follicle count (AFC) doubled or at the end of follow-up.
Main Outcome Measure(s): The primary outcome was the number of metaphase II (MII) oocytes obtained. Antral follicle count, antimüllerian hormone level, and reproductive outcomes were recorded as secondary outcomes. Exploratory outcomes included surgical results and analysis of protein and gene expression.
Result(s): Ovarian fragmentation for follicular activation resulted in an increase in AFC in the intervention ovary compared with the control ovary and an increase in total AFC in the OFFA group compared with controls. Serum antimüllerian hormone and follicle-stimulating–hormone levels did not improve in the OFFA group throughout the follow-up period. Fifteen patients from each arm underwent IVF. In the control group, 33 MII oocytes were retrieved and 18 embryo transfers were performed, with a 20% pregnancy rate and an 18.7% live birth rate per cycle. In the OFFA group, 23 MII oocytes were retrieved and 11 embryo transfers were performed, with a 13.3% pregnancy rate and a 6.7% live birth rate per cycle. Reproductive outcomes did not significantly differ between the groups. Hippo pathway inhibition was confirmed by an 18.8% reduction in the phospho-YAP/YAP (Yes-associated protein 1) ratio and *BIRC* and *CCN* overexpression after fragmentation.
Conclusion(s): Ovarian fragmentation for follicular activation in women with POR resulted in an increase in AFC but did not modify IVF outcomes when compared with controls.

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Authors' roles: C.D.-G. was responsible for the design of the experiment, surgical procedures, evaluation of the results, and writing of the article. L.P. and J.S. participated in the follow-up of the patients. S.H. participated in the histologic evaluation of the samples, processing and fragmentation of the ovarian cortex, data analysis, and writing of the article. M.J.S. participated in the analysis of gene and protein expression. A.P. and C.S. participated in the design of the study and analysis of the results. E.S. participated in analysis of the data and writing of the manuscript. All authors participated in revision of the manuscript.
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El resumen está disponible en Español al final del artículo.

Key Words: Follicular activation, low ovarian reserve, ovarian fragmentation, poor ovarian response, premature ovarian insufficiency

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Ovarian aging is playing an increasingly important role in infertility and is associated with a decrease in the ovarian follicular pool and impaired oocyte quality. During the reproductive life of a woman, a number of dormant primordial follicles are activated during each menstrual cycle. From each follicular wave, 1 follicle will be ovulated while the rest will undergo atresia (1). Ovarian reserve declines throughout the reproductive lifespan, and a smaller follicular pool is associated with a lower number of antral follicles available in each cycle (2).

In women undergoing infertility treatment with in vitro fertilization (IVF), response to controlled ovarian stimulation (COS) is considered an important determinant of outcome. Women who demonstrate (or are expected to demonstrate) a lower-than-expected response to COS are grouped under the diagnostic term of poor ovarian response (POR) or diminished ovarian reserve (DOR). Several attempts have been made to standardize the definition of POR/DOR. The most commonly used diagnostic algorithm has been proposed as the European Society of Human Reproduction and Embryology (ESHRE) Bologna criteria (3). More recently, an alternative classification was proposed by the Patient Oriented Strategies Encompassing IndividualizeD Oocyte Number (POSEIDON) group, taking into account the woman's age, antral follicle count (AFC), serum antimüllerian hormone (AMH) levels, and past response to COS (4). Poor ovarian response/DOR should be distinguished from premature ovarian insufficiency (POI), which is defined by ESHRE as the presence of oligomenorrhea or amenorrhea for at least 4 months, together with a serum follicle-stimulating–hormone (FSH) level >25 IU/L on 2 separate occasions in women younger than 40 years (5).

Independently of the diagnostic criteria that are used, POR/DOR seems to be an increasingly more common diagnosis for women undergoing IVF. Indeed, in the United States, the prevalence of the diagnosis of DOR in women undergoing IVF has increased by almost 3 times in 10 years, from 12% of all cycles (n = 16,111) in 2005 to 31% (n = 81,709) in 2016 (6). Equally important are the lower success rates achieved with IVF in patients with a diagnosis of DOR. In 2016, the live birth rates after the first transfer per intended retrieval in women <35, 35–37, and 38–40 years old who were undergoing IVF with their own eggs were 39.7%, 29.1%, and 19.5% for women with all diagnoses and 25.4%, 20.6%, and 15.1% for women with DOR, respectively. Consequently, women with POR/DOR are more likely to search for alternative treatment modalities. Within the past decade, a number of experimental treatment options aimed at activating the limited number of available follicles in the ovary have been tested in women with POR as well as those with POI (7, 8).

In 2013, a study by Kawamura et al. (9) described for the first time a technique called in vitro activation (IVA). The investigators used mechanical fragmentation of the ovarian tissue to activate the pool of dormant follicles remaining in the ovaries of women with POI (9). Tissue fragmentation increases actin polymerization, leading to an interruption in intracellular Hippo signaling (10). The Hippo signaling pathway is responsible for maintaining the proper size of organs (11). Disruption of the Hippo pathway leads to increased cell proliferation and decreased apoptosis by decreasing the degree of phosphorylation of Yes-associated protein 1 (YAP). Migration of phosphorylated YAP to the nucleus promotes the expression of *CCNs* (growth promoters) and *BIRCs* (apoptosis inhibitors) and promotes the activation of the primordial follicles (9). In addition to the fragmentation of ovarian tissue to achieve Hippo pathway inhibition, Kawamura et al. (9, 12) incubated ovarian tissue for 48 hours with phosphatase and tensin homolog (PTEN) inhibitors and Akt-stimulating molecules to further promote follicular growth.

Since the first publication describing this technique, a number of case series and cohort studies using the same approach reported varying degrees of success in women with POR and POI (9, 13, 14). More recently, an alternative approach using ovarian fragmentation for follicular activation (OFFA) without PTEN inhibitors or Akt-stimulating molecules (also called drug-free IVA) has been reported (15–19). This drug-free approach is more readily applicable to clinical practice because of the known carcinogenic effects of PTEN inhibitors (20). Despite encouraging results with the use of OFFA and IVA in early studies, the efficacy of these interventions has not yet been tested in a randomized controlled trial (RCT) with a nonintervention control group.

The aim of our study was to determine whether OFFA increases the number of antral follicles and subsequently increases the number of metaphase II (MII) oocytes collected after ovarian stimulation and also to determine the reproductive outcomes of women with POR undergoing IVF. We therefore designed a RCT with an intervention group in which 1 ovary underwent cortical removal and fragmentation and a control group did not undergo surgery.

MATERIALS AND METHODS

Study Design and Outcome Variables

Women with a diagnosis of POR on the basis of the ESHRE Bologna criteria (3) who were younger than 40 years were randomly allocated to undergo OFFA or no intervention (parallel arms design). In the intervention group, only 1 of the ovaries underwent OFFA, and the other ovary remained intact

as an internal control. The women in both arms of the study were followed for 6 months, and the ovarian reserve biomarkers AMH and AFC were monitored. In vitro fertilization cycles were initiated when the number of antral follicles doubled or at the end of the follow-up period if it did not.

Patients

All patients were recruited between March 2016 and February 2019 at La Fe University Hospital IVF Unit, Valencia, Spain.

Inclusion criteria. Patients were included in the study if they met at least 1 of the following ESHRE criteria of POR (3): at least 2 episodes of POR ($\leq$3 oocytes retrieved) with a standard protocol; a previous IVF cycle with $\leq$3 oocytes retrieved (after a standard stimulation protocol); or the presence of an abnormal ovarian reserve test: AFC $\leq$5 or AMH $\leq$5 pM (0.7 ng/mL).

Exclusion criteria. Patients were excluded if at least 1 of the following characteristics was present: age over 39 years, clinical signs of endometriosis, history of ovarian surgery, genital tract malformations not suitable for correction, partner with severe male factor (total motile sperm <5 million), contraindications for laparoscopic surgery, and desire to use donated eggs.

Ethical Approval

All patients signed informed consent before inclusion. The study was approved by the Ethical Review Board of La Fe University Hospital (no. 2014/0004), and the protocol was registered in clinicaltrials.gov (NCT02354963).

Allocation, Blinding, and Power Analysis

A randomly generated 1:1 assignment list was created using the software provided by www.random.org. The sequence was not accessible to the researchers participating in the study. The patients were allocated to the study groups through a phone call from a dedicated contact center. The sample size was estimated using the mean and SD of the number of MII oocytes obtained from patients with a diagnosis of POR treated in our unit as a reference (2.90 SD 2.54). To demonstrate differences of at least twice the number of oocytes and on the basis of an alpha error of 5% and a beta error of 10%, the number of patients to be randomized would be 32. Assuming a 15% loss, the number of patients to include in the study would be 36.

Ovarian Fragmentation for Follicular Activation Surgery

Patients allocated to the OFFA group underwent laparoscopy to retrieve a 1- to 2-cm^2 ovarian cortical biopsy specimen from the posterior side of the intervention ovary, using cold scissors and avoiding the use of electrocautery to achieve hemostasis. After retrieval, the ovarian medulla was removed with the use of the cold scalpel friction method (21) in M199 media (Sigma, St. Louis, MI) at 4°C, and the ovarian cortex was fragmented into small pieces of approximately 1 mm^3. The tissue fragments were then placed in a Gynetics Probet endometrial catheter (Gynetics, Lommel, Belgium) and handed to the surgeon.

Subcortical pockets were developed for tissue reimplantation by blunt dissection on the anterior side of the same ovary by creating a tunnel between the remaining cortex and the medulla. Each pocket was filled with tissue fragments using the plunge of the Probet catheter. The tunnel was closed with a single suture, using Monosyn 4-0 (Braun Surgical SA, Rubi, Spain) by intracorporeal knot tying.

The ovary with lower AFC at recruitment was established as the intervention ovary in both study arms, and the ovary with higher AFC was the control ovary for the duration of the study.

Postoperative Follow-Up and COS

After the surgical procedure, or after randomization to the control group, all patients started the follow-up phase of the study, consisting of hormonal analysis (AMH, FSH, and estradiol) and AFC determination every 2 weeks, as described in the Supplemental Methods (available online). If the number of antral follicles increased to at least twice the number at inclusion, COS was started. When such an increase was not observed, ovarian stimulation was started after the completion of the follow-up period, regardless of the AFC count. A short protocol with 300 IU/d of menotropin and cetrorelix acetate was used for the stimulation. More detailed information can be found in the Supplemental Methods.

Assessment of Follicular Density and Disruption of the Hippo Pathway

Ovarian biopsies were kept for calculating follicular density. The degree of disruption of the Hippo pathway was measured by assessing the degree of phosphorylation of YAP and the gene expression profile of the target genes of YAP. The method has been previously described (22, 23), and a full description can be found in the Supplemental Methods.

Primary and Secondary Outcomes

The primary outcome was the number of MII oocytes retrieved. The secondary outcomes included the AFC and AMH levels and the laboratory and clinical outcomes after IVF: fertilization rate, number of embryos obtained, canceled cycles, cycles with and without embryo transfer (ET), implantation rate, pregnancy rate, and live birth rate. Other exploratory outcomes included surgical variables (size of the retrieved ovarian cortex, number of fragments reimplanted, and presence of follicles at histologic analysis) and the degree of inhibition of the Hippo pathway (phosphorylated YAP and YAP protein expression were assessed by Western blotting, and *BIRC* and *CCN* gene expression were assessed by real-time quantitative polymerase chain reaction).

Statistical Analysis

Variables with normal distribution were expressed as means and standard deviations, and nonnormal variables were expressed as medians and ranks. Categorical variables were expressed as absolute values and percentages. Differences were considered statistically significant if $P<.05$. Quantitative

variables were compared by Student's *t*-test or the Mann-Whitney *U* test, as appropriate. Paired statistics were compared by the Wilcoxon rank test. Categorical variables were compared by the chi-squared or Fisher's exact test, as appropriate. Statistical analysis was performed with the statistical package Statistical Package for the Social Sciences (SPSS) 20.0.

RESULTS

Study Population

A total of 382 infertile women were screened for inclusion in the study. Of these, 302 did not meet the inclusion criteria, 23 declined to participate, 15 were spontaneously pregnant, and 7 did not reply. Among the 35 eligible patients, 1 withdrew consent before randomization and 34 were randomly allocated: 16 to the OFFA group and 18 to the control group. The patient flowchart is shown in Supplemental Figure 1 (available online).

The baseline characteristics of the patients included in the control and OFFA groups are shown in Table 1. There were no differences between the 2 groups in age, body mass index, duration of infertility, diagnosis, AFC before starting IVF, or any of the examined variables, confirming the homogeneity of our study population and effective randomization. Only 13 of 34 randomized women (38.9%) had previously been pregnant, with an overall live birth rate of 5.8% (5.6% in the control group and 6.2% in the OFFA group).

Surgical Outcomes

In the OFFA group, 1 randomized patient did not undergo surgery for work-related reasons. Ovarian fragmentation was successfully completed in 15 women. The number of grafted fragments ranged from 30 to 100, depending on the size of the ovary and the retrieved biopsy specimen. The main surgical outcomes are shown in Supplemental Table 1 (available online). After the surgery, 1 patient experienced a 2-day episode of fever of unknown origin and was treated with paracetamol without requiring antibiotic treatment. This was noted as a postsurgical complication. Interestingly, although clinical endometriosis was among the exclusion criteria, 26.6% of patients showed macroscopic signs of ovarian endometriosis during the surgery. The zones affected by endometriotic lesions were excluded and not grafted back to the patients.

Ovarian Reserve Biomarkers Follow-Up

The median duration of follow-up before IVF was 155.5 (121.0–163.0) days in the control arm and 97.0 (71.0–196.0) days in the OFFA arm; there was no statistically significant difference between the groups. Two patients from the control arm withdrew from the study during the follow-up, whereas all patients who underwent OFFA completed the follow-up (Supplemental Fig. 1).

The antral follicle count (AFC) was not significantly different in either of the ovaries at inclusion in either of the groups (Table 2). However, after OFFA, the total AFC of the OFFA group was higher than the total AFC of the control group ($P=.021$) (Table 2). The increase in the total AFC was due to an increase in the number of follicles in the ovary in which OFFA was performed, resulting in more antral follicles than in the control group ($P=.008$). There were no differences in the AFC of the ovaries that were not operated. When the percentage increase in the number of antral follicles in the ovaries with lower AFC at baseline (intervention ovaries) was compared between the 2 groups, the increase was greater in the OFFA group ($P=.048$). The AMH, estradiol, and FSH levels did not differ between the groups at baseline or during follow-up (Table 2 and Supplemental Fig. 2-Supplemental Fig. 4, available online).

In Vitro Fertilization Outcomes

The patients in the 2 groups were treated with similar doses of gonadotropins. The time required to initiate the IVF cycles was slightly less in the OFFA group, although there were no significant differences. A total of 15 IVF cycles were initiated in the OFFA group and 16 in the control group (Table 3).

TABLE 1

Baseline characteristics of the randomized women with POR.

Characteristic	Control group (n = 18)	Surgery group (n = 16)	*P* value
Patient's age (y)	36.5 (35.0–38.0)	36.5 (34.0–37.7)	.746
Partner's age (y)	37.0 (33.0–42.0)	37.5 (37.0–39.7)	.422
BMI (kg/m^2)	21.0 (19.8–23.6)	22.4 (20.2–24.0)	.746
Previous gestations	7/18 (38.9%)	6/16 (37.5%)	.342
Previous deliveries	1/18 (5.6%)	1/16 (6.2%)	.365
Previous miscarriages	6/18 (33.3%)	5/16 (31.2%)	.563
Infertility etiology			.632
Only POR	16/18 (88.8%)	15/16 (93.8%)	
Tubal factor	1/18 (5.6%)	1/16 (6.2%)	
MFI	1/18 (5.6%)	0/16 (0.0%)	
Infertility duration (mo)	36.0 (36.0–72.0)	48.0 (36.0–87.0)	.365
Baseline AFC	5.0 (2.0–5.0)	4.0 (2.0–4.0)	.325
Baseline AMH (ng/mL)	0.36 (0.08–0.56)	0.44 (0.18–0.50)	1.000
No. of previous IVF cycles	1.0 (1.0–2.0)	1.0 (1.0–3.0)	.365
No. of previous POR cycles	1.0 (1.0–1.0)	1.0 (1.0–2.0)	.088

Note: Values are shown as median (p25–p75) or raw numbers (proportions). AFC = antral follicle count; AMH = antimüllerian hormone; BMI = body mass index; IVF = in vitro fertilization; MFI = male factor infertility; POR = poor ovarian response.

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TABLE 2

Patients' follow-up of ovarian reserve markers.

Variable	Control group (n = 16)	Surgery group (n = 15)	*P* value
Time between treatment and IVF (d)	155.5 (121.0–163.0)	97.0 (71.0–196.0)	.340
AFC control ovary			
Initial	3.0 (1.0–4.0)	2.0 (2.0–3.0)	.582
Final	3.0 (2.0–4.0)	2.0 (2.0–4.0)	.221
Increase (%)	50.0 (0.0–100.0)	66.7 (0.0–150.0)	.753
AFC intervention ovary			
Initial	1.0 (0.0–2.0)	1.0 (0.0–2.0)	.604
Final	1.0 (1.0–2.0)	3.0 (1.0–4.0)	.008
Increase (%)	100.0 (50.0–100.0)	100.0 (100.0–200.0)	.048
Total AFC			
Initial	5.0 (2.0–5.0)	4.0 (2.0–4.0)	.449
Final	5.0 (3.0–6.0)	5.0 (3.0–7.0)	.021
Increase (%)	20.0 (25.0–50.0)	75.0 (45.8–200.0)	.134
AMH (ng/mL)			
Initial	0.36 (0.08–0.56)	0.44 (0.18–0.50)	.427
Final	0.35 (0.11–0.66)	0.29 (0.20–0.69)	.496
Increase (%)	48.7 ([−56.6]–144.8)	26.8([−45.5]–112.1)	1.000

Note: Values are shown as median (p25–p75) or raw numbers (proportions). Comparison between control and surgery group. Wilcoxon rank test. Comparison between the initial and the final value within the same individual are shown. AFC = antral follicle count; AMH = antimüllerian hormone; IVF = in vitro fertilization.

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No differences were observed between the groups when the primary outcome was analyzed: the number of MII oocytes obtained during egg collection did not differ between the groups (OFFA, 4.0 [1.0–8.0] vs Control, 2.0 [1.0–3.0], *P*=.302). In the control group, 33 MII oocytes were retrieved, 28 cleavage stage embryos developed, and 18 ETswere performed. In the OFFA group, 23 MII oocytes were retrieved, 12 cleavage stage embryos developed, and 11 ETs were performed. No significant differences were detected in the number of punctured follicles, retrieved oocytes, transferred embryos, and cycles ending in an ET (Table 3). The rates of cancelation (relative risk [RR] 0.71 [0.13–3.68], *P*=.684), implantation (RR 1.14 [0.27–4.91], *P*=.857), clinical pregnancy (RR 0.71 [0.13–3.68], *P*=.684), and live birth (RR 0.36 [0.04–3.05], *P*=.942) did not differ between the groups. Three pregnancies and 4 live births (one twin pregnancy) were recorded in the control group, and 2 pregnancies and 1 live birth were recorded in the OFFA group.

All patients were contacted when the study was closed, 3 years after starting. No spontaneous pregnancies were reported outside the study.

Histologic Analysis of Ovarian Biopsy Specimens

Primordial follicles were identified in hematoxylin and eosin-stained sections from 6 of the 15 women who underwent OFFA. In 2 biopsy specimens, primary follicles were also identified. In addition, a corpus luteum was identified in sections obtained from another woman, giving an overall rate of 46.6% follicle activity/presence detected in the OFFA group.

TABLE 3

Reproductive outcomes and IVF cycle characteristics.

Variable	Control group (n = 16)	Surgery group (n = 15)	*P* value
Total gonadotropin dose (IU)	2,700.0 (2,100.0–3,900.0)	2,400.0 (2,100.0–3,000.0)	.306
AFC first day of COS	5.0 (3.0–6.0)	5.0 (3.0–7.0)	.356
Peak E2 level (pg/mL)	996.0 (602.0–1,743.0)	657.0 (451.0–1,126.0)	.322
Follicles >16 mm	2.0 (2.0–5.0)	2.0 (1.0–3.0)	.318
No. of punctured follicles	4.0 (2.0–8.0)	5.0 (2.5–6.5)	1.000
No. of retrieved oocytes	4.0 (2.0–10.0)	2.0 (1.0–5.0)	.303
No. of retrieved MII oocytes	4.0 (1.0–8.0)	2.0 (1.0–3.0)	.302
Fertilization rate (%)	100.0 (50.0–100.0)	33.3 (0.0–81.3)	.480
Cycles with embryo transfer	8/16 (50.0%)	4/15 (26.6%)	.236
No. of transferred embryos	2.0 (1.0–2.0)	1.0 (0.0–2.0)	.172
Implantation rate	4/14 (28.6%)	2/8 (25.0%)	.966
Clinical pregnancy rate	3/16 (18.7%)	2/15 (13.3%)	.684
Live birth rate	3/16 (18.7%)	1/15 (6.7%)	.512
Canceled cycles	3/16 (18.7%)	2/15 (13.3%)	.684

Note: Values are shown as median (p25–p75) or raw numbers (proportions). AFC = antral follicle count; COS = controlled ovarian stimulation; E2 = estradiol; IVF = in vitro fertilization; MII = metaphase II.

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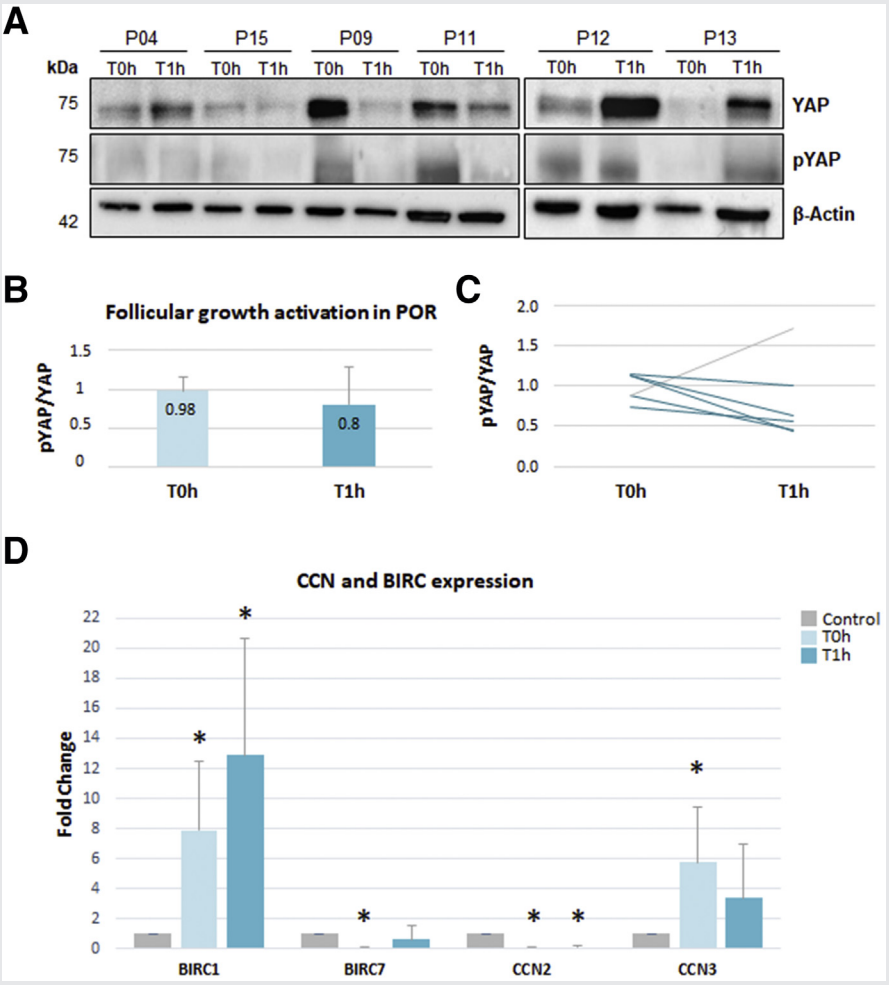
FIGURE 1



Hippo pathway inhibition after ovarian fragmentation in women with poor ovarian response. (**A**) Representative Western blots showing the levels of Yes-associated protein 1 (YAP) and phospho-YAP (pYAP) in ovarian fragments at the time of fragmentation and 1 hour later. (**B**) Ovarian fragmentation decreased the phospho-YAP/YAP ratio, showing inhibition of the Hippo pathway. (**C**) Individual assessment of the phospho-YAP/YAP ratio showed that the ratio decreased 1 hour after the fragmentation procedure in all but one patient allocated to the surgery group. (**D**) Messenger ribonucleic acid expression levels of the downstream Hippo pathway genes *BIRC1*, *BIRC7*, and *CCN2* were higher 1 hour after ovarian fragmentation. When compared with reference ovarian control tissue, *BIRC1* and *CCN2* were found to be overexpressed in women with POR at both time points analyzed. *$P<.05$. POR = poor ovarian response.

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Inhibition of the Hippo Pathway

Assessment of Hippo pathway inhibition by determining phosphorylated YAP levels showed that the phospho-YAP/YAP ratio was reduced by 18.8% between samples at the time of tissue retrieval and 1 hour after fragmentation (1.0 ± 0.2 vs 0.8 ± 0.5) (Fig. 1A to C). When patients were individually examined, all but 1 showed increased phosphorylation levels at t = 1 hour and therefore a successful Hippo pathway inhibition.

The relative gene expression of downstream Hippo factors showed up-regulation of *BIRC1*, *BIRC7*, and *CCN2* 1 hour after fragmentation when compared with basal levels (0 hours) (Fig. 1D). Interestingly, an overall increased expression of *BIRC1* was found in women with POR compared with reference ovarian tissues from healthy women (fold change t0h = 7.9 ± 4.5, $P=.004$, and t1h = 12.9 ± 7.7; $P=.003$) (Fig. 1D), which may have been induced by the procedure of tissue retrieval and decortication.

DISCUSSION

To our knowledge, this is the first RCT investigating whether OFFA improves IVF outcomes in women with POR compared with an untreated control group. Women in the OFFA group experienced an increase in total AFC compared with its baseline level due to a specific increase in the ovary that underwent surgery. Ovarian fragmentation also resulted in earlier

initiation of ovarian stimulation. This was possibly due to the earlier detection of a 100% increase in AFC in the treated ovary, which was one of the study criteria for initiation of IVF during the follow-up. However, AMH levels and IVF outcomes showed no statistically significant differences between the control and the OFFA groups.

In the case of a complex intervention such as OFFA, it is important to determine whether the procedure achieved its goal, which was to suppress the Hippo pathway. In the current study, the assessment of Hippo pathway inhibition at the molecular level confirmed the efficacy of the fragmentation procedure (Fig. 1A to D). We found both YAP phosphorylation and downstream expression of *CCN* and *BIRC* factors, as previously described (9, 24). Interestingly, when these variables were compared in the POR biopsy specimens after decortication and fragmentation, significant differences were detected between t = 0h samples from ovaries of women with POR and control reference tissue, indicating that ovarian cortex preparation steps can also have an additional effect on Hippo pathway inhibition (21).

During the past decade, a number of case series and small cohort studies in women with POI reported the outcomes of this innovative approach (9). These studies reported an overall cumulative pregnancy rate ranging from an eventual success rate as high as 30%, reported by Kawamura (9) when first describing the IVA technique in a well-characterized POI population, to 60%, reported by Andersen (19) in women with DOR whose inclusion criteria were solely based on repeated serum AMH levels ≤5 pM (0.7 ng/mL) (19). The heterogeneity in the reported results could be in part due to differences in the definition of the primary endpoints between studies; whereas some used the number of follicles in response to stimulation (19), others did not use predefined outcomes to assess the success of the technique (9, 13, 14). The protocols used to activate the follicles also varied between experimental settings. Initial studies attempted to achieve IVA by using a combination of ovarian fragmentation and treatment of fragmented ovarian cortical fragments with PTEN inhibitors and Akt stimulators to further promote follicle development (9, 13, 14). Subsequently, several modifications of the original IVA method have been proposed, mainly focused on avoiding the use of PTEN inhibitors and Akt stimulators because of the possibility of harmful effects when transplanted into the patient (15–19). Avoidance of the 2-day drug incubation also eliminated the need for cortex cryopreservation (and potential cryodamage) and the need for a second surgical intervention. In addition, because the tissue was retrieved and grafted during the same surgery, the highly vascularized medullar bed created a better orthotopic grafting site for ovarian fragments. Orthotopic grafting on a tunnel created between the remaining cortex and medulla could also have indirect beneficial effects on resident follicles near the grafting site, because the activated fragments secrete biochemical signals including growth factors (25), which, in a paracrine manner, can reach nearby resident follicles. To date, OFFA, or drug-free IVA, has been tested with varying results in women with POI and POR and in patients of advanced maternal age (9, 13, 15, 16, 18, 19). However, most of these studies were case series or cohort studies that applied the IVA or drug-free IVA to both ovaries and compared outcomes with the prior cycles in the same patients. This approach is prone to significant bias because of the regression to the mean phenomenon (26), especially in younger women.

The study by Andersen et al. (19) using OFFA is noteworthy, because 1 ovary was randomized to surgery while the other ovary served as the control; 20 women with DOR were included. During a 10-week follow-up period, AMH levels and total AFC did not increase. In fact, AFC was found to be higher in the control ovary than in the biopsied ovary, perhaps because of the cortex retrieval, as fragments were grafted into peritoneal pockets instead of the ovary. The main inclusion criteria were based only on AMH levels ≤5 pM in a population of women between the ages of 30 and 39. On the basis of this criterion, the selected population could include diagnoses of both POI and POR, and therefore the effect of treatment for specific diagnoses cannot be elucidated. In fact, the investigators reported 12 pregnancies in these women, including 3 spontaneous pregnancies, 2 at the first IVF attempt during the 10-week follow-up and the remaining 1 resulting from an IVF cycle performed within a year after the surgery. A 60% pregnancy rate has never been described for properly stratified and selected women with POR or POI, although the pregnancy rate per treatment was only 21.8% (12/55), close to those reported in our study. Moreover, the time distribution of the pregnancies in this study suggests that some pregnancies that occurred earlier in the follow-up period resulted from activation of antral follicles (consistent with POR), whereas others (pregnancies that occurred later in the follow-up period) were derived from follicles that were at the primordial stage at the time of the surgery (this would be more consistent with POI, because primordial follicles need several months from activation to antral stage).

One of the strengths of our study is that, to our knowledge, it is the only trial that includes a control group. This allows us to assess the effects of continuous follow-up and ovarian stimulation in patients not allocated to the intervention. Such effects should be carefully considered when establishing the effectiveness of new treatments involving exhaustive monitoring of the ovaries in women with a reproductive aging phenotype.

It is noteworthy that we observed pregnancies and live births in both study arms (pregnancy rate, 18.7% in the control and 13.3% in the OFFA group). It seems very unlikely that OFFA could have any impact on the reproductive outcomes of patients with POR, given that we could not demonstrate any difference in the number of retrieved MII oocytes (4.0 [1.0–8.0] vs 2.0 [1.0–3.0] in the control and OFFA groups, respectively). If OFFA had an impact on oocyte quality, the rates of fertilization, pregnancy, or live birth might be different. Unfortunately, this study was not designed to detect differences in such variables. Assuming that differences in live birth rates due to OFFA existed, and based on a post hoc analysis, the power of the present analysis to detect the differences with an alpha error of 5% would be only 5.9%. A sample size of more than 1,048 patients per group would be necessary to demonstrate a 5% increase in live birth rates compared with the control group, which may reflect the limited clinical relevance of OFFA in this specific population.

Assessing the efficacy of therapeutic interventions in women with POR is complicated by the lack of well-established and universally agreed-upon criteria to diagnose this entity. Arguably, the most commonly used diagnostic criteria for POR are those proposed by the ESHRE Bologna group (3). These criteria have been criticized (27–31) because they group age-related POR together with POR at a younger age, 2 entities that may have different etiopathologies. In the present study, we adopted a modified understanding of the ESHRE Bologna criteria. To be considered for admission to our study, women had to be under 40 years of age and still meet the Bologna criteria. Using this approach, we achieved increased homogeneity in the study group and limited the effect of age-related (and arguably physiologic) POR and the associated increased rate of aneuploidy in our outcomes. Thus, the conclusion of our study is quite specific: although OFFA increases the number of antral follicles, it does not seem to improve IVF outcomes in women younger than 40 years who have a diagnosis of POR according to the ESHRE Bologna criteria, and therefore it should not be used in such a population.

DIALOG: You can discuss this article with its authors and other readers at https://www.fertstertdialog.com/posts/32882

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Activación folicular en mujeres diagnosticadas previamente como pobres respondedoras: estudio controlado y aleatorizado.

Objetivo: investigar si la fragmentación ovárica para la activación folicular (OFFA) mejora los marcadores de reserva ovárica y resultados de fertilización in vitro (FIV) en mujeres con pobre respuesta ovárica (POR).

Diseño: Estudio controlado, aleatorizado, con asignaciones paralelas.

Escenario: Hospital universitario.

Paciente(s): Treinta y cuatro mujeres con POR según los criterios de la Sociedad Europea de Reproducción Humana y Embriología.

Intervención: Mujeres con POR fueron aleatoriamente asignadas a recibir fragmentación ovárica en un ovario o ninguna intervención (grupo control). Se hizo un seguimiento de marcadores de reserva ovárica cada 2 semanas durante 6 meses. Se iniciaron ciclos de fertilización in vitro al duplicarse el recuento de folículos antrales (AFC) o una vez finalizado el seguimiento.

Medición(es) del resultado principal: El desenlace principal fue el número de ovocitos metafase II (MII) obtenidos. Como desenlaces secundarios se registraron el recuento de folículos antrales, niveles de hormona antimulleriana, y resultados reproductivos. Los desenlaces exploratorios incluyeron resultados quirúrgicos y análisis de proteinas y expresión génica.

Resultados: La fragmentación ovárica para activación folicular resultó en un aumento del AFC en el ovario intervenido comparado con el ovario control y en un aumento del AFC total en el grupo OFFA comparado con controles. Los niveles séricos de hormona antimulleriana y hormona folículo estimulante no mejoraron en el grupo OFFA a lo largo del período de seguimiento. Quince pacientes de cada rama se sometieron a FIV. En el grupo control, se recuperaron 33 ovocitos MII y se realizaron 18 transferencias embrionarias, con una tasa de embarazo de 20% y una tasa de recién nacido vivo de 18.7% por ciclo. En el grupo OFFA, se obtuvieron 23 ovocitos MII y se transfirieron 11 embriones, con un 13.3% de tasa de embarazo y 6.7% de recién nacido vivo por ciclo. Los resultados reproductivos no difirieron significativamente entre los grupos. La inhibición del paso hippo se confirmó por una reducción del 18.8% en la razón fosfo-YAP/YAP y por la sobreexpresión de BIRC y CCN después de la fragmentación.

Conclusión(es): La fragmentación ovárica para activación folicular en mujeres con POR resultó en un aumento de AFC pero no modificó los resultados de IVF al compararlos con controles.