Arch Gynecol Obstet (2012) 286:1061–1064
DOI 10.1007/s00404-012-2424-1

REPRODUCTIVE MEDICINE

Is follicular flushing really effective? A clinical study

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Received: 2 January 2012 / Accepted: 7 June 2012 / Published online: 20 June 2012

Abstract
Purpose Oocyte retrieval under transvaginal ultrasonographic guidance has been used for in vitro fertilization-intracytoplasmic sperm injection. Despite considerable advances in the assisted reproductive techniques, the efficacy of follicular flushing during egg collection remains controversial. The aim of this study was to compare the follicular aspiration only and aspiration + flushing methods in terms of retrieved oocyte number and clinical pregnancy rates.
Materials and methods A total of 200 patients were randomly divided into the intervention and control groups. All the patients underwent long protocol. Oocyte retrieval was performed when the dominant follicle reached 17 mm. Aspiration was performed using a single- or double-lumen catheter. Follicular flushing was performed after follicular aspiration in 100 patients of the intervention group. In the control group, only follicular aspiration was performed.
Results There were no detected differences in the retrieved oocyte number. Although the clinical pregnancy rate in the intervention group was higher than the control group (40 vs. 33 %), the difference was not statistically significant. Cycle cancelation rate was lower in follicular flushing group (8 %) than control group (11 %) but, this difference was not statistically significant. Metaphase I (MI), germinal vesicle numbers were higher in group 1 than in group 2 and the differences were not statistically significant, either. Total operation time was longer in aspiration + flushing group (group 2) than aspiration only group (group 1) and the difference was statistically significant ($p = 0.02$).
Conclusion In conclusion, our results indicate that follicular flushing during oocyte retrieval does not improve the retrieved oocyte number or clinical pregnancy rate but, it significantly increases the duration of procedure.

Keywords In vitro fertilization · Oocyte retrieval · Flush medium · Pregnancy rate

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Introduction

In vitro fertilization-intra cytoplasmic sperm injection (IVF-ICSI) has been widely used in gynecology practice for more than three decades and oocyte retrieval is essential as much as embryo transfer for a successful treatment [1]. Oocyte pick-up (OPU) procedure has been performed using transvaginal ultrasonography. Double- or single-lumen retrieval needles are utilized to collect the oocytes. Double-lumen aspiration needles have a capacity to flush and reaspirate the ovarian follicles and have been used for this purpose for a long time [2]. Today, in most clinics single-lumen transvaginal oocyte retrieval needles are usually used for oocyte pick-up and these needles are capable of flushing ovarian follicles. Follicular flushing was used in poor responder women via a double-lumen catheter because oocyte retrieval is very important in these patients due to poor ovarian reserve. However, there were no differences in the number of oocytes retrieved between aspiration + flushing and aspiration only groups [3]. There are conflicting data about the efficiency of follicular flushing

performed during OPU. Some authors reported that the use of follicular flushing using 2 mL flush medium resulted an increase in the number of oocytes collected [2, 4]. On the contrary, other studies detected no change [3, 5]. Therefore, we planned a randomized prospective clinical study aimed to assess the effect of follicular flushing during egg collection for oocyte quality, fertilization rate and pregnancy rate.

Materials and methods

This is a prospective randomized study. The study protocol was reviewed and approved by the Ethical Committee of Bozok University Medical Faculty. To randomly allocate the patients into groups, all women were initially randomly numbered. Then, computer-assisted randomization was utilized according to the instructions at www.randomization.com. Patients were allocated randomly to groups 1 and 2 on the day of the study.

Study design

A total of 200 patients were included into the study. The subjects were recruited from the patients referred to our clinic between June 2010 and June 2011. In group 1, a single-lumen transvaginal oocyte retrieval needle (Otrieva® Tapered Ovum Aspiration Needle K-TIVM-172035-US, Cook Medical, Spencer, IN, USA) was used. In group 2, a double-lumen transvaginal oocyte retrieval needle (Echo Tip Double Lumen Aspiration Needle, K-OPSD-1635-A-L, Cook Medical, Spencer, IN, USA) was used. Oocytes were retrieved via a single puncture of ovary using a 6.5 MHz transvaginal ultrasonography probe (GE Logiq 200 Alpha® Ultrasound Machine, General Electric, USA). During oocyte retrieval, in group 1 (single-lumen group) follicles were aspirated and flushing was not performed. In group 2 (double-lumen group), follicles were aspirated then each aspirated follicle was washed with 2 mL flush medium and reaspirated (follicular flushing). During OPU, women were anesthetized using Propofol® 1,000 mg/100 ml, Abbott, USA). Total intervention time from the beginning of the procedure into the first ovary up to finishing it in the second ovary was noted.

All the patients underwent long protocol. In all cases, pituitary was down-regulated with Leuprolide acetate (Lucrin® daily 0.25 mg Abbott, USA). Leuprolide acetate was started at a dose of 0.5 mg on the 21th day of the previous cycle and when the pituitary supression started the dose was reduced to 0.25 mg and was continued until the day of the hCG. Controlled ovarian stimulation (COS) was performed with FSH starting on cycle day 3. Average FSH starting dose was 300 iu and the dose was individually adjusted according to the previous treatment cycles, body mass index (BMI), and age. Follicular development was monitored and dose adjusted according to E_2 level and ultrasonographic measurements. When 1 or 2 follicles reached 17 mm size, hCG (Pregnyl® 5,000 IU × 2, Schering-Plough, USA) was administered for final maturation. Transvaginal ultrasound-guided needle aspiration of follicular fluid was carried out 35–36 h after hCG administration.

In all cases, ICSI was performed. Semen samples were washed using gradient method. Isolate Sperm seperation medium (Irvine Scientific, Santa Ana, California) and Quinn's Sperm washing medium (Sage, Trumbull, CT, USA) were used for sperm preparation. G-MOPS plus, G-IVFplus, G1-plus and G2-plus (Vitrolife, Sweden AB, Kungsbacka, Sweden) were the mediums which were used for embryo culturing. Embryos were classified according to the number of blastomeres, percentage of fragmentation and blastomere appearences as type I, II, III or IV on 1st, 3rd and 5th days. Up to four embryos were transferred into the uterine cavity on days 2, 3 or 5 after oocyte retrieval. All transfers were made using Rocket Thin wall Transfer set (Rocket Medical, Hingham, MA,USA). Luteal phase support was done by transvaginal progesterone administration (Crinone 8 % vaginal gel® Merck-Serono, Switzerland). Progesterone administration was initiated on the oocyte pick-up day and continued for 12 days (until the serum beta hCG measurement day). In cases of pregnancy, progesterone was given until the 12th gestational week. OHSS was not developed.

Statistical analysis

Statistical analyses were performed using the Statistical Package for the Social Sciences (17.00 SPSS Inc., Chicago). The Chi-square test was used for categorical variables and an independent sample t test was used for continuous variables that were normally distributed. P value < 0.05 was considered significant.

Results

The groups were homogenous according to the patient characteristics. Age, duration of infertility, basal FSH level, and body mass index (BMI) were evaluated but, there was no statistical difference. Mean age of the patients was 28.1 ± 5.5 and 30.1 ± 5.3 in group 1 and group 2, respectively (Table 1).

Retrieved oocyte number (RON) was lower in flushing group than in control group (10.8 ± 6.8 vs. 11.5 ± 6.2) but, the difference was not statistically significant ($p = 0.42$). Metaphase II oocyte (M II) and metaphase I oocyte

Table 1 Characteristics of the patients

	Group 1 ($n = 100$)	Group 2 ($n = 100$)	P
Age	28.1 ± 5.5	30.1 ± 5.3	0.42
Duration of infertility (year)	4.9 ± 1.8	5.3 ± 2.0	0.81
Basal FSH level (iu/l)	5.83 ± 1.1	6.232 ± 1.4	0.54
BMI	25.4 ± 4.5	27.1 ± 4.9	0.29
TSD	3,093.4 ± 986.1	3,545.3 ± 1,251.2	0.12

BMI body mass index

Table 2 IVF-ICSI outcomes of the patients

	Group 1 ($n = 100$)	Group 2 ($n = 100$)	P
RON	11.5 ± 6.2	10.8 ± 6.8	0.42
M II	8.6 ± 5.0	8.4 ± 6.1	0.81
M I	0.9 ± 1.1	0.8 ± 1.2	0.37
GV	1.9 ± 2.4	1.5 ± 1.9	0.30
EZ (%)	94	98	0.30
DEG (%)	98	100	–
FR (%)	46.0	50	0.79
CCR (%)	11	8	0.31
CPR (%)	33	40	0.18
OPR (%)	29	35	0.23
PT (min)	7.6 ± 2.7	12.2 ± 4.1	0.02[a]

RON retrieved oocyte number, *M II* metaphase II oocyte, *M I* metaphase I oocyte, *GV* germinal vesicle, *EZ* empty zona, *PT* procedure time, *min* minute, *DEG* degenerated oocyte, *FR* fetilization rate, *CCR* cycle cancelation rate, *CPR* clinical pregnancy rate, *OPR* ongoing pregnancy rate

[a] Statistical difference between groups 1 and 2

(M I) numbers were higher in group 1 than in group 2 (8.6 ± 5.0 vs. 8.4 ± 6.1 and 0.9 ± 1.1 vs. 0.8 ± 1.2, respectively) but, these differences were not statistically significant. Fertilization rate, clinical pregnancy rate, and ongoing pregnancy rate were better in flushing group than in control but these differences were not statistically significant. On the other hand, cycle cancelation rate was better in control group than in flushing group (11 vs. 8 %, $p = 0.31$). The duration of the procedure was 7.6 ± 2.7 and 12.2 ± 4.1 in group 1 and group 1, respectively, and this difference was found as statistically significant ($p = 0.002$). All these data were shown in Table 2.

Discussion

In this randomized clinical study, the effect of follicular flushing during oocyte retrieval on IVF-ICSI outcome was studied. Our results indicate that there are no increases in the oocytes yielded and clinical pregnancy rates with follicular flushing during egg collection. To our best knowledge, there is no study indicating whether follicular flushing increases the retrieved oocyte number. For this reason, this prospective randomized clinical trial is designed to detect the efficacy of follicular flushing performed during OPU.

Although oocyte retrieval technique which is essential for IVF-ICSI has been well described, the issue whether to perform follicular flushing during OPU is controversial. In most studies, follicle flushing after aspiration has not improved the RON and CPR [3, 5, 6]. Scott et al. [7] compared the efficacy of single and double-lumen needles used for aspiration and flushing and reported no significant difference between two groups. Haydardedeoglu et al. [8] compared the retrieval efficiency of aspiration + flushing and aspiration only groups. They demonstrated no beneficial effect of double-lumen retrieval needles compared with single-needle in relation with RON and CPR. In our study, there were no statistically significant difference between single-lumen retrieval needle group and double-lumen needle group. Our findings were similar with the results of previous studies.

On the other hand, Bagtharia et al. assessed the effect of repeated follicular flushing on the RON. They reported that the rate of RON was 40 % with direct aspiration without flushing of the follicle, 82 % with two flushes, and 97 % with four flushes [9]. However, there is no comparison group in this study and it is not randomized. The possible causes of these conflicting results in the previous studies could be the usage of different techniques, utilization of different length and diameter of retrieval needles and different experiences of the clinicians who performed oocyte retrieval. Duration of procedure was reported as significantly increased in aspiration + flushing groups than in aspiration only groups [3, 5, 10]. In our study, operation time was almost two times more in the intervention group than control group (12.2 ± 4.1 vs. 7.6 ± 2.7, $p = 0.02$).

In this prospective clinical study, it was aimed to assess whether follicular aspiration and flushing increases the RON, CPR over aspiration alone in women who underwent IVF-ICSI. Our study was designed because there is no consensus about the utilization of single or double-lumen oocyte retrieval needles. Our results indicate that follicular flushing performed after aspiration is not associated with increased number of oocytes or improved clinical or ongoing pregnancy rates. As a conclusion, the present available data do not support routine flushing of the follicles after aspiration. Live birth rates were not evaluated in this study. To our knowledge, there is no study comparing the effect of flushing on live birth rate or miscarriages. Large prospective and randomized trials are required to determine the differences between oocyte retrieval techniques.

Acknowledgments We are grateful to Turhan Aran, Asist. Prof. Dr. (Karadeniz Technical University, Department of Obstetrics and Gynecology) for helping us to evaluate the statistical data.

Conflict of interest The authors declare no conflict of interest.

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