What is the optimum number of follicular flushes in mono-follicular in-vitro fertilization cycles in a poor responder population?

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Abstract

Objective: Assessment of the optimal number of follicular flushes on retrieval rate and quality of oocytes in mono-follicular in-vitro fertilization (IVF) cycles.

Material and Methods: A retrospective analysis of 246 oocyte pick-up procedures in mono-follicular IVF cycles of 226 poor responder women was performed. The primary endpoint was oocyte retrieval rate in the initial aspirate versus subsequent flushing episodes. The secondary endpoints were oocyte maturity, fertilization rates and embryo cleavage.

Results: The procedure was successful in 187 cycles (76%), of which 160 metaphase-II oocytes were retrieved. Retrieval rates were similar for natural and modified natural cycles (p=0.595). The initial aspirate provided 54% of the total yield and the rest was obtained from up to four episodes of flushing. Follicular flushing increased oocyte recovery rate from 41.1% to 76%. None of the oocytes retrieved after three flushes fertilized. Oocyte maturity, fertilization and embryo cleavage rates were comparable for oocytes from the initial aspirate and one or two episodes of flushing. Oocytes obtained after the third flushing episode developed into poor quality embryos.

Conclusion: Flushing confers a benefit for oocyte recover rates in mono-follicular IVF cycles in poor responder women. However, more than three attempts at flushing were not associated with good outcome. (J Turk Ger Gynecol Assoc 2022; 23: 33-7)

Keywords: Oocyte retrieval, in-vitro fertilization, assisted reproductive technologies

Received: 20 January, 2021 **Accepted:** 29 April, 2021

Introduction

Since the early days of human in-vitro fertilization (IVF), ovarian stimulation cycles have gradually replaced natural cycles owing to the benefits of increased oocyte yield and improved pregnancy rate (1). Although natural cycle IVF has regained attention in parallel with the increased interest in minimal ovarian stimulation strategies, in many clinics it is considered as the last resort for women who do not respond to ovarian stimulation with more than a single follicle. Despite a bleak prognosis, a considerable number of women opt for multiple treatment attempts with their own oocytes before convincing themselves to proceed with oocyte donation and some others do not or cannot consider this option due to personal, religious, or legislative reasons.

The success of natural cycle IVF is impeded, however, by high cancellation rates because of premature ovulation, failed oocyte retrieval, and fertilization or cleavage problems (2). As the success is dependent on the retrieval of the oocyte presumed to be in the single growing follicle, flushing is commonly performed when the initial aspirate is negative. However, data regarding the benefit of flushing during oocyte retrieval is not conclusive and is mainly derived from stimulated cycles with multiple growing follicles (3,4). Excessive flushing is associated with long operative times and wasted flushing medium. Prompted by the scarcity of data, we retrospectively

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Journal of the Turkish-German Gynecological Association published by Galenos Publishing House.

DOI: 10.4274/jtgga.galenos.2021.2021-0016

analyzed our mono-follicular IVF cycles to assess the optimal number of flushes.

Material and Methods

Study Population and Participants

This was a retrospective analysis of 279 oocyte pick-up (OPU) procedures performed in a tertiary care infertility center between January 2016 and December 2018 for natural (n=126) and modified natural (n=153) IVF cycles. Data regarding female age, body mass index (BMI), serum estradiol (E_2) level and diameter of the follicle at the time of ovulation trigger, number of flushes, oocyte maturity, fertilization and embryo quality were extracted from an electronic database. At the beginning of IVF treatment, all patients gave informed consent that their anonymized data to be used for research projects in the future. Treatment cycles of patients with more than one growing follicle (n=8) and premature ovulation (n=25) were excluded from the analysis. The study group included 99 natural and 147 modified natural IVF cycles.

During natural and modified natural IVF cycles, ultrasonographic monitoring was started on the second or third day of menstruation to exclude the presence of ovarian cysts that may be confused with a growing follicle. In the presence of a sonolucent structure >10 mm in size, serum estradiol was measured to differentiate between a growing follicle and a cyst. Ovulation was triggered with 250 μg of recombinant human chorionic gonadotropin (Ovitrelle®, Merck-Serono, Italy) when the mean follicle diameter reached or exceeded 16 mm. In modified natural IVF cycles, 75 IU recombinant FSH (Gonal F®, Merck-Serono, Italy) and gonadotropin-releasing hormone antagonist (Cetrotide®, Merck-Serono, Italy) was started when the follicle reached 12 mm in diameter. Ovulation was triggered with 250 μg of recombinant human chorionic gonadotropin (Ovitrelle®, Merck-Serono, Italy) when the mean follicle diameter reached or exceeded 16 mm. Indomethacin suppositories (Endol sup®, 100 mg, Deva, Turkey) were administered every 12 hours, starting with the ovulation trigger and continued until egg collection. Oocyte retrieval was performed under local anesthesia 34-36 hours after triggering ovulation, using a 17-gauge double-lumen needle (K-OPSD-1735-B-L, Cook, Australia), connected to a vacuum pump (K-MAR-5200, Cook, Australia). The aspiration pressure was set at 150 mmHg. The follicle was aspirated and an additional 1.5 cc (this is the volume of the aspiration tubing of the needle) of flushing medium was given and aspirated again to retrieve the oocyte-cumulus corona complex (OCCC) if trapped in the aspiration tubing. This was referred to as the initial aspirate. If no OCCC was observed, flushing was affected using a specifically formulated medium (ASP, Vitrolife, Sweden) that was prewarmed to 37 ˚C. The maximum number of flushes was six. OCCCs were denuded after at least two hours of incubation. Following maturation assessment, all metaphase-II (M-II) oocytes were fertilized by standard intracytoplasmic sperm injection (ICSI). Fertilization was assessed 16-17 hours after ICSI, and the presence of two pronuclei represented normal fertilization. Embryos were cultured for 3-5 days, depending on the primary physician's preference. Figure 1 shows the flowchart of the inclusion and exclusion of patients from the study.

The Koç University Local Research Ethics Committee approved the study (approval number: 2020.181.IRB1.049). Informed consent was obtained.

Statistical analysis

The Kolmogorov-Smirnov test was used to check for normality of distribution. All continuous variables displayed a normal distribution. Continuous variables are represented as mean ± standard deviation while categorical variables are described as frequency with rate. The Student's t-test for normally distributed continuous data and chi-square or Fisher's exact tests for categorical data were used for statistical comparison, as appropriate.

The primary endpoint was oocyte retrieval rate in the initial aspirate versus subsequent flushing episodes. The secondary endpoints were oocyte maturity, fertilization and embryo

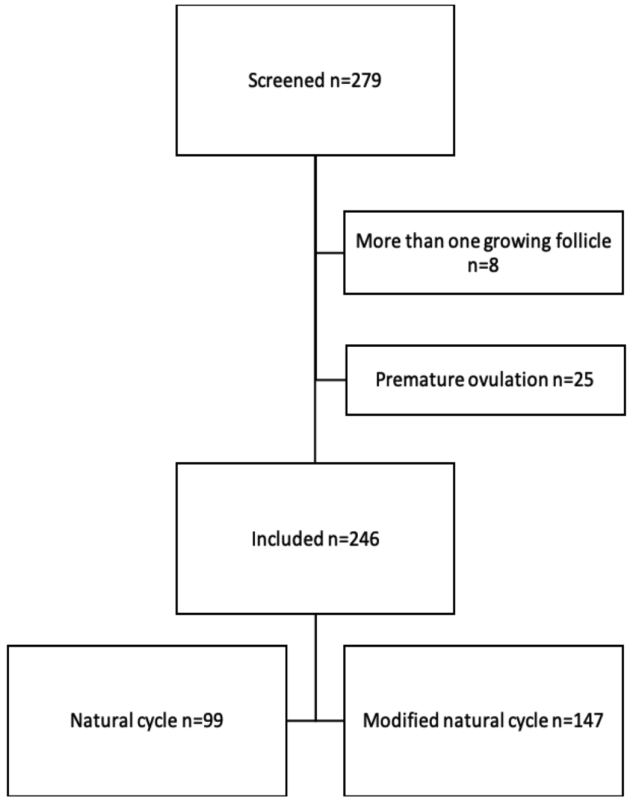

Figure 1. Flowchart of the study population

cleavage rates. Correlation and logistic regression analyses were used to assess the factors related to oocyte retrieval. Several literature-derived and biologically plausible confounders were identified including maternal age, BMI, natural or modified natural cycle, peak E_2 level, and diameter of the follicle at the time of triggering ovulation. All p-values were two-sided and $p<0.05$ was considered significant. Statistical analyses were carried out using the SPSS, version 24.0 (IBM, Chicago, IL, USA).

Results

The study group included 246 OPU procedures in 226 women, for 99 natural cycles and 147 modified natural IVF cycles. Baseline characteristics of all cycles and their outcomes are reported in Table 1. Seven women had multiple treatment attempts. The procedure was successful in 187 (76%) cycles, from which 160 M-II oocytes were retrieved (including five that were developed in vitro from M-I oocytes). The fertilization rate was 53.1% (85/160). On the third day of in vitro culture, these 85 zygotes developed into 23 (27.1%) grade 1 and 55 (64.7%) grade 2 embryos, whereas seven (8.2%) showed cleavage arrest.

Table 2 shows the number of oocytes, M-II oocytes, zygotes and cleaved embryos generated from the oocytes collected from the initial aspirate and subsequent flushing episodes. The initial aspirate contained approximately half (54%) of the total oocyte yield (101/187). The first, second, third and fourth flushes provided 46 (24.5%), 19 (10%), 14 (7.5%) and 7 (4%) oocytes, respectively. No oocytes were recovered thereafter.

Table 1. Characteristics of all cycles and their outcomes

Variable	All
Number	246
Female age (years)	40.1±4.6 (27-49)
Body mass index (kg/m^2)	27.5±3.9 (18.7-43)
Number of previously failed cycles	2.3±1.6 (1-8)
Follicle diameter on hCG day (mm)	17.9±0.9 (16.5-19.5)
Peak E_2 level (mIU/L)	245.2±56.8 (139-413)
*Values are represented as number or mean ± standard deviation (range)	

Figure 2 depicts cumulative percentages of the oocytes retrieved. The odds of retrieving an oocyte were 0.07 [95% confidence interval (CI): 0.05-0.11], if no flushing was performed (p=0.0001).

Among a priori selected confounders, the follicle diameter was positively correlated with the chance of retrieving an oocyte (r=0.185, p=0.040). None of the other factors were related with success in oocyte retrieval (female age: r=-0.030, p=0.635; BMI: r=0.043, p=0.503; peak E_2 level: r=-0.099, p=0.126; natural vs modified natural cycle p=0.595). The lowest E_2 level in a cycle with an M-II oocyte retrieval was 139 pg/mL.

Oocyte maturity was gradually decreased in subsequent flushing episodes, but the difference was not statistically significant (p=0.577). Fertilization rates of M-II oocytes obtained from the initial aspirate and one to three episodes of flushing were comparable (p=0.971). None of the five oocytes obtained from the fourth flush was fertilized.

Cleavage rate of embryos derived from oocytes retrieved from the initial aspirate and one to two episodes of flushing, however, was significantly higher compared to those of embryos derived from the oocytes obtained from the third flushing episode (50%, 3/6, p=0.006).

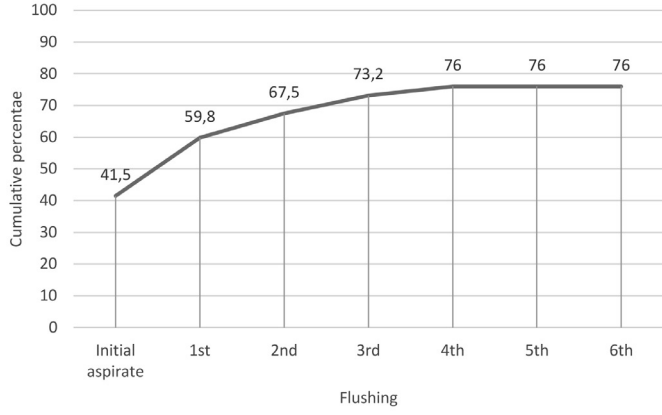

Figure 2. Cumulative percentage of oocyte recovery in 246 procedures

Table 2. Oocyte yield, M-II oocytes, zygotes and cleaved embryos generated from the oocytes collected from the initial aspirate and subsequent flushing episodes

Flushing episode	Oocyte yield	M-II oocyte	Fertilization	Cleavage
Initial aspirate	101 (54)	90 (89.1)	50 (55.5)	47 (94)
1st	46 (24.5)	39 (84.8)	20 (51.3)	20 (100)
2nd	19 (10)	15 (78.9)	9 (60)	8 (88.9)
3rd	14 (7.5)	11 (78.6)	6 (54.5)	3 (50)
4th	7 (4)	5 (71.4)	0	0
Total	187	160 (85.6)	85 (53.1)	78 (91.8)
All data are shown as n (%), M-II: Metaphase-II				

Discussion

This study has shown that follicular flushing increased oocyte recovery rate in mono-follicular IVF cycles. However, no oocytes were retrieved after four flushing episodes. Oocytes obtained from the third flush onward either failed to be fertilized or developed into poor quality embryos.

The benefit of routine flushing in OPU is controversial (3,4). Published reports have concentrated mainly on data derived from multi-follicular growth in stimulated cycles. The latest Cochrane meta-analysis, including 10 randomized controlled trials in 928 women, reported no difference in oocyte yield between direct aspiration versus follicular flushing of multiple follicles (5). Observational studies suggest a potential benefit in cycles with only a few growing follicles (3,6,7). However, data on natural IVF cycles are very limited. Our study showed a clear benefit from flushing in natural and modified natural cycles, as flushing increased the oocyte retrieval rate from 41.1% (101/246) to 76% (187/246). Similarly, Mendez Lozano et al. (8) showed an increase in the oocyte yield from 46.8% to 84.6% in minimally stimulated cycles and von Wolff et al. (9) reported an increase from 44.5% to 80.5% in mono-follicular cycles. A recent randomized trial showed significant increase in the mature oocyte retrieval rate by flushing (77.1% versus 59.3%) (10). Compared to previous reports, the rate of mature oocyte retrieval was lower in our study (65%, 160/246). This might stem from the differences in patient characteristics, as the study groups were much younger in these three earlier studies as the mean female age was 33.5 (20-37), 37.0 (28-45) and 35.0 (18-42), respectively (8-10) compared to 40.1±4.6 years in our population.

Despite the suggested benefit of flushing in minimally stimulated or natural IVF cycles, there is no consensus on the optimal number of flushing attempts. When the initial aspirate does not contain the oocyte, it is likely that the very first flushing would drive the oocyte that remains in the dead space within the lumen of the needle or connecting system into the collecting tube. In a prospective study on stimulated IVF cycles, 40% of the oocytes were obtained in the primary aspirate and 41.3% in the dead space of the collecting system (11). We observed that the last flush that yielded an M-II oocyte was the fourth and an oocyte with fertilization capacity was the third. No oocytes were retrieved after the fourth flushing episode and these findings are comparable with previously published reports. Mendez Lozano et al. (8) harvested 55.5% of oocytes in the direct aspirate, and 44.5% from follicular flushing (80.3% in the first, 10.7% in the second, 5.8% in the third and 2.9% in the fourth flushing). Bagtharia and Haloob (12) reported that direct aspiration provided 40% of the oocytes and the rate was increased to 97% after two to four flushes. von Wolff et al. (9) retrieved 44.5% of oocytes in the primary aspirate, 20.7% in the first, 10.4% in the second and 4.3% in the third flush. Xiao et al. (13) was able to collect an oocyte from the ninth flushing episode but suggested that a reasonable maximum number of flushes was four. Kohl Schwartz et al. (10) reported that the majority of mature oocytes were retrieved in three flushing episodes.

Another concern related with oocytes obtained with flushing is their quality. A prospective study of 300 embryos generated from oocytes retrieved either in initial aspirate or flushing episodes showed that viability, fertilization capacity and cleavage rates were lower in oocytes harvested through flushing (11). During flushing the increase in intra-follicular pressure, longer procedure time, and change in paracrine milieu due to dilution may cause damage to the oocyte, either fracturing the zona or stripping the cumulus mass (4,14). In contrast, Kohl Schwartz et al. (10) showed no association between the number of flushes and quality of embryos [odds ratio (OR): 1.39; 95% CI: 0.93-2.11]. We observed that the last flushing episode that yielded an M-II oocyte was the fourth and for an oocyte with fertilization capacity this was the third. However, fertilization rates in oocytes obtained from the first three flushing episodes were comparable and cleavage rates in embryos generated from the oocytes retrieved in the first two flushing episodes were similar.

Study limitation

Our study has limitations due to its retrospective data collection design. As the study was based on a heterogenous group of poor responder women, the results cannot be generalized to women with good ovarian reserve undergoing natural cycle IVF.

Conclusion

Flushing confers a benefit for oocyte recovery rates in mono-follicular IVF cycles in poor responder women. However, more than three attempts at flushing were not associated with good outcome.

Acknowledgments: *The authors are grateful to the embryology unit for their valuable support during data acquisition.*

Ethics Committee Approval: *The study was approved by the Ethical Committee of the Koç University Faculty of Medicine (approval number: 2020.181.IRB1.049).*

Informed Consent: *It was obtained.*

Peer-review: *Externally peer-reviewed.*

Author Contributions: *Surgical and Medical Practices: S.E., B.B., B.U., K.Y.; Concept: K.Y.; Design: K.Y., S.E.; Data Collection or Processing: S.E., B.B., K.Y.; Analysis or Interpretation: K.Y.; Literature Search: S.E., K.Y.; Writing: K.Y.*

Conflict of Interest: *No conflict of interest is declared by the authors.*

Financial Disclosure: *The authors declared that this study received no financial support.*

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