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Direct aspiration versus follicular flushing in poor responders undergoing intracytoplasmic sperm injection: a randomised controlled trial

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Objective To compare follicle flushing three times with direct follicular aspiration in poor responders. Our hypothesis was that follicle flushing three times in poor responders would result in similar oocyte yield compared with direct aspiration in poor responders.

Design A randomised controlled trial performed between January 2015 and June 2015.

Setting University hospital.

Population or sample Eighty eligible poor responders, who were defined as having five or fewer follicles ≥13 mm in average diameter with at least two follicles having maximum diameters >17 mm on the day of human chorionic gonadotrophin administration. Monofollicular cycles, including natural cycles, were excluded from the current trial.

Methods In the double-lumen needle group, oocyte retrieval was performed by flushing three times with 2 ml in each follicle and in the single-lumen group direct follicle aspiration was performed.

Main outcome measure Number of metaphase II oocytes retrieved.

Results The mean number of metaphase II oocytes was similar in both groups (1.9 ± 0.1 versus 2.1 ± 0.1, respectively). The clinical pregnancy and live birth rates were similar in both groups (32.5% versus 25% and 25% versus 22.5%, respectively). The only significant difference between the two groups was the duration of oocyte retrieval (178.4 ± 13.4 versus 236.3 ± 24.1 seconds, respectively, $P = 0.01$).

Conclusion Follicular flushing is time consuming and has similar results compared with direct follicle aspiration in poor responders.

Keywords follicular flushing, *in vitro* fertilisation/ intracytoplasmic sperm injection, live birth rate, metaphase II oocyte, poor responders.

Tweetable abstract Direct follicle aspiration versus flushing in poor responders yields similar metaphase II oocytes.

Linked article This article is commented on by JM Franasiak, p 1197. To view this mini commentary visit https://dx.doi.org/10.1111/1471-0528.14628.

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Introduction

Transvaginal oocyte retrieval for assisted reproductive technologies (ART) is a routine procedure performed under ultrasound guidance. A 2001 survey reported that >50% of ART clinics performed routine follicular flushing.[1] In theory, double-lumen retrieval needles are more effective than direct aspiration, although two recent meta-analyses found that both needles were similar and capable of collecting oocytes within follicles.[2,3] Previously, we showed that follicular flushing is not superior to direct aspiration in normally responding patients in the largest reported series.[4] Numerous studies have shown that direct follicle aspiration shortens the oocyte retrieval procedure time and has similar oocyte yield compared with follicular flushing in normally responding patients.[5–8] Based on these findings, most ART clinics have changed to direct aspiration needles. However, the efficacy of follicular flushing was studied in normally responding

Trial registration number NCT 02391155.

patients. There was no proper randomised study in poor responders for the evaluation of oocyte retrieval needle comparison until the trial by Mok-Lin et al.[9]

The randomised study of Mok-Lin et al. examined the effects of follicular flushing in poor responders and showed that flushing may lead to detrimental effects in terms of a lower pregnancy rate because of the collection of poor-quality oocytes, although the total number of retrieved oocytes was similar.[9] Therefore, we aimed to compare in a randomised trial the oocyte retrieval rate after follicular flushing using a double-lumen retrieval needle with direct follicle aspiration needle in poor responders. Our hypothesis was to accept that follicle flushing three times in poor responders would result in a similar oocyte yield compared with direct aspiration in poor responders.

Methods

We conducted an open-label, randomised trial in the Division of Reproductive Endocrinology and IVF Unit, Department of Obstetrics and Gynaecology, Baskent University Adana, Turkey, from January 2015 to June 2015. The study was approved by the Ethics Committee of Baskent University (project number 13/161) and by the Turkish Ministry of Health, Drug and Medical Equipment Department. The study was registered at clinicaltrials.gov (NCT 02391155) with the official title of 'Follicular Flushing Outcomes in Single- versus Double-lumen Oocyte Retrieval Needles in Poor Responding Patients.' Informed consent was obtained from each patient on the day of oocyte retrieval. The first patient was enrolled to the study at 11 February 2015.

The study inclusion criteria were women aged 20–43 years; poor ovarian response was defined as having five or fewer follicles ≥13 mm in size on the day of human chorionic gonadotrophin (hCG) administration; serum progesterone level <1.5 ng/ml on the day of hCG administration; and known poor functional ovarian reserve to predict a poor ovarian response to gonadotrophin stimulation diagnosed by an antral follicle count (AFC) <6 in both ovaries together with an anti-Müllerian hormone (AMH) level <0.8 ng/ml.

The study exclusion criteria were monofollicular ovarian response; natural *in vitro* fertilisation (IVF) cycle programme, and presence of ovarian endometrioma.

The controlled ovarian stimulation protocols were based on the women's age, weight, AFC, AMH level and previous cycle characteristics. Approximately half of the poorly resonding women (poor responders) took part in a low-dose luteal gonadotrophin-releasing hormone (GnRH) agonist programme, which consisted of a luteal start with 0.5 mg leuprorelin acetate (Lucrin; Abbott, Paris, France) until day 2 or 3 following menses, when baseline ultrasound scanning and blood testing were performed. After ovarian suppression was achieved, the dose was reduced to 0.25 mg until the day of 10 000 IU of hCG administration. If there were no cysts ≥2 cm and the estradiol (E_2) level was <50 pg/ml, then gonadotrophin stimulation with 300 IU (Puregon; MSD, Oss, the Netherlands) was performed. Ultrasound and blood E_2 monitoring continued until the administration of 10 000 IU of hCG when at least two follicles have reached maximum diameters >17 mm.

The GnRH antagonist protocol involved the administration of letrozole + recombinant follicle-stimulating hormone (FSH) group. On day 2 or 3 of menses, letrozole 5 mg/day (Femara; Novartis, Basel, Switzerland) was started and continued for 5 days. The administration of gonadotrophins was started on the same day with 150 IU recombinant FSH (Puregon; MSD) plus 150 IU pure human menopausal gonadotrophin (hMG) (Menopur; Ferring Pharmaceuticals, Lozan Saint-Prex, Switzerland). A GnRH antagonist (Orgalutran; MSD) was added to this regimen when the leading follicle had reached 14 mm. Ultrasound and blood E_2 monitoring continued until the hCG administration criterion was met, with at least two follicles having a maximum diameter of >17 mm.

Only four patients were placed on the FSH + pure hMG/GnRH antagonist protocol, which administered gonadotrophins and started on day 2 or 3 of menses with 150 IU recombinant FSH (Puregon; MSD) plus 150 IU pure hMG (Menopur; Ferring Pharmaceuticals). A GnRH antagonist (Orgalutran; MSD) was added to this regimen when the leading follicle reached 14 mm. After the leading follicle reached >17 mm, 10 000 IU of hCG (Pregnyl ampoule; MSD) and 0.2 mg/ml triptorelin were injected.

Using a random numbers table, the 80 eligible patients were assigned randomly to the single-lumen (direct aspiration) or double-lumen needle groups using consecutively numbered opaque, sealed envelopes on the day of oocyte retrieval. The random allocation sequence and enrolment of the participants were performed by the corresponding author. The envelopes were opened by an individual who was not associated with the study, generally the attending nurse. All patients were blinded to the randomisation for the duration of the study. Clinicians performing the oocyte retrieval procedure were notified of the treatment allocation on the day of the retrieval to record duration of procedure and given anaesthetic drug amounts.

Transvaginal ultrasound-guided oocyte retrieval was performed 36 hours after this dual trigger under sedation with 1% propofol (Fresenius Kabi, Homburg, Germany). For the single-lumen needle group ($n = 40$), a 17-gauge needle (Cook Ireland, Limerick, Ireland) was used to aspirate follicles. A 17-gauge needle (Cook Ireland) was also used in the double-lumen needle group ($n = 40$), and 2 ml was injected into each follicle using a manually pressed syringe containing 10 ml of culture medium warmed to 37°C and re-aspirated and re-injected three times for each punctured

follicle. The pressure at which we aspirated the follicles was strictly maintained at 80 mmHg.

The oocyte–corona complexes were denuded, and intracytoplasmic sperm injection was performed after a 2-hour incubation. Embryos were transferred on day 3. Embryo grading was performed by Hardarson et al.[10] All patients received luteal support with daily intravaginal 90 mg progesterone (Crinone 8% gel; Merck Serono, Darmstadt, Germany) and 0.1 mg/ml triptorelin on the third day after embryo transfer.[11,12] Clinical pregnancy was defined as the presence of at least one gestational sac with detectable fetal cardiac activity by transvaginal ultrasonography. Live birth was defined as the woman delivered a healthy newborn baby. The live birth rate is the percentage of all cycles that lead to live birth.

Our hypothesis was to accept that follicular flushing three times in poor responders results in similar oocyte yield compared with direct aspiration in poor responders. The primary end point of the study was the number of metaphase II oocytes retrieved. Sample size estimation determined that 40 women in each group would be required to demonstrate a one-oocyte increase from three to four between the two groups with 80% power ($\beta = 0.2$ and $\alpha = 0.05$) with a standard deviation of 1.48 oocytes, according to Mok-Lin et al.[9] Secondary outcomes included the total number of punctured follicles and retrieved oocytes, fertilisation rates and implantation rates, duration of the procedure, and total use of anaesthetic. Additional outcomes were clinical pregnancy and live birth rate. The procedure duration was recorded from the injection of propofol to removal of the needle from the patient after finishing the last follicle puncture. The data are expressed as the mean ± SD. The baseline differences between the two groups were analysed using Student's *t*-test. Pearson's chi-square test and Fisher's exact test were used to compare the ratios between groups. A *p*-value of <0.05 was considered statistically significant. The data were analysed using SPSS for Windows (ver. 17.0; SPSS, Chicago, IL, USA).

Results

During the 6-month study period, 867 cycles were performed in our IVF centre, and 156 women were diagnosed as poor responders. Twelve women were excluded due to monofollicular/ natural cycles, 16 women were excluded due to premature progesterone elevation (i.e. progesterone levels ≥1.5 ng/ml on the day of hCG administration), and 48 women declined to take part in the trial. Eighty women consented and were randomised (Figure 1). Table 1 gives the baseline characteristics of participants, which were similar in both groups. The mean number of metaphase II oocytes was similar in the two groups (1.9 ± 0.1 versus 2.1 ± 0.1). The relative risk for metaphase II oocyte retrieval in the single-lumen group was estimated as 0.963 (95% CI 0.725–1.279) and the relative risk for metaphase oocyte retrieval in the double-lumen group was 1.04 (95% CI 0.772–1.401). The only significant difference between the two groups was the duration of oocyte retrieval (178.4 ± 13.4 versus 236.3 ± 24.1 seconds, $P = 0.01$) (Table 2). The clinical pregnancy and live birth rates were similar (32.5% versus 25% and 25% versus 22.5, respectively). The implantation rates were also similar (33.3 ± 8.0% versus 29.6 ± 8.1%) (Table 3). Embryo transfer procedure was cancelled in 26 women. Of the 13 women in the single-lumen group, no oocytes were retrieved in four, total fertilisation failure was detected in four, and embryo cleavage arrest was found in five women (Table 4). Of the 13 women in the flushing group, no oocytes were retrieved in two, total fertilisation failure was detected in six, and cleavage arrest was found in five (Table 4).

Discussion

Main findings

This is the second randomised controlled trial (RCT) to show that follicular flushing is time consuming and has similar results to direct aspiration in poor responders. Although it is difficult to overcome general beliefs and routine practice, this trial showed that there is no need to be anxious when direct aspiration of follicles is used in poor responders. Our study also showed that there are no detrimental effects of flushing of follicles, contrary to Mok-Lin et al.[9]

Strength and limitations

The previous RCT suggested that the high intrafollicular pressure of flushing and longer procedure time resulted in collection of poor-quality oocytes, which led to lower implantation and clinical pregnancy rates.[9] Although the previous RCT found no difference in embryo quality in the two groups, these explanations seemed to be logical; however, we should remember that these patients were poor responders, and could therefore produce poor-quality oocytes. If the high intrafollicular pressure of flushing affected the oocytes, we would expect to observe poor embryo quality together with lower implantation and clinical pregnancy rates in normally responding patients.[4–7] However, the longer procedure time and anaesthetic agent effects would not impair oocyte quality in poor responders in the follicular flushing group because the anaesthetic cannot reach a sufficiently high intrafollicular concentration during the approximately 5-minute operation; or impair oocyte quality in such a short period. We did not have enough power to conclude that flushing has detrimental effects on oocyte quality in terms of pregnancy or live birth rates due to

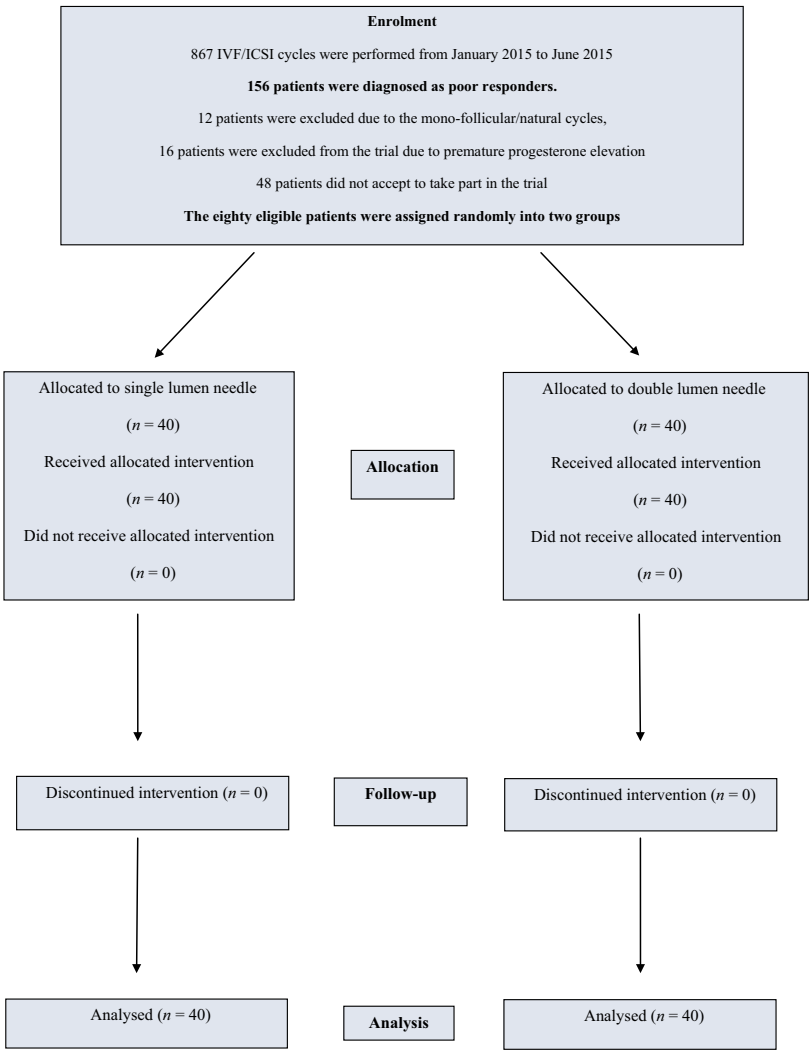

Figure 1. Flow diagram.

Table 1. Basal characteristics of single-lumen and double-lumen needle groups poor responders

	Single-lumen needle group (*n* = 40)	Double-lumen needle group (*n* = 40)	*P*
Age (years) (mean ± SD) (Median and IQR)	34.3 ± 5.4 (35.5–9.5)	36.2 ± 3.9 (36–16)	0.7
BMI (kg/m^2) (mean ± SD) (Median and IQR)	25.2 ± 0.7 (24.5–6.7)	27.01 ± 1.0 (25.7–9)	0.1
Duration of infertility (years) (mean ± SD) (Median and IQR)	5.2 ± 2.9 (4.0–3.0)	5.51 ± 3.3 (4–5)	0.7
AMH (mIU/l) (mean ± SD) (Median and IQR)	0.63 ± 0.10.38–0.54	0.61 ± 0.1 (0.71–0.69)	0.8
Follicle count >14 mm on hCG day (mean ± SD) (Median and IQR)	3.3 ± 0.1 (3–1.25)	3.07 ± 0.1 (3–2)	0.1
E_2 levels (pg/ml) on hCG day (mean ± SD) (Median and IQR)	528.8 ± 54.8 (483–604.7)	476.4 ± 49.9 (441–504)	0.4
Endometrial thickness (mm) (mean ± SD) (Median and IQR)	9.9 ± 0.3 (9.6–2.5)	9.4 ± 0.3 (9.8–3.4)	0.2
Total FSH dose (IU) (mean ± SD) (Median and IQR)	2636.8 ± 141.0 (2700–900)	2631.2 ± 179.9 (2662.5–1425)	0.9
GnRH agonist cycles	47.5% (19/40)	57.5% (23/40)	0.5
GnRH antagonist cycles	5% (2/40)	5% (2/40)	0.9
Letrozol + FSH/GnRH Antagonist Cycles	47.5% (19/40)	37.5% (15/40)	0.6

BMI, body mass index; GnRH, gonadotrophin-releasing hormone; IQR, interquartile range; SD, standard deviation.

Table 2. Oocyte retrieval and embryology laboratory outcomes of poor responder women in single- and double-lumen needle groups

	Single-lumen needle group (n = 40)	Double-lumen needle group (n = 40)	P	95% CI
Total follicles aspirated	124	123	0.1	
Numbers of total oocytes	93	94	0.1	
Retrieved oocytes (mean ± SD) (Median and IQR)	2.3 ± 0.2 (2.5–1)	2.3 ± 0.2 (3–1)	0.1	−0.4 to 0.5
M II oocyte numbers (mean ± SD) (Median and IQR)	1.9 ± 0.1 (2–1.25)	2.1 ± 0.1 (3–1)	0.2	−0.66 to 0.26
Numbers of GV (mean ± SD)	0.07 ± 0.04	0.05 ± 0.03	0.6	
Duration of OR (seconds) (mean ± SD) (Median and IQR)	178.4 ± 13.4 (138.5–102.5)	236.3 ± 24.1 (230–130)	0.01	−113.3 to –2.5
Total anaesthetics used (mean ± SD) (Median and IQR)	11.08 ± 3.8 (5–1)	11.8 ± 5 (5–0)	0.1	−1.3 to 0.2
Fertilisation rates (%) (mean ± SD) (Median and IQR)	66.1 ± 5.7 (100–50)	63.1 ± 5.9 (100–50)	0.7	−13.8 to 12.6
Numbers of cleavage (mean ± SD) (Median and IQR)	1.5 ± 0.1 (1.5–1)	1.5 ± 0.1 (2–2)	0.6	−0.45 to 0.27
Embryo numbers (mean ± SD) (Median and IQR)	1.5 ± 0.1 (1.5–1)	1.5 ± 0.1 (2–2)	0.8	−0.4 to 0.3
Number of transferred embryos (mean ± SD) (Median and IQR)	1.3 ± 0.1 (1–1)	1.3 ± 0.09 (1–1)	0.7	−0.23 to 0.3
Number of Grade 1 ET (mean ± SD) (Median and IQR)	0.6 ± 0.1 (0–1)	0.5 ± 0.1 (0–1)	0.5	−0.25 to 0.48
Number of Grade 2 ET (mean ± SD) (Median and IQR)	0.8 ± 0.1 (1–1)	0.9 ± 0.1 (2–1)	0.2	−0.52 to 0.3

ET, embryo transferred; GV, germinal vesicle; IQR, interquartile range.

Table 3. IVF/intracytoplasmic sperm injection outcomes of poor responders who received direct aspiration and flushing at oocyte retrieval

	Single-lumen needle group (n = 40)	Double-lumen needle group (n = 40)	P	RR with 95% CI
Positive β-hCG	15 (37.5%)	10 (25%)	0.3	0.76–1.58
Clinical pregnancy rate	13 (32.5%)	10 (25%)	0.8	0.74–1.49
Implantation rate (%)	33.3 ± 8.0	29.6 ± 8.12	0.7	0.82–1.27
Singleton pregnancy rate	12 /13 (92.3%)	10 (100%)	1	0.73–1.46
Twin pregnancy rate	1/13 (7.69%)	0	1	0.74–1.38
Live birth rate	10/40 (25%)	9/40 (22.5%)	1	0.72–1.42
Cancel rate	13 (32.5%)	13 (32.5%)	1	0.71–1.41

the small numbers of patients. Much larger studies (including 200–800 per group) would be needed to properly assess clinical pregnancy and live birth outcomes.

There was no difference in flushing pressure between our study and the first RCT, but Mok-Lin et al. used larger needles (16-gauge), which might aspirate greater numbers of immature oocytes. Furthermore, they used four flushes, which was a greater number of injections and re-aspirations. We used three flushes in the double-lumen group. These were main the differences between the studies. In fact, Mok-Lin et al. reported contradictory results compared with RCTs of normally responding women and needle type. However, similar to our study, a recent trial on follicular flushing and ART outcomes demonstrated that flushing has no detrimental effects on fertilisation rate, embryo quality and pregnancy rate.[13]

In the current RCT, we excluded poor reserve women with endometrioma. The ART outcomes of women with endometrioma differ from those in women without endometrioma.[14] Although the AFC is reduced in ovaries with endometrioma, the oocyte retrieval rate is similar in ovaries without endometrioma.[15,16] We measured both AMH and AFC, which seemed to be lower in endometrioma patients who were excluded from the current RCT. We selected patients with five or fewer follicles ≥13 mm in size on the day of hCG administration to obtain a homogeneous sample size for fertilisation and cleavage rates. Although follicular flushing seemed to be effective in small-diameter follicles in the trial of Mehri et al.,[17] the fertilisation and cleavage rates were reduced in small diameter follicles when compared with larger (>18 mm) diameter follicles.[17]

As with the previous RCT, our study has a limitation in terms of evaluating pregnancy and implantation rates because of the relatively small sample size. Another limitation was the exclusion of monofollicular/natural cycles. A previous study demonstrated that follicular flushing in monofollicular/natural cycles resulted in a significant

Table 4. The reasons of cancelled cycles among poor responders who received direct aspiration and flushing at oocyte retrieval

	Single-lumen needle group (n = 40)	Double-lumen needle group (n = 40)	P
No oocyte in OPU	4 (10%)	2 (5%)	0.6
Total fertilisation failure	4 (10%)	6 (15%)	0.7
Cleavage arrest	5 (7.5%)	5 (7.5)	1

OPU, oocyte pick-up.

increase in oocyte yield and transferrable embryos.[18] Actually, this is for the poorest responders and candidates for donor programmes. Furthermore, these patients have the lowest clinical pregnancy rates and implantation rates. If we considered clinical pregnancy/implantation rates in the poorest responders, we would not be able to achieve our main goal of evaluating technique efficacy.

Interpretation

As in our previous RCT and the meta-analysis, our current RCT found neither detrimental nor beneficial effect of follicular flushing three times on mature oocyte yield in poor responders.[2–4] An animal study showed that needle type did not affect oocyte yield, although twisting the needle within the follicle seemed to be effective in a single-lumen aspiration group in cattle.[19]

Conclusion

This is the second RCT to indicate that there is no need for anxiety about using a single-lumen needle in patients with few follicles (poor responders) in terms of mature oocyte yield. Additional RCTs are needed to draw conclusions in terms of pregnancy and live birth rates.

Disclosure of interest

None declared. Completed disclosure of interests form available to view online as supporting information.

Details of ethics approval

The study was approved by the Ethics Committee of Baskent University (project number 13/161) and by the Turkish Ministry of Health, Drug and Medical Equipment Department at 23 January 2015. The release of approval was on 3 February 2015.

Contribution to authorship

BH designed the trial, performed the procedure, analysed the data and wrote the manuscript. FG obtained the approval of the Ethics Committee of Baskent University and Turkish Ministry of Health, Drug and Medical Equipment Department, and also worked on the literature review, data collection and obtaining patient informed consent. PCA performed procedures in this trial, and EBK helped to design the trial and performed procedures in this trial.

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