

Follicular Flushing Versus Direct Aspiration at Oocyte Retrieval in Poor Responders Undergoing *in vitro* Fertilization: A Randomized Controlled Trial

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ABSTRACT

Aim: This study aims to study the effect of follicular flushing at oocyte retrieval on Assisted Reproductive Technique (ART) outcomes in poor responders undergoing *in vitro* fertilization. **Settings and Design:** A prospective randomized controlled trial was conducted in the ART center of our hospital. **Materials and Methods:** A total of 71 patients who responded poorly during controlled ovarian stimulation were recruited. Patients were randomized to follicular flushing or to direct aspiration group. The primary outcomes of the study were the total number of oocytes retrieved and the number of metaphase (M II) oocytes retrieved. Secondary outcomes were anesthesia time, procedure time, fertilization rate, cleavage rate, total number of embryos, number of embryos transferred, number of Grade 1 embryos, failed oocyte recovery, failed fertilization, implantation rate, clinical pregnancy rate, miscarriage rate, and live birth rate. **Statistical Analysis Used:** Chi-square test and Student's *t*-test. **Results:** The total number of oocytes retrieved, number of M II oocytes, fertilization rate, cleavage rate, total number of embryos, number of Grade 1 embryos, failed oocyte recovery, failed fertilization, implantation rate, miscarriage rate, and live birth rate were comparable between the two groups. The anesthesia and procedure time was significantly higher in the flushing group. **Conclusions:** Follicular flushing does not result in a significant improvement in the ART outcomes despite increasing procedure and anesthesia times. Trial registration number CTRI/2017/07/009062.

KEYWORDS: Double lumen needle, follicular flushing, oocyte retrieval, poor responders

INTRODUCTION

Oocyte retrieval is the most crucial step in the process of *in vitro* fertilization (IVF). It is extremely important that every attempted follicular aspiration result in the recovery of an undamaged oocyte and to retrieve as many oocytes as possible since the overall success rate of IVF depends on the number of oocytes obtained. Despite advances in assisted reproductive techniques, poor ovarian response is still a challenge for clinicians and embryologists alike. Strategies to improve pregnancy rates in poor responders include modifying stimulation

protocols, improving techniques of oocyte recovery, or advanced techniques in handling gametes or embryos. Follicular flushing has been suggested in poor responders to overcome the chances for oocyte retention within ovarian follicles and the retrieval collection system. Data from randomized studies suggested that follicular

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flushing did not improve oocyte recovery in patients with optimal follicular development. On the contrary, it increased operative and anesthesia time.^[1,2] The largest of the studies have excluded women with poor response to controlled ovarian stimulation (COS).^[3] Results on follicular flushing in poor responders are limited as there are fewer studies as compared to normal responders and further, there were limitations in defining the so-called poor responders. The recent meta-analysis including three trials, suggests that there is little benefit as regards improvement in the number of oocytes retrieved or pregnancy rates. There was a paucity of randomized trials as regards flushing with a need for further trials to conclude on the same. To address the lack of evidence on the utility of follicle flushing, we performed this randomized trial on a defined group of poor responders in the hope of adding further data to the literature.

MATERIALS AND METHODS

The study was conducted in the Reproductive Medicine Unit of our hospital over 3 years from January 2014 to December 2016. The study was approved by the Institute's Ethics Committee (Institute Review Board), and all participating women consented prior to the study after a written informed consent. Women aged between 22 and 38 years, having 3–5 follicles ≥ 14 mm on the day of human chorionic gonadotropin (hCG) trigger and a normal uterine cavity were included in the study. Patients with \leq two follicles on the day of trigger or those with ovarian endometrioma were excluded from the study. All participants underwent COS by long agonist or antagonist protocol. Patients who underwent long protocol were started on GnRH-a (leuprolide) 0.5 mg on day 21 of the previous cycle. Once downregulated (serum estradiol < 40 pg/ml, LH < 3 IU/l, no follicles > 10 mm and endometrial thickness < 4 mm), gonadotropin (Recombinant follicle stimulating hormone [FSH]-Gonal F; Merck Serono) was started at doses ranging from 375 to 450 IU per day. Patients who underwent antagonist protocol were started on Recombinant FSH (Gonal F, Merck Serono) from day 2 of the menstrual cycle. Follicle monitoring was started from day 5 of stimulation. GnRH antagonist (Cetrotide, Serono laboratories) was initiated when lead follicle measured 14 mm. Serial follicle tracking was done to assess the ovarian response to stimulation, and accordingly, gonadotropin doses were adjusted. All patients were triggered with recombinant hCG (250 mcg, Ovitrel; Merck Serono) when there were at least 2 follicles ≥ 18 mm. The number and the size of all the follicles were documented on the day of the trigger. Serum estradiol and progesterone were estimated and patients were randomized to undergo follicular

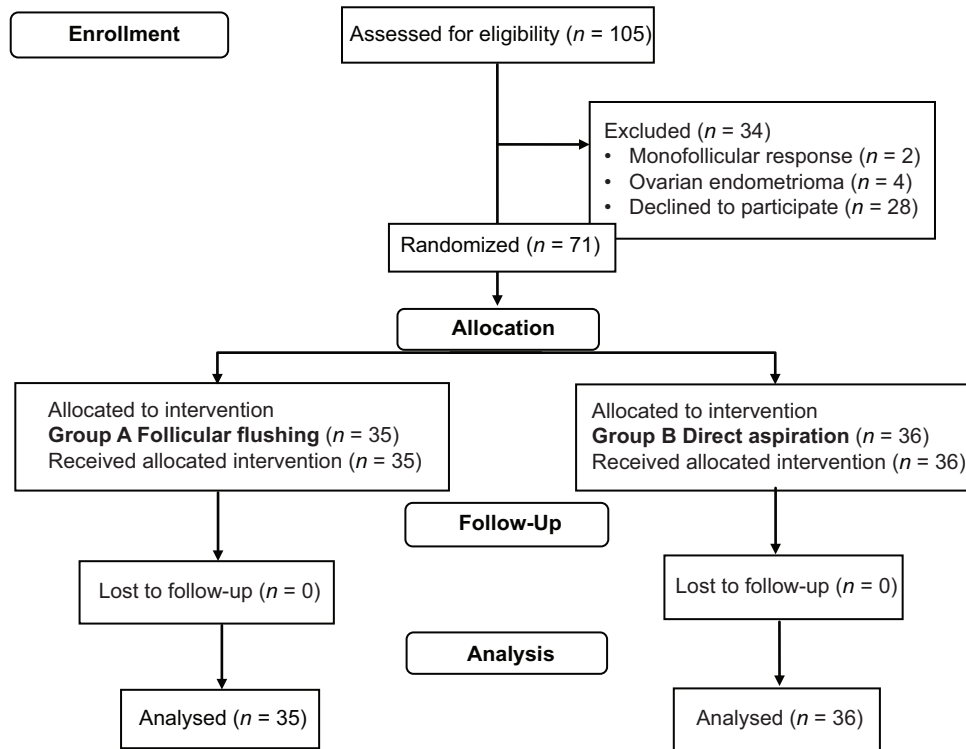
flushing (Group A) and direct aspiration (Group B), through computer-generated random numbers and allocations with concealment were done.

Oocyte retrieval was done 34–36 h after hCG trigger under short general anesthesia. In patients who were randomized to the flushing group, a double lumen needle of 17-gauge was used. Oocyte retrieval was done under transvaginal ultrasound guidance, with a suction pressure of 160–180 mm Hg. Two milliliter of flush with culture medium (Vitrolife Sweden AB Göteborg, Sweden) was used each time if no oocyte was retrieved at the direct aspiration. In case no oocyte was retrieved at first flush, further flushes were done up to a maximum of three flushes before moving to the next follicle. In women randomized to direct aspiration, oocyte retrieval was done as the standard procedure using a single lumen needle of 17 gauge with a suction pressure of 100–110 mm Hg. Retrieved oocytes were inseminated or injected with husband's spermatozoa by conventional IVF or intracytoplasmic sperm injection. Fertilization check was done 16–18 h after insemination. Normal fertilization was defined as the presence of two pronuclei and extrusion of the second polar body. Further cleavage was assessed, and embryos graded as per Istanbul's consensus.^[4]

All women underwent fresh embryo transfer. None of the patients had supernumerary embryos for cryopreservation, considering the patient profile in our study. Up to a maximum of two good quality embryos were transferred on day 3 or 5 under ultrasound guidance using a soft embryo transfer catheter (Cook's medical Sydney, Australia). Luteal support was given in the form of micronized progesterone 100 mg daily intramuscular injections (Injection Susten, Sun Pharma, India). Serum β hCG was checked 16 days after embryo transfer, and those with a positive β hCG were confirmed for clinical pregnancy by sonography 4 weeks after embryo transfer.

The primary outcome measures of the study were the total number of oocytes retrieved and the number of M II oocytes retrieved. Secondary outcomes were anesthesia time, procedure time, fertilization rate, cleavage rate, the total number of embryos, number of embryos transferred, number of Grade 1 embryos, failed oocyte recovery, failed fertilization, implantation rate, clinical pregnancy rate, miscarriage rate, and live birth rate.

Fertilization rate was defined as the number of fertilized oocytes to the total number of oocytes retrieved. Implantation rate was defined as the number of gestational sacs determined by ultrasound by the number of embryos transferred. The clinical pregnancy rate was defined as the presence of a gestational sac with a fetal pole with cardiac activity on transvaginal ultrasound at



Flow diagram 1: Consort flow diagram

6 weeks. Miscarriage rate was defined as the number of pregnancy losses < 20 weeks of gestation. Live birth rate was defined as the percentage of all cycles that lead to live birth and is the pregnancy rate adjusted for miscarriages and stillbirths.

Considering the previous study by Moklin *et al.*, with a power of 80% and 5% level of significance, the sample size was calculated to be 35 in each group.

Statistical analysis

Statistical analysis was carried out using Stata 12a. 0 (Stata Corp LP, College Station, Texas, USA). Data were presented as number (percentage), mean \pm standard deviation, or median (min-max) as appropriate. The baseline categorical and continuous variables were compared between the two groups using Chi-square test and Student's *t*-test for independent samples/Wilcoxon rank-sum test, respectively. The primary outcomes were compared between the two groups using Wilcoxon rank-sum test since the data were not following a normal distribution. The secondary outcomes were compared using the Chi-square test and Fisher's exact test for categorical variables and Wilcoxon rank-sum test for continuous variables. The *p* value < 0.05 was considered statistically significant.

RESULTS

During the 3-year study, a total of 105 poor responder patients were assessed for eligibility. Twenty-eight

patients were not willing to participate in the study. Two patients had monofollicular response, and four had ovarian endometrioma. Seventy-one patients consented and were randomized into follicular flushing (Group-A) and direct aspiration (Group-B) with 35 patients in Group A and 36 patients in Group B. Of the 71 women, 25 were defined poor responders (as per Bologna criteria) while 46 presented with unexpected poor response (tubal and unexplained with normal ovarian reserves) [Flow diagram 1].

Baseline characteristics

The two groups were comparable with respect to baseline characteristics such as age, BMI, the cause of infertility, day 2 serum FSH, serum anti-Mullerian hormone, ovarian antral follicle count, and response to COS [Table 1].

Primary outcome measures

The total number of oocytes retrieved and the number of metaphase II (M II) oocytes were not significantly different between the groups [Table 2].

Secondary outcome measures

The follicular flushing group required more duration of anesthesia and procedure time ($p < 0.001$). The fertilization rate was not significantly different between the two groups. The total number of embryos, cleavage rate, the number of Grade 1 embryos, and the number of embryos transferred were comparable between the

Table 1: Baseline characteristics

Population characteristics	Follicular flushing (n=35)	Direct aspiration (n=36)	p
Age (years) ^a	32.0 ± 3.9	32.5 ± 4.1	0.616
BMI (kg/m ²) ^a	25.4 ± 3.4	24.7 ± 2.7	0.242
Day 2 FSH (mIU/ml) ^a	7.4 ± 1.8	7.6 ± 1.8	0.713
AMH (ng/ml) ^a	1.7 ± 0.7	1.7 ± 0.7	0.676
AFC ^a	8.6 ± 4.4	8.1 ± 4.5	0.643
Cause of infertility, n (%) ^b			
Diminished ovarian reserve	11 (31.4)	14 (38.9)	0.796
Tubal	19 (54.3)	18 (50)	
Unexplained	5 (14.3)	4 (11.1)	
Poor ovarian response, n (%) ^b			
Expected	11 (31.4)	14 (38.9)	0.511
Unexpected	24 (68.6)	22 (61.1)	
Protocol, n (%) ^b			
Agonist	22 (62.9)	24 (66.7)	0.737
Antagonist	13 (37.1)	12 (33.3)	
Starting dose of gonadotropins (IU) ^a	346.4 ± 48.4	340.6 ± 59.3	0.654
Total dose of gonadotropins (IU) ^a	4492.3 ± 1126	4087.6 ± 1075.7	0.126
Total duration of stimulation (days) ^a	11.8 ± 1.3	11.6 ± 1.3	0.608
Follicles on the day of trigger ^a	5.6 ± 1.1	5.5 ± 1.3	0.806
Serum estradiol on day of trigger (pg/ml) ^a	1542.6 ± 645.2	1693.9 ± 786.9	0.379
Serum progesterone on day of trigger (ng/ml) ^a	1.5 ± 1.2	2.2 ± 1.8	0.068
ET on day of trigger (mm) ^a	9.3 ± 1.7	9.0 ± 1.6	0.558

Data were presented as ^aMean±SD, ^bn (%). BMI=Body mass index, FSH=Follicle stimulating hormone, AMH=Anti-Müllerian hormone, AFC=Antral follicle count, SD=Standard deviation, ET=Endometrial thickness

two groups. The occurrence of failed oocyte recovery and failed fertilization was not significantly different between the groups. The clinical pregnancy rate was significantly higher in Group A. The implantation rate, miscarriage rate, and live birth rates were comparable between the two groups [Table 2].

DISCUSSION

It has been hypothesized that patients who perform poorly to COS might benefit from follicular flushing to retrieve more oocytes. This benefit is thought to occur because cumulus-oocyte complexes in poor responding women have less luteinizing hormone receptor responsiveness and oocyte may not be released from the follicle wall as easily compared to women who are normal responders.^[5] Failure of retrieval of

cumulus-oocyte complex from a given follicle is most likely a problem of maturity and healthiness of oocyte, cumulus cells, and mural granulosa cells, rather than technically insufficient aspiration procedure.^[6] So even if one or two more oocytes are retrieved as a result of the flushing process, it is not necessary that the extra oocytes retrieved are mature and are capable of fertilization. Our study has shown that follicular flushing does not result in a significant improvement in the assisted reproductive technique outcomes in poor responders. Flushing did not result in any significant improvement in the total number of oocytes retrieved and the number of M II oocytes. The significantly higher clinical pregnancy rates in the flushing group may be due to mere chance. The results of our study were similar to those observed in the previous studies.^[6,7] The significant increase in the procedure duration and anesthesia requirement noted in our study is in agreement with numerous previous studies and is undebatable.^[6-9] Increase in the anesthesia and procedure times can also be detrimental to the oocyte quality.^[9] Follicular flushing might remove some of the granulosa cells, which might serve as a potential source of hormones for luteal support. The use of high pressures during flushing with double lumen needles might result in cracking of the zona pellucida. The presence of residual flushing fluid in the pelvis might result in an altered endometrial milieu at the time of implantation as well as a focus of infection. Flushing also unnecessarily increase the cost of the cycle by the use of double lumen needles and the use of much more flushing media.

The strengths of the present study are that it is a randomized controlled trial and that we have followed the patients up to live birth. Furthermore, we have included women who had normal ovarian reserve tests but had an ongoing poor response, the so-called unexpected poor responders which made both the groups inhomogeneous in their profile to begin with but were ultimately similar with their inclusion criteria.

The limitations of our study were small sample size. Further, the sample size was calculated based on the number of oocytes retrieved. A large sample size calculated based on live birth would make more robust evidence but would require a huge sample size. This may not have been accomplished at a single center and in the given time frame in which we conducted our study.

CONCLUSIONS

Follicular flushing should not be considered in poor responders as it does not result in a significant improvement in the live birth rates but may be tried as a

Table 2: Assisted reproductive technology outcomes in poor responders undergoing follicular flushing and direct aspiration

Primary outcomes	Follicular flushing Group A (n=35)	Direct aspiration Group B (n=36)	p	Difference between means or proportion	95% confidence limits	
					Lower limit	Upper limit
Total number of oocytes retrieved ^c	4.5 ± 1.7	3.7 ± 1.9	0.066	0.79	-0.05	1.6
Total number of MII oocytes ^c	2 (2-3)	2.5 (1-3)	0.907	-	-	-
Secondary outcomes						
Fertilization rate ^a	0.73 ± 0.29	0.68 ± 0.34	0.537	0.05	-0.10	0.19
Cleavage rate ^c	1 (0-1)	1 (0-1)	0.716	-	-	-
Total number of embryos ^c	4 (2-4)	3 (2-4)	0.073	-	-	-
Number of Grade 1 embryos ^c	3 (2-3)	2 (1-3)	0.075	-	-	-
Number of embryos transferred ^a	2.6±1.1	2.2 ± 1.2	0.148	0.41	-0.15	0.96
Failed oocyte recovery, n (%) ^b	2/35 (5.7)	4/36 (11.4)	0.421	-0.054	-0.187	0.079
Failed fertilization, n (%) ^b	1/35 (2.8)	0/36 (0)	0.328	-0.028	-0.084	0.028
Implantation rate, n (%) ^b	8/74 (10.8)	2/72 (2.8)	0.098	8	0	17.4
Clinical pregnancy rate, n (%) ^b	8/35 (22.9)	2/36 (5.6)	0.046	17.3	0.007	34.0
Miscarriage rate, n (%) ^b	4/35 (11.4)	0/36 (0)	0.054	11.4	0	26.0
Live birth rate, n (%) ^b	4/35 (11.4)	2/36 (5.6)	0.429	5.9	0	20.9
Anesthesia time ^a	36.7 ± 8.6	19.1 ± 3.7	<0.001	17.6	14.5	20.7
Procedure time ^a	8.2 ± 3.4	3.8 ± 1.5	<0.001	4.4	3.2	5.6

Data were presented as ^aMean±SD, ^bn (%), ^cMedian (minimum–maximum). SD=Standard deviation, MII=Metaphase II

last resort before declaring the patient of a total failure of oocyte recovery for the psychological satisfaction of the clinician and the patient. More studies are required with a large sample size and live birth rate as the primary outcome, which can be accomplished only by a multicenter trial.

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Conflicts of interest

There are no conflicts of interest.

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