J Gynecol Obstet Hum Reprod 50 (2021) 102150

Contents lists available at ScienceDirect

Journal of Gynecology Obstetrics and Human Reproduction

journal homepage: www.elsevier.com

Original Article

Follicular flushing using double lumen needle yields more oocytes in mono-follicular poor responders

Emre G. Pabuccu[a,*], Tufan Arslanca[a], Ugras Ucar[b], Gurhan Keles[d], Ozgul Celikel[c], Recai Pabuccu[a,d]

[a] Ufuk University School of Medicine, Department of Obstetrics and Gynecology, Ankara, Turkey
[b] Dogu Fertil IVF Center, Malatya, Turkey
[c] Yenimahalle Education and Research Hospital, Ankara, Turkey
[d] Centrum Clinic Assisted Reproduction Center, Ankara, Turkey

ARTICLE INFO

Article History:
Received 3 December 2020
Revised 19 February 2021
Accepted 14 April 2021
Available online 16 April 2021

Keywords:
Double-lumen needle
Follicular flushing
Mono-follicular
Oocyte retrieval
Poor-responder
IVF

ABSTRACT

Objective: To compare the efficacy of follicular flushing (FF) with double lumen needle and direct follicular aspiration with single lumen needle in terms of oocyte yield in mono-follicular responder patients undergoing assisted reproduction techniques (ART).

Materials and Methods: Prospective 'quasi-experimental' study was carried out in an IVF center. Infertile women 18–42 years of age with diminished ovarian reserve who revealed a single follicle >17 mm on the day of oocyte retrieval were included in the study. Follicular flushing up to 8 times was performed in FF group using an 17-G double lumen needle. Direct follicular aspiration using a 17-G single lumen needle without FF was performed in direct aspiration group. Total numbers of collected oocytes, metaphase 2 oocytes, fertilization and pregnancy rates were compared among groups.

Results: A total of 206 women underwent oocyte retrieval procedure; 106 women were assigned to FF and 100 women into direct aspiration arm. Overall oocyte retrieval rate was 50.4% in all cases. The total oocyte retrieval rate was higher (65/106, 61.9%) in FF group, than in direct aspiration group (39/100, 39%, $p = 0.001$). Metaphase 2 oocyte rate was also higher in FF group (47/106, 44.3% vs 29/100, 29% $p = 0.04$). Fertilization rates, total number of patients with a cleavage stage embryo and grade 1 cleavage embryo were similar among the groups ($p>0.5$). Numbers of positive hCG, ongoing pregnancy and miscarriage rates were similar among groups.

Conclusion: Follicular flushing using double lumen needle in mono-follicular responder patients with diminished ovarian reserve yields more oocytes and mature oocytes than direct aspiration of follicles.

Introduction

Assisted reproductive technology has evolved to a great extent since it was first introduced. Historically, laparoscopy was used to obtain oocytes and beginning from the 1980s, transvaginal retrieval procedure by specialized needles under sonographic guidance was adopted [1,2]. For this purpose, double-lumen needles were developed which allow for aspiration of the follicle through one lumen and flushing the follicle through a separate lumen avoiding mixture of the fluids. Another option for vaginal oocyte retrieval is single lumen needles allowing both aspiration and flushing procedure through a same lumen.

Since ART treatment comes with a huge economic burden, oocyte yield is utmost important for treatment success. Within this scope, several studies compared double lumen needles by assessing the beneficial impact of FF with that of single lumen in terms of oocyte yield. Even though some prospective data suggested enhanced oocyte yield by FF [3,4], several randomized controlled trials (RCTs) in unselected ART populations have failed to demonstrate favorable results and reported comparable outcomes [5-7]. Similarly, FF was not found to enhance oocyte yield and pregnancies in poor responder patients, yet considerable heterogeneity in patient populations and oocyte retrieval procedures was present in these RCTs [8,9]. Accordingly, all these data have justified implementation of a shift in clinical practice from double lumen needle to a single lumen needle in many centers particularly for costs and simplicity.

Mono-follicular responder patients represent another special population undergoing oocyte retrieval either following natural cycle or standard ovarian stimulation mostly due to severely diminished ovarian reserve. Despite few reports evaluating the efficacy of FF in mono-follicular IVF patients [10-12], there has been no solid data directly comparing double lumen needle and FF with a single lumen needle and direct follicular aspiration.

* Corresponding author: Ufuk University School of Medicine, Department of Obstetrics and Gynecology, Address: Mevlana Bulvarı No 86, Balgat, Ankara, phone: +90 532 414 7844.
E-mail address: emregpabuccu@gmail.com (E.G. Pabuccu).

https://doi.org/10.1016/j.jogoh.2021.102150
2468-7847/

Since obtaining an oocyte in mono-follicular responders is crucial, we aimed to compare the efficacy of FF using double lumen needle with that of direct aspiration using a single lumen needle in patients with a mono-follicle on the day of OPU. Primary outcome of the study is the number of collected oocytes in each study arm. Secondary outcomes include mature oocytes, fertilization, positive hCG, ongoing pregnancy and miscarriage rates.

Material and methods

This observational study was conducted in a referral IVF clinic between April 2019 and October 2020. The study was approved by the Ethics Committee (2019/04) and informed consents were provided from all participants. Study inclusion criteria were women aged between 20 and 42 years defined as poor-responders (serum AMH < 1 ng/ml) with mono-follicular growth despite standard ovarian stimulation; as having a single follicle >17 mm on the day of oocyte retrieval.

Exclusion criteria were natural cycle IVF, poor responders with more than one follicle on the day of ovulation trigger, pre-implantation genetic screening cycles, presence of hydrosalpinx or endometriosis, severe male factor infertility (oligo-astheno-teratozoospermia) and frozen-thawed embryo transfer cycles. Ovarian stimulation was carried out with antagonist protocol based on the women's age, weight, basal ovarian reserve tests and previous cycle characteristics. All participants received daily gonadotropins (rec FSH/Gonal-F, Merck-Serono with or without human menopausal gonadotropin/Menopur; Ferring) ranging from 225 IU to 450 IU/day. Ovulation was triggered with recombinant human chorionic gonadotropin (HCG) 250 mcg (Ovitrelle, Merck-Serono) when the leading follicle >17 mm.

Oocyte retrieval procedure and patient assignments into groups

The procedure was performed 34–36 h after the ovulation trigger under intravenous general anesthesia with 1% propofol (Fresenius Kabi, Homburg, Germany). **Prior to the procedure, we confirmed mono-follicular appearance for all subjects by vaginal sonography. Then, if a woman was assigned to flushing group (physician A-double lumen needle), every other person was assigned to direct aspiration (physician B-single lumen needle) in a systematic manner controlled by operating nurse. All participants were recorded in a database to overcome possible disturbances. In order to overcome inter-observer discrepancy, all oocyte pick-ups were performed by the same physician in each intervention.** A17-gauge double-lumen needle (Wallace, Cooper Surgical, Denmark) was used by physician A for follicle aspiration followed by serial flushing (3.0 ml each time) until oocyte recovery or up to eight times using a manually pressed syringe containing 50 ml of culture media pre-warmed to 37C **(FF group).** Likewise, an 17-gauge single-lumen needle (Wallace, Cooper Surgical, Denmark) was used by physician B for direct follicle aspiration without any flushing (**aspiration group**). The aspiration pressure was 140 mmHg for all procedures. Aspirated follicular fluid was collected in tubes stacked into a pre-warmed aluminum warming block. Oocyte pick-up procedures were performed by one of the two senior physicians to overcome interobserver bias.

Collected cumulus-oocyte complexes (COC) were denuded and intracytoplasmic sperm injection was performed after a 2-hour incubation. Oocyte yield, fertilization rate (proportion of pronuclear stages per women), and good quality day 3 embryo rate per women were analyzed for both groups. A fresh embryo transfer was performed on day 3 under sonographic guidance with soft catheter. Grading of the cleavage embryos was performed according to the Atlas of Human Blastocysts [13]. Luteal support was initiated on the day of oocyte retrieval and consisted of vaginal progesterone capsules 200 mg thrice daily. (Progestan, Kocak Farma). Following a positive pregnancy test, ongoing pregnancies were confirmed by presence of gestational sac with fetal heart rate on ultrasound in 6–8 weeks gestation.

Sample size calculation and statistical method

Based on a collected numbers of oocytes as primary end-point, chi-squared test estimated total of 212 patients (106 in each arm) in order to detect a risk difference (RD) of 20% between the two groups depending on a recent data [11] (two-sided, adjusted alpha-level of 0.049). The data are expressed as mean and standard deviation (SD). Baseline differences between the two groups were analyzed using Student's *t*-test. Pearson's chi-square test and Fisher's exact test were used to compare ratios between the groups. A p-value of <0.05 was considered statistically significant. The data were analyzed using SPSS for Windows (ver. 17.0; SPSS, Chicago, IL, USA)

Results

Total of 220 patients were assessed for eligibility assuming drop-outs. A total of 212 patients started ovarian stimulation and total of 206 patients were included in the final analysis (oocyte retrieval procedure); 106 in FF and 100 in direct aspiration arm. Demographic characteristics (table 1) including mean woman age, mean serum AMH level, mean BMI, TMSC ($x10^6$) and number of previous failed cycles were similar ($p>0.5$). Infertility duration was longer in direct aspiration group ($p = 0.02$).

The total oocyte retrieval rate was higher (65/106, 61.9%) in FF group, than in direct aspiration (39/100, 39%, $p = 0.001$). Metaphase 2 oocyte rate was also higher in FF group (47/106, 44.3% vs 29/100, 29% $p = 0.04$). Fertilization rates, total number of patients with a cleavage stage embryo and grade 1 cleavage embryo were also similar among the groups ($p>0.5$) (Table 2).

Positive hCG rates per oocyte retrieval was similar between the two groups (9.4% vs 7%, $P = 0.9$) (Table 2). Ongoing pregnancies per oocyte retrieval and per ET were also similar among both groups ($p>0.5$). Finally, there were two miscarriages in the flushing group (Table 3). No major complications including hemoperitoneum which requires blood transfusion, severe infection or major pain were encountered in both groups.

Discussion

This observational study suggests that FF with double lumen needle yields to more recovered oocytes and mature oocytes in mono-follicular responder patients when compared to direct follicular aspiration.

In IVF practice, it is crucial to optimize oocyte yield on the day of retrieval as more oocytes are generally correlated with increased treatment success to some extent [14]. In theory, it can be assumed

Table 1
Basic demographics of the groups.

	Flushing (*N* = 106)	Direct aspiration (*N* = 100)	P value
Age (years)	36.4 ± 4	36.7 ± 4.4	0.82
BMI (kg/m^2)	21.9 ± 2.3	22.4 ± 1.7	0.10
Infertility duration	6.8 ± 4.2	8.4 ± 5.4	**0.02**
Previous failed ART cycle	1 (0–6)	1(0–9)	0.32
Serum AMH (ng/ml)	0.49 (0.03–1)	0.5 (0.01–1)	0.64
Antral follicle count (AFC)	1 (1–3)	1 (1–3)	0.5
TMSC ($x10^6$)	14 (1–154)	18 (1–232)	0.12

The data are expressed as mean and standard deviation (SD) or median (min-max).
BMI: body mass index.
TMSC: total motile sperm count.

Table 2
Ovarian stimulation outcomes of the groups.

	Flushing (*N* = 106)	Direct aspiration (*N* = 100)	P value
Total stimulation (days)	10.1 ± 2.7	10.5 ± 2.5	0.27
Cumulative gonadotropin dose (IU)	3896±1378	4055±974	0.33
Progesterone on the day of hCG (ng/dl)	0.68±0.2	0.72±0.3	0.26
Total No of patients with an oocyte (%)	65 (61.9)	39 (39)	**0.001**
Total No of patients with M2 oocyte (%)	47 (44.3)	29 (29)	**0.04**
COC after first flushing (%)	34 (32)	NA	
No of follicular flushing needed in patients with COC	3 (1–8) 2.6 ± 1.4	NA	
Total No of fertilized oocytes (%)	38/47 (80)	23/29 (79.3)	0.13
Total No of patients with cleavage embryo (%)	30 (25.4)	20 (20)	0.15
Total No of patients with grade 1 cleavage embryo (%)	19 (17.9)	10 (10)	0.09

The data are expressed as mean and standard deviation (SD) or median (min-max).
COC: cumulus-oocyte complex.
M2: metaphase 2 oocytes.

that by FF with a proper pressure, it might be possible to detach the cumulus-oocyte complex from the follicular wall. However, this theory has not been translated into practice as studies comparing the FF with double lumen needles with direct follicular aspiration through a single lumen needle failed to prove superiority of the concept at least in unselected patient population [5-7].

Poor responders account up to 24% of patients undergoing ovarian stimulation for IVF meaning that up to one in four patients conceals a poor reproductive prognosis [15,16]. After introduction of the Bologna criteria in order to attempt to reduce the heterogeneity of the cohort, much lower (5–7%) live birth rates were reported [17]. Oocyte yield is quite critical in this population as one additional oocyte is associated with 2-fold higher pregnancy rates per started IVF cycle [14,18]. In this context, efficacy of FF was questioned in heterogeneous poor responder patients. Retrospective studies reported favorable outcomes with FF [19,20], however RCTs [8,9] and a meta-analysis [21] failed to reveal any beneficial effects. Despite proper study designs, there was considerable heterogeneity with respect to the type of aspiration needle used, the volume of flushing medium, application pressures and the number of flushes in above mentioned studies.

Table 3
Pregnancy outcomes of the groups.

	Flushing	Direct aspiration	P value
Positive hCG per started cycle (%)	10/109 (9.1)	7/103 (6.8)	0.85
Positive hCG per oocyte retrieval (%)	10/106 (9.4)	7/100 (7)	0.91
Ongoing pregnancies per oocyte retrieval (%)	8/106 (7.5)	7/100 (7)	0.54
Ongoing pregnancies per ET (%)	8/30 (26.6)	7/20 (35)	0.62
Miscarriage per ET (%)	2/30 (6.6)	0	0.16
Cycle cancelation before oocyte retrieval	3 (2.7)	3 (3)	0.5

ET: embryo transfer.

A group of patients with a mono-follicular growth despite conventional ovarian stimulation obviously represent the group with poorest prognosis. In our patient population, only one adequate follicle was achieved despite very long stimulation and high gonadotropin consumption as nearly 4000 IUs in each study group. It is obvious that such patient population is suffering from severely diminished ovarian reserve. Our stimulation policy with higher doses and duration in the light of recent recommendation in favor of mild stimulations [22] can be criticized. However, every poor prognosis patient should be counselled and considered individually, particularly in private IVF settings, moreover this discussion is beyond the scope of this study. It can be assumed that, this subset of poor responders represent a challenging cohort and should be separated from other poor responder groups

In the literature, there are some studies evaluating those with mono-follicular IVF (Table 4). Mendez-Lozano et al. reported higher oocyte yield following FF in poor responder patients managed with semi natural cycle IVF [12]. Likewise, Van Wolff et al. reported two fold higher oocyte yield and transferrable embryos with FF [10]. The same group conducted a well-designed adequately powered RCT directly comparing aspiration and FF in their mono-follicular IVF patients after gonadotropin free ovarian stimulation [11]. Higher oocyte yield, mature oocytes and fertilization rates were noted with FF. In all of the above mentioned studies and in the present study, a great proportion of oocytes were obtained after first flushing. In our study, higher oocyte yield was noted with FF with mean of 3 flushing and more than 80% of oocytes were collected after initial aspiration and first flushing as this finding is consistent with the recent data [23]. However the FF system was slightly different in our study from the recent RCT [11] as; we used double lumen needle for FF whereas they used single lumen needle either for direct aspiration or for FF. Flushing the follicle by a single lumen needle obviously comes with a risk of retained oocyte in the aspiration needle or in the tubbing during the suction-flushing. However, authors explained this point by clearing the dead space in the needle and tubing system by using additional fluid. Whether FF with a single lumen needle with a proper technique or FF using double lumen needle is effective for oocyte retrieval is another point to be discussed.

Initial reports revealed decreased fertilization and cleavage rates, and viability of oocytes obtained through FF [3,4]. Latter studies did not support these findings as they reported similar fertilization rates for mature oocytes [8,9,22,24]. Likewise, fertilization and cleavage rates were not compromised in mono-follicular IVF patients, on the contrary better outcomes were reported [11,12]. We also demonstrated similar fertilization and grade 1 cleavage embryo rates among the groups. Better oocyte yield in the FF group did not translate into better pregnancy rates despite comparable sperm analysis characteristics in our analysis. Relatively lower pregnancy rates in our cohort can be explained by severely diminished ovarian reserve of patients.

Our study was limited by the lack of proper randomization. Furthermore, we did not record the duration of oocyte aspiration systematically and not measured overall pain scores. Moreover, infertility duration of patients in the single lumen needle arm is longer than the other group. It is obvious that homogenous populations should be compared to clarify the hypothesis. On the other hand, the two groups were statistically similar with regard to all other basic parameters such as women age, ovarian reserve tests, sperm parameters and BMI.

In conclusion, FF using double lumen needle in mono-follicular responder patients with severely diminished ovarian reserve yield more oocytes and mature oocytes than direct aspiration of follicles.

Declaration of Competing Interest

None

Table 4
Effect of follicular flushing in mono-follicular IVF patients.

	Design	Population	Outcomes
Lozano et al. 2008	Observational	Poor responders, mild stimulation	Oocyte yield increased with FF (46% to 84%)
Von Wolff et al. 2012	Observational	mild stimulation	Oocyte yield increased with FF (44.5% to 80.5%)
Kohl Schwartz et al. 2020	RCT	Natural cycle	Oocyte yield increased with FF (20.6% risk difference)

RCT: randomized controlled trial.
FF: follicular flushing.

Acknowledgements

None

References

[1] Lenz S, Lauritsen JG, Kjellow M. Collection of human oocytes for in vitro fertilisation by ultrasonically guided follicular puncture. Lancet 1981;1:1163–4.
[2] Wikland M, Enk L, Hamberger L. Transvesical and transvaginal approaches for the aspiration of follicles by use of ultrasound. Ann N Y Acad Sci 1985;442:182–94.
[3] El Hussein E, Balen AH, Tan SL. A prospective study comparing the outcome of oocytes retrieved in the aspirate with those retrieved in the flush during transvaginal ultrasound directed oocyte recovery for in-vitro fertilization. Br J Obstet Gynaecol 1992;99:841–4.
[4] Waterstone JJ, Parsons JH. A prospective study to investigate the value of flushing follicles during transvaginal ultrasound-directed follicle aspiration. Fertil Steril 1992;57:221–3.
[5] Haydardedeoglu B, Cok T, Kilicdag EB, Parlakgumus AH, Simsek E, Bagis T. In vitro fertilization- intracytoplasmic sperm injection outcomes in single- versus double-lumen oocyte retrieval needles in normally responding patients: a randomized trial. Fertil Steril 2011;95:812–4.
[6] Wongtra-Ngan S, Vutyavanich T, Brown J. Follicular flushing during oocyte retrieval in assisted reproductive techniques. Cochrane Database Syst Rev 2010;9:CD004634.
[7] Roque M, Sampaio M, Geber S. Follicular flushing during oocyte retrieval: a systematic review and meta-analysis. J Assist Reprod Genet 2012;29:1249–54.
[8] Haydardedeoglu B, Gjemalaj F, Aytac P, Kılıcdag E. Direct aspiration versus follicular flushing in poor responders undergoing intracytoplasmic sperm injection: a randomized controlled trial. BJOG 2017;124:1190–6.
[9] Mok-Lin E, Brauer AA, Schatmann G, Zaninovic N, Rosenwaks Z. Follicular flushing and in vitro fertilization outcomes in the poorest responders: a randomized controlled trial. Hum Reprod 2013;28(11):2990–5.
[10] von Wolff M, Hua Y-ZZ, Santi A, Ocon E, Weiss B. Follicle flushing in monofollicular in vitro fertilization almost doubles the number of transferable embryos. Acta Obstet Gynecol Scand 2013;92(3):346–8. doi: 10.1111/aogs.12054.
[11] Kohl Schwartz AS, Calzaferri I, Roumet M, Limacher A, Fink A, Wueest A, et al. Follicular flushing leads to higher oocyte yield in monofollicular IVF: a randomized controlled trial. Hum Reprod 2020;35(10):2253–61 Oct 1. doi: 10.1093/humrep/deaa165.
[12] Mẽndez Lozano DH, Fanchin R, Chevalier N, Feyereisen E, Hesters L, Frydman N, et al. The follicular flushing duplicate the pregnancy rate on semi natural cycle IVF. J Gynecol Obstet Biol Reprod (Paris) 2007;36:36–41.
[13] Veeck LL, Zaninovic N. An atlas of human blastocysts. 1st edn. FL, USA: CRC Press; 2003.
[14] Sunkara SK, Rittenberg V, Raine-Fenning N, Bhattacharya S, Zamora J, Coomarasamy A. Association between the number of eggs and live birth in IVF treatment: an analysis of 400 135 treatment cycles. Hum Reprod 2011;26(7):1768–74 Juldoi: 10.1093/humrep/der106. Epub 2011 May 10.
[15] Ubaldi FM, Rienzi L, Ferrero S, et al. Management of poor responders in IVF. Reprod Biomed Online 2005;10:235–46.
[16] Vaiarelli A, Cimadomo D, Ubaldi N, et al. What is new in the management of poor ovarian response in IVF. Curr Opin Obstet Gynecol 2018;30:155–62.
[17] Polyzos NP, Nwoye M, Corona R, et al. Live birth rates in Bologna poor responders treated with ovarian stimulation for IVF/ICSI. Reprod Biomed Online 2014;28 469 74.
[18] Oudendijk JF, Yarde F, Eijkemans MJ, Broekmans FJ, Broer SL. The poor responder in IVF: is the prognosis always poor? a systematic review. Hum Reprod Update 2012;18(1):1–11. doi: 10.1093/humupd/dmr037.
[19] Souza A, Sampaio M, Noronha G, Coster L, de Oliveira R, Geber S. Effect of follicular flushing on reproductive outcomes in patients with poor ovarian response undergoing assisted reproductive technology. J Assist Reprod Genet 2017;34:1353–7.
[20] Xiao Yu, Wang Yong, Wang Min, Liu Kai. Follicular Flushing Increases the Number of Oocytes Retrieved in Poor Ovarian Responders Undergoing in Vitro Fertilization: a Retrospective Cohort Study. BMC Womens Health 2018;18(1):186. Nov 16.
[21] Neumann K, Griesinger G. Follicular flushing in patients with poor ovarian response: a systematic review and meta-analysis. Reprod Biomed Online 2018;36(4):408–15. doi: 10.1016/j.rbmo.2017.12.014.
[22] Comparison of pregnancy rates for poor responders using IVF with mild ovarian stimulation versus conventional IVF: a guideline. Practice Committee of the American Society for Reproductive Medicine. Electronic address: aSRM@asrm.org. Fertil Steril 2018;109(6):993–9 Jundoi: 10.1016/j.fertnstert.2018.03.019.
[23] Neyens S, De Neubourg D, Peeraer K, De Jaegher N, Spiessens C, Debrock S, et al. Is There a Correlation between the Number of Follicular Flushings, Oocyte/Embryo Quality and Pregnancy Rate in Assisted Reproductive Technology Cycles? Results from a Prospective Study. Gynecol Obstet Invest 2016;81(1):34–40. doi: 10.1159/000434750.
[24] von Horn K, Depenbusch M, Schultze-Mosgau A, Griesinger G. Randomized, open trial comparing a modified double-lumen needle follicular flushing system with a single-lumen aspiration needle in IVF patients with poor ovarian response. Hum Reprod 2017;32:832–5.