

Follicular flushing increases the number of oocytes retrieved in women with diminished ovarian reserve and serum progesterone levels of ≤ 5.0 ng/ml post surge



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ABSTRACT

Objective

To evaluate the utility of follicular flushing increase the number of oocytes retrieved in women with diminished ovarian reserve (DOR) and serum progesterone of ≤ 5.0 ng/ml the day after final oocyte maturation induction.

Design

Retrospective study.

Subjects

Women <40 years, with DOR, underwent controlled ovarian stimulation for an invitro fertilization cycle (IVF) were separated into two groups: Group 1: follicular flushing (n=35) Group 2: direct aspiration (n=33).

Main outcomes measured

Our primary outcome was the number of retrieved oocytes. Secondary outcome measures included the total procedure time, mean oocyte/follicle retrieval rate, mean number of metaphase II (MII) oocytes, MII rate, mean number of fertilized oocytes, and blastulation rate.

Results

A total of 288 oocytes were retrieved: 59.0% from the flushing group and 41.0% from the direct aspiration group. The mean number of oocytes retrieved by aspiration and subsequent flushes was significantly higher than the number retrieved from direct aspiration (4.4 ± 0.96 versus 2.64 ± 1.00 , $P = 0.003$). The oocyte/follicle rate was also significantly higher in women in Group 1 vs Group 2 (68.3% vs 48.7%, $p=0.001$). No significant differences were observed in the total number of MII, oocyte maturation rate, fertilization rate nor blastulation rate. The retrieval procedure time was higher among those who underwent follicular flushing, with an estimated increase of 6.5 minutes (14.0 ± 2.5 versus 7.5 ± 1.7 , $P = 0.003$).

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Conclusions

The results of this analysis in the poor responders found that women with DOR and post surge P4 levels of ≤ 5.0 ng/ml may benefit from follicular flushing on the number of oocytes retrieved.

KEYWORDS: Follicular flushing, Oocyte yield, DOR.

MANUSCRIPT

Introduction

As the number of oocytes available for in vitro fertilization (IVF) is a determinant of the cumulative chance of a patient to achieve pregnancy¹, it is important to retrieve the maximum number of oocytes from a given number of follicles that have developed in response to medication. Accordingly, in patients with poor ovarian response, the use of follicular flushing and double lumen needles has become popular, the purpose of follicular flushing is to increase the oocyte yield, possibly by improving the detachment of the cumulus–oocyte complex (COC) from the follicular wall and decreasing the risk of oocyte retention within the follicle^{2,3}.

Oocyte maturation and embryo development are controlled by intra-ovarian factors such as steroid hormones. Progesterone (P4) exists in the follicular fluid, and it is known to mediate luteinizing hormone (LH)-initiated periovulatory events through autocrine/paracrine mechanisms that help mediate granulosa cell luteinization and oocyte maturation⁴. More importantly, a rise in P4 levels is associated with an adequate follicular rupture⁴. We hypothesize that lower P4 levels (≤ 5.0 ng/ml) after oocyte maturation induction is a reflection of impaired physiological periovulatory mechanisms required for the oocyte release from the follicular wall, and, by flushing the follicle it may facilitate oocyte detachment from the COC. The aim of this study is to determine the utility of using P4 levels the day after surge as a cutoff point to establish if woman with diminished ovarian reserve (DOR) undergoing an oocyte retrieval could benefit from this practice.

Materials and Methods

Study design and patient population

This retrospective, single center study included women <40 years, with DOR diagnosis according to ESHRE Bologna criteria described poor ovarian response as the presence of two out of three of the following criteria: advanced maternal age, previous poor ovarian response (three oocytes or fewer after conventional stimulation), an abnormal ovarian reserve test (AFC 5–7, AMH <0.5–1.1 ng/mL)⁵ who underwent IVF from October 2019 through January 2022 and had serum P4 levels of ≤ 5.0 ng/ml the day after trigger.

Cohorts were separated into two groups: Group 1: follicular flushing; Group 2: direct aspiration (control).

Cases of patients harboring chromosomal rearrangements, undergoing preimplantation genetic testing for monogenic defects (PGT-M) and/or using donor gametes were excluded from the analysis.

Stimulation protocol

Patients underwent controlled ovarian hyperstimulation (COH) for IVF as previously described⁶. Briefly, the COH protocol was selected at the discretion of the reproductive endocrinologist and involved the administration of follicle-stimulating hormone (FSH) and human menopausal gonadotropin (hMG) with a gonadotropin-releasing hormone (GnRH) agonist downregulation protocol with leuprolide, a GnRH antagonist protocol, or a microflare protocol with leuprolide acetate. These protocols have been described previously⁷. Follicular development was monitored using transvaginal ultrasonography. When at least two follicles reached 18 mm in diameter, final oocyte maturation was induced with either hCG (5000–10,000 IU, Choragon, Ferring Pharmaceuticals, Mexico, CDMX), recombinant human chorionic gonadotropin (250–500 μ g, Ovidrel, EMD Serono, Mexico, CDMX) or a dual trigger with 2 mg of leuprolide acetate (Lucrin, AbbVie Laboratories, Mexico, CDMX) and 1000–5000 IU of hCG. For all cases, the level of serum progesterone the day after trigger was measured. All P4 serum levels were measured with electro-chemi-luminescence analysis utilizing an in-site 'Cobas e-601'VR (Roche Diagnostics, Mexico) (measuring range =0.03–60 ng/ml, Intra-assay variation = 1.1% and Inter-assay variation = 0.99). Thereafter, patients underwent vaginal oocyte retrieval under transvaginal ultrasound guidance 36 h after oocyte maturation was triggered.

Oocyte retrieval

The follicular flushing group puncture was performed with aspiration and follicular flushing using a 35 cm double-lumen 17-gauge needle (Cook1 EchoTip1 Double Lumen Aspiration Needle K-OPSD-1735-B-L). The flushing system inclusive of the needle was pre-filled with pre-warmed and equilibrated flushing medium before each procedure. The initial aspirated follicular fluid was collected in a tube, if no oocyte was identified, follicular flushing was repeated

until an oocyte was retrieved or up to a maximum of three times.

The direct aspiration group puncture was performed following the department's standard protocol, with a 35 cm single-lumen 17-gauge needle (Cook1 EchoTip1 Single Lumen Aspiration Needle 1735). The follicular fluid was collected in tubes without differentiating between the follicles. For both groups aspiration of the follicular fluid was performed using a vacuum pump set at -130 mmHg). All retrievals were performed by one physician to minimize surgical variability. The physician was experienced in both flushing and direct aspiration techniques.

Laboratory procedures

The oocytes and embryos were cultured in the same type of media as that used for follicular flushing. All metaphase II (MII) oocytes underwent either intracytoplasmic sperm injection (ICSI) or conventional insemination (CI). Embryos were cultured to the blastocyst stage as previously described⁶.

Outcome measures

The primary outcome was the total number of retrieved oocytes. Secondary outcome measures included the total procedure time (time of transvaginal insertion of the retrieval needle into the first ovary to the removal of the needle from the second ovary), mean oocyte/follicle retrieval rate (number of COC seen by ultrasound divided by the number of aspirated follicles),

mean number of MII oocytes, MII rate, mean number of fertilized oocytes, and blastulation rate.

Statistical analysis

Groups were compared using Student's T, Mann-Whitney U and Chi-squared when appropriate. Results were expressed as percentages, means and SDs. Adjusted odds ratios (aORs) with 95% CI's were calculated using a multivariate logistic regression analysis to adjust for confounding variables. Statistical analyses were performed using SAS version 9.4 (SAS institute Inc., Cary, NC, USA). All p-values were two-sided and were considered significant if less than < 0.05.

Regulatory approval

This retrospective study was approved by the local institution (RMA) Institutional Review Board, Inc.

Results

In total 68 women were included in the cohort (group 1, n= 35 women; group 2, n= 33). Patient demographic characteristics and COH parameters are described in [Table 1](#). No significant differences were found in mean patient's age, BMI, baseline FSH, AMH, baseline antral follicle count dose of gonadotropins used, serum progesterone and estradiol levels the day of ovulation trigger, day of ovulation trigger, serum progesterone and estradiol levels post trigger, and COC among cohorts.

	Group 1 (n=35)	Group 2 (n=33)	P value
Age at retrieval (years)	38.6 ± 3.5	38.4 ± 3.1	0.66
BMI (Kg/m ²)	24.1 ± 3.5	23.3 ± 3.9	0.52
AMH	0.5 ± 0.2	0.6 ± 0.3	0.11
Baseline FSH (IU/mL)	10 ± 4.0	9.5 ± 3.5	0.44
Baseline antral follicle count	12.4 ± 6.8	11.9 ± 6.0	0.39
Male age	40.6 ± 2.5	41.6 ± 2.5	0.68
Cycle characteristics			
Cumulative GND dose (Units)	3690 ± 1301	3166 ± 1339	0.35
Day of ovulation trigger	13.0 ± 1.6	12.6 ± 2.4	0.22
Surge E2 (pg/mL)	1295.1 ± 1036.8	1214.3 ± 1148.7	0.09
Surge P4 (ng/mL)	0.9 ± 0.5	0.9 ± 0.4	0.08
Post surge E2 (pg/mL)	1174.1 ± 1236.3	1017.5 ± 1154.3	0.07
Post surge P4 (ng/mL)	3.6 ± 1.2	3.8 ± 1.1	0.65

Table 1. Demographic and cycle characteristics of patients.

Note: Data presented as percentages, mean and ± standard deviations, unless stated otherwise. BMI= body mass index; AMH = anti Mullerian hormone; FSH = Follicle stimulating hormone; GND = gonadotropins; E2 = estradiol; P4= progesterone; COC=cumulus-oocyte complex.

A total of 288 oocytes were retrieved: 170 (59.0%) from the flushing group and 118 (41.0%) from the direct aspiration group. All patients who underwent retrieval had oocytes retrieved (Table 2). The mean number of oocytes retrieved by aspiration and subsequent flushes was significantly higher than the number retrieved from direct aspiration (4.4 ± 0.96 versus 2.64 ± 1.00 , $P = 0.003$). In the flushing group, 21 oocytes (12.3%) were retrieved at the initial aspiration, 115 (67.6%) in the first flush, 27 (15.9%) in the second, and 7 (4.2%) in the

third. The oocyte/follicle rate was also significantly higher in women in Group 1 vs Group 2 (68.3% vs 48.7%, $p=0.001$). No significant differences were observed in the total number of MII, oocyte maturation rate, fertilization rate nor blastulation rate (Table 2). The retrieval procedure time was higher among those who underwent follicular flushing, with an estimated increase of 6.5 minutes (14.0 ± 2.5 versus 7.5 ± 1.7 , $P = 0.003$).

	Group 1	Group 2	P value
Average oocytes retrieved	4.4 ± 0.9	2.64 ± 1.0	0.003 *
Oocyte/follicle retrieval rate %	68.3	48.7	0.001 *
Number of MII	3.3 ± 1.3	1.9 ± 2.3	0.18
Oocyte maturity rate %	77.1 (132/170)	70.3 (83/118)	0.46
Fertilization rate %	78.0 (103/132)	77.1 (64/83)	0.08
Blastulation rate%	67.9 (70/103)	65.6 (42/64)	0.66

Table 2. Cycle outcomes among poor responders who received direct aspiration versus follicular flushing at oocyte retrieval.

Note: Data presented as percentages, mean and \pm standard deviations, unless stated otherwise. MII= Metaphase II oocytes.

*= Statistical significance is defined as $p < .05$.

Discussion

This is the first study to establish post surge P4 levels as a cutoff point to determine whether patients with DOR could benefit from follicular flushing. Our findings suggest that patients with P4 levels of ≤ 5.0 ng/ml who underwent follicular flushing had higher oocyte yield compared with direct aspiration. It is also the first to suggest that lower P4 levels (≤ 5.0 ng/ml) after final oocyte maturation induction may be a result of compromised mechanisms associated to the release of the oocyte from the follicular wall as demonstrated by lower oocyte recovery rate.

The use of follicular flushing remains controversial. Prior trials and meta-analyses found no benefit of flushing on the number of oocytes retrieved.⁸⁻¹⁰ However, these studies are limited by not having a control group, each follicle was punctured a single time and aspiration occurred followed by flushing. Oocytes retrieved early were attributed to direct aspiration and oocytes retrieved later were attributed to flushing. Some of these trials attempted to address this issue by counting oocytes retrieved in the first flush as resulting from aspiration alone. Randomizing patients to flushing versus non-flushing is the best way to compare these approaches, and to address the potential for inaccurate attribution of oocytes retrieved to aspiration or flushing. Our study found that women with DOR and lower P4 levels (≤ 5.0 ng/ml) after final oocyte maturation

induction benefit from follicular flushing, possibly by detaching the oocyte from the COC.

Several studies have reported higher oocyte yield following follicular flushing in poor responder patients managed with semi natural cycle IVF¹⁴. Likewise, Van Wolff et al. reported two-fold higher oocyte yield and transferrable embryos with follicular flushing¹⁵. The same group conducted a well-designed adequately powered RCT directly comparing aspiration and follicular flushing in their mono-follicular IVF patients after gonadotropin free ovarian stimulation. Higher oocyte yield, mature oocytes and fertilization rates were noted with follicular flushing¹⁶. Similar to our findings, we demonstrated higher number of oocytes retrieved, however, no differences were noted in the MII nor blastulation rates.

For the quantitative outcome procedure duration, our findings show a significantly prolonged procedure time for follicular aspiration when using flushing. This finding comes as no surprise and confirms previous meta-analyses for normal response IVF patients¹¹⁻¹³. The mean increase is only 6.5 minutes, which is likely of no clinical relevance for the patient in anesthesia time. Follicular flushing, however, increases the effort for the team (preparation and equilibration of the flushing medium), as well as the financial burden, as double lumen needles and flushing media are costly.

Our study distinguishes itself as it was performed at a single, high-volume academic center with a team

of embryologists all uniformly trained, thereby reducing the inherent variability that may arise from multicenter studies. Oocytes retrievals were carried out by one physician, reducing the inherent intra-operator variability that may arise from distinct surgical techniques.

Notwithstanding our best efforts to avoid biases, some shortcomings and limitations exist in the analysis. The most notable limitation is its retrospective design, which increases the chance of selection bias, the small sample size, selected progesterone cutoff value and progesterone assay techniques compared to other ART centers may limit the external validity of our findings. A prospective randomized controlled trial is urged in order to analyze the real benefit of this novel intervention in patients with DOR. As for now, this study serves as a proof of concept and description of this technique for follicular flushing in women with serum P4 levels of ≤ 5.0 ng/ml the day after trigger aiming to improve ART outcomes in this group of patients with adverse prognosis during their family-building journey.

CONCLUSION

The results of this analysis in the poor responders found that women with DOR and post surge P4 levels of ≤ 5.0 ng/ml may benefit from follicular flushing on the number of oocytes retrieved.

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CONFLICT OF INTEREST

The authors declare they have no conflict of interest.

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