


Research Article

Flushing of the Follicles in Ovum Pick-up Procedures gives a Better Chance for Pregnancy in Low ovarian Reserve Patients

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Abstract

Background: This study aims compare the pregnancy and live birth rates between the oocytes retrieved without follicular flushing FF(-) in the oocyte pick-up (OPU) procedure performed in women with diminished ovarian reserve (DOR) and those retrieved by follicular flushing FF(+).

Results: The study was conducted among patients diagnosed with DOR according to Bologna criteria and applied to the clinic for IVF between 2017-2020. A total of 358 infertile women with follicles three and below on the hCG day, between the ages of 21 and 42, without severe male factor, without uterine anomaly, without uterine surgery, and who did not undergo PGD were included in the study. Each follicle was aspirated once in the OPU procedure, and if a follicle was retrieved, it was moved to the other follicle. If the follicle could not be retrieved, the oocyte was tried to be retrieved by flushing a maximum of 3 times. The number of oocytes retrieved, clinical pregnancy rate, and live birth rate were compared. Since all the oocytes retrieved in 143 patients were retrieved directly without the need for FF, it was named FF(-) group. Since at least one oocyte of the remaining 215 patients was retrieved by performing FF, it was named FF(+) group. Since some of the oocytes retrieved from 112 patients in the FF(+) group were retrieved with FF and some without FF, they were excluded from the study, and the remaining 103 cases formed the FF(+) group a total of 246 patients were compared. The mean number of MII oocytes ,the pregnancy rates , rates of live births and the abortion rates between two groups did not show any statistical difference.

Conclusion: FF applied during oocyte retrieval in DOR did not positively affect the number of retrieved oocytes, clinical pregnancy, and live birth rates even doing this may decrease the pregnancy rate because of the probable low quality egg but we should not forget that if we did not do flushing after once we aspirated the follicle we would not be able to obtain any pregnancy at all in this patients.

Keywords: oocyte retrieval, follicular flushing, diminished ovarian reserve, pregnancy, live birth

Introduction

Since its first application assisted reproductive technology (ART) has progressed significantly [1]. In ART, transvaginal follicular aspiration is accepted as the standard approach for oocyte retrieval [2,3]. Changes have been made to the original technique over time to maximize oocyte yield during oocyte retrieval [3,4]. In order to reduce the risk of possible oocyte retention in the follicle, the “follicle flushing” technique, which means

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allowing the follicles to be “washed” with medium with the help of a double-lumen needle, has been developed.

Various attempts have been made to reduce the economic burden of ART therapy and increase oocyte yield. Several studies have evaluated the beneficial effect of Follicle Flushing (FF) with a double-lumen needle in oocyte yield, and these data have been compared with procedures with a single-lumen needle [5,6]. Although it was claimed in the first studies that FF increased the numerical efficiency in oocyte retrieval, several randomized controlled studies (RCTs) did not show positive results, and it was found that FF did not affect live birth rates [7,8]. Numerous studies have shown that direct follicular aspiration shortens the oocyte retrieval procedure time and has a similar oocyte yield compared to follicular flushing in normal responding patients.

However, in studies evaluating the effect of FF in patients with poor response, it was found that this group showed low pregnancy rates since FF also allowed the collection of poor quality oocytes [9,10]. In addition, these studies have shown that follicular flushing increases the procedure time by about 10 minutes. In the Cochrane review published in 2018, it was stated that FF did not provide any increase in oocyte yield in both normal and poor responding patients, and with this, oocyte retrieval times were prolonged. More importantly, the effect of FF on live birth did not appear to be beneficial in either normal or poor responders [11]. Although we have data from publications evaluating groups with or without FF, no satisfactory data compares the treatment success of oocytes retrieved by FF in DOR group patients with oocytes retrieved directly without FF.

Our study aims to compare the pregnancy and live birth rates between oocytes retrieved without the need for FF and those that could be retrieved only by FF in the oocyte pick-up (OPU) procedure performed in women with diminished ovarian reserve (DOR).

Method

Our study was conducted among patients who applied to a private IVF clinic and were diagnosed with DOR according to the Bologna criteria [12]. The approval of the study was obtained from the ethics committee of Haliç University (29.12.2021/218), and written consent was obtained from each patient. The study evaluated the data of 358 patients who underwent egg retrieval at Private Şişli Kolan Hospital IVF Center between January 2017 and December 2020. Infertile women with follicles of 3 or less on hCG day, between the ages of 21 and 42, without severe male factor, without uterine anomaly, without a history of uterine surgery, and whose embryos were not subjected to PGD were included in the study.

Three hundred fifty-eight cases were processed under anesthesia using a 17G double-lumen Wallace (Cooper

Surgical, US) needle during OPU. In the OPU procedure, first, each follicle was aspirated once, and the embryologist checked the follicle fluid. If the oocyte was retrieved, it was passed to the other follicle. If an oocyte could not be retrieved, the oocyte was tried to be retrieved by flushing at most three times. Since all the oocytes retrieved in 143 of 358 patients were retrieved directly without the need for FF, it was named FF(-) group. Since at least one oocyte of the remaining 215 patients was retrieved by performing FF, it was named FF(+) group. Since some of the oocytes retrieved from 112 patients in the FF(+) group were retrieved by making FF, and some were retrieved without FF, these 112 cases were excluded from the study, and the remaining 103 cases formed the FF(+) group. Freeze-all was applied to all patients included in the study due to the stimulation protocol where progesterone pills were used to suppress the premature luteinization of the follicles.. Then, the frozen embryo transfer (FET) procedure was performed after 2-3 months.

After frozen embryo transfer (FET) procedures, patients' pregnancy rates and live birth rates were compared in both groups. The number of embryos at the blastocyst level in which cryopreservation could be performed in all patients was compared. More than one FET procedure could be applied to some of the patients. After transferring all cryopreserved and transferable embryos, the study was completed. A single outcome was included if patients became pregnant more than once.

Stimulation Protocol

The patients used recombinant FSH (Gonal-F, Merck Serono, Switzerland) at a dose determined according to age and BMI values, following the transvaginal USG control performed on the second or third day of menstruation. When the leading follicle of all of them reached 12-13 mm, medroxyprogesterone acetate tablet 10 mg (Tarlusal 5mg, Deva, Turkey) was added to prevent LH surge. When the leading follicle was 17-18 mm, it was triggered with recombinant hCG (Ovitrelle, Merck Serono, Switzerland), and OPU was performed 35 hours later under anesthesia. Freeze-all was applied to all cases, and frozen embryo transfer protocol was applied.

ICSI Procedure

Oocyte-cumulus complexes (OCC) retrieved after the OPU procedure were stripped, and intracytoplasmic sperm injection was performed after incubation. Embryo grading was done according to the Gardner & Schoolcraft Grading System [13,14]. All embryos were cryopreserved by vitrification using the Cryotop method when they reached the blastocyst stage on the fifth or sixth day.

Frozen Embryo Transfer

All patients started taking estradiol tablets 4 mg

(Estraferm, Novo Nordisk, Denmark) from the second day of menstruation. Estradiol was increased by 2 mg every four days. Progesterone treatment was started if the endometrial thickness was above 7 mm on the 15th day of the cycle. For this, dydrogesterone tablet (Duphaston 10 mg, Abbott, Switzerland) was used as 3x1. Although the freezing day of the embryos to be transferred was the fifth or sixth day, the transfer process was carried out on the sixth day of the progesterone initiation. 30 mg Duphaston tablet and 8 mg estradiol tablet were used as luteal phase support. A beta hCG test was performed 12 days later. Clinical pregnancy was defined as the presence of at least one gestational sac in which fetal cardiac activity was seen on imaging with transvaginal ultrasonography. Live birth was defined as a baby born healthy. The live birth rate was calculated as the percentage of live births to all cycles in that group.

Statistical Analysis

Data with normal distribution were given as mean and standard deviation, and differences between groups were compared with an independent sample t-test. For the data without normal distribution, the differences between groups were compared with the Mann-Whitney U test by giving the median and interquartile ranges. Pearson chi-square tests were used to compare categorical variables. IBM SPSS version 25.0 (NY, USA) was used for all statistical analyses. $P < 0.05$ was considered statistically significant.

Results

The mean age of the patients was $31(\pm 3,61)$, mean BMI was $28(\pm 1.75)$, mean infertility duration was $9(\pm 0.73)$ years. The mean AMH levels of the patients were $0.61(\pm 0.30)$, and the mean MII oocyte count was 2.27 ± 0.63 . The rate of FET at least once in all patients was 90.2%, the clinical pregnancy rate was 55.2%, and the live birth rate was 35.6%. Demographic characteristics of the patients are presented in (Table 1).

The mean age of the patients in the FF(+) group was 31

(± 2.65), and the mean age of the patients in the FF(-) group was $31(\pm 3.11)$, and it was found to be similar in both groups ($p: 0.657$). Body mass indexes and infertility durations were observed as similar in both groups; BMI was $31(\pm 3.11)$ and infertility duration was $8(\pm 0.32)$ in the FF(+) group; BMI was $28(\pm 0.54)$ and infertility duration was $9(\pm 0.15)$ in the FF(-) group, respectively ($p > 0.05$) (Table 2). When the AMH levels were compared, the mean AMH level in the FF(+) group was 0.62 ± 0.29 , and it was observed as 0.64 ± 0.31 in the FF(-) group in which direct oocyte was retrieved ($p: 0.682$). The median oocyte count of FF(+) group patients was 2 (2-3), and the median oocyte count of FF(-) patients was 3 (2-3) ($p: 0.413$). The mean number of MII oocytes was $2.14(\pm 0.687)$ in FF(+) patients and $2.36(\pm 0.564)$ in FF(-) patients ($p: 0.005$). In both groups, when the cryopreserved embryos were thawed in some of the patients, they were not found suitable for transfer and were not included in the transfer. Thus, when the number of cryopreserved embryos in both groups was compared by excluding the patients who did not have FET, the mean number of cryopreserved embryos in the FF(+) group was 1.46 ± 0.563 , and the mean number of cryopreserved embryos in the FF(-) group was 1.78 ± 0.674 ($p < 0.001$). While the median number of embryo transfers was 1 (1-2) in the FF(+) group, it was 2 (1-2) in the group that was not performed ($p: 0.160$) (Table 2). Embryo transfer numbers were performed according to the age of the patients and the number of previous trials.

The proportions of patients who could undergo FET at least once in both groups were similar (89.3% vs. 90.9%, $p: 0.679$). When the rates of pregnancy in transferred cases in both groups were compared, the pregnancy rate was 45.7% (42 of 92 patients) in the FF(+) group and 62% (80 of 129 patients) in the FF(-) group ($p: 0.016$). Although the live birth rates tended to be higher in the FF(-) group, where oocytes could be retrieved without flushing, there was no statistically significant difference between the two groups. Numbers and rates of live births in FF(+) and FF(-) groups were 26 (28.3%) vs 52 (40.3%), $p: 0.065$, respectively. Abortion rates were similar in both groups (%38.1 vs. %35, $p: 0.735$)

Table-1: Demographic Characteristics of the patients

Age of the patients (mean \pm SD) years	31($\pm 3,61$)
Body Mass Index of the patients (mean \pm SD)	28(± 1.75)
infertility period of the infertile couple (mean \pm SD) years	9(± 0.73)
AMH levels of the patients (mean \pm SD) ng/mL	0.61(± 0.30)
Mean Oocyte per patient (mean \pm SD)	2(± 0.23)
MII Oocyte per patient (mean \pm SD)	2.27(± 0.63)
Frozen embryos per patient (mean \pm SD)	1.61(± 0.64)
Number of embryos transferred per patient (mean \pm SD)	1.49(± 0.50)
Frozen embryo cycle rate (%)	90.20%
Pregnancy rate (%)	55.20%
Live birth rate (%)	35.60%

Table-2: Comparison of the group with and without FF

	Flushing (+) (n:103)	Flushing (-) (n:143)	p
Age, mean ±SD	31 (±2.65)	31 (±3.11)	p>0.05
Body Mass Index mean ±SD	28 (±0.76)	28 (±0.54)	
Infertility time, mean±SD	8 (±0.32)	9 (±0.15)	
AMH levels, mean±SD	0.61±0.29	0.61±0.31	0.931
Oocyte numbers, median (Q1, Q3)	2 (2, 3)	3 (2, 3)	0.413
MII oocyte numbers, mean±SD	2.14±0.687	2.36±0.564	0.005
Frozen embryo numbers, mean±SD	1.46±0.563	1.78±0.674	<0.001
Number of transferred embryos, median (Q1, Q3)	1 (1, 2)	2 (1, 2)	0.16
Frozen embryo cycles (%)	89.30%	90.90%	0.679
Pregnancy, (%)	45.70%	62%	0.016
Live birth (%)	61.90%	64.60%	0.773

For regression analysis, when FF, age (under/over 35 years of age), MII number, and the number of cryopreserved embryos were evaluated as factors affecting pregnancy, the probability of getting pregnant in patients in the FF (-) group was 1.9 times (p:0.03). In patients under 35 years of age, it was 2.3 times higher (p:0.015). No statistically significant correlation was observed between the number of cryopreserved embryos and the number of MII embryos and pregnancy (p>0.05).

Discussion

As a result of the study, it was determined that FF during the oocyte retrieval procedure for ART positively affect pregnancy and live birth in patients with DOR because otherwise, if flushing had not been performed in these patients, there would be no chance of pregnancy because there would be no eggs for fertilization. The pregnancy rate was found to be lower in the group that needed FF compared to the group where oocytes were collected without flushing but this difference did not reach significant level. Maybe in larger study this difference would be significantly different however, since the eggs were able to be obtained only by flushing in these patients, there will be still a chance of pregnancy and a better result will be obtained than not having any eggs if flushing was not performed. When the factors affecting pregnancy were examined, it was found that oocyte retrieval directly without FF increased pregnancy 1.6 times, this reminds us that oocytes may be more capable of obtaining the pregnancy than the oocytes obtained without flushing. With flushing, perhaps we obtain an egg that cannot be obtained otherwise and we are forcing a lower quality egg for a pregnancy, but if we do not do this flushing it seems that, this patient would not have a chance at all because there will be no any oocytes. So it seems that by flushing the follicles in DOR patient we are giving them a chance for a pregnancy.

Due to the increase in the planned age of maternity [15], the age of women giving birth is increasing, and the demand for assisted reproductive technologies is increasing from year to year. Advanced maternal age is a risk factor for poor response to IVF [12], and various methods are used to reduce this risk factor. With direct puncture to the follicle, only 60-80% of the oocytes in the follicle can be reached [16]. The purpose of FF is to increase the yield for oocyte retrieval, as it allows the excretion of residual contents.

Haydardedeoğlu et al. evaluated the benefit of FF in their study with a large-scale number of patients and found no significant difference [9]. However, in the study that included only normal responding patients, there were 13.09 oocytes in direct aspiration, while there were 12.25 oocytes in the FF group. In cases that are thought to be related to an intrinsic gametogenesis problem, significant results cannot be obtained with FF, and the duration of the oocyte retrieval process will be prolonged [17].

The findings obtained in the study support the meta-analyses that found that FF did not affect oocyte retrieval [17]. Non-randomized studies with a small sample size published with the debut of the FF have shown that the FF has an effect [5,6,18]. However, in randomized controlled studies with a strong methodology, it was found that FF had no effects on oocyte retrieval, clinical pregnancy, and live birth [9,19-21].

There is no evaluation of the effect of oocytes retrieved by FF on pregnancy and live birth in women with DOR in the literature. A recently published meta-analysis reported that FF did not significantly affect live birth and pregnancy and it prolonged procedure duration [17]. Although a significant difference was observed in the number of oocytes in the study of Calabre et al., no significant difference was found in live birth and pregnancy rates [22].

In previous studies in the literature, it has been suggested that FF causes retrieval of poor-quality oocytes with the effect of high intra-follicular pressure, which leads to lower implantation and clinical pregnancy rates [10]. In addition, the change in the paracrine environment due to the dilution process during FF may cause damage to the oocyte, which may break the shingles and strip the OCC [23]. The patient population in our study was those diagnosed with DOR, and the presence of poor-quality oocytes is possible. In the study of Mok-Lin et al. [10], although FF was performed with a larger needle (16 G), it was suggested that the immature oocyte retrieval might be higher with the FF, and low fertilization and pregnancy rates were found. In our study, a smaller needle was used, and as a result, it was determined that the pregnancy rate was lower in the group that needed FF, and the live birth rates were found to be lower than the group that did not require FF, although it was not statistically significant.

In the light of this information, the effect of oocyte retrieval with the need for FF on pregnancy and live birth in DOR patients where it is aimed to retrieve the high-quality oocyte was evaluated. While no effect was observed on the number of oocytes collected, clinical pregnancy, and live birth in this group of patients, on the contrary, it was determined that oocyte retrieval without the need for FF showed a higher pregnancy rate. Although this high pregnancy rate was not statistically significant in live birth rates in our study, it is still thought-provoking that it was observed higher in those who did not need FF. The fact that there was no difference in abortion rates between the groups supports the fact that the live birth rate is in favor of the FF(-) group, which had higher pregnancy rates.

The study has some limitations. The first is that it was a non-randomized controlled study. The lack of standardization of FF can be stated as another limitation.

Conclusion

In conclusion, the oocytes collected with FF are capable of obtaining pregnancy at the same level as the eggs collected without follicular flushing. FF applied during oocyte retrieval in DOR cases gives a chance for a pregnancy similar to those eggs collected without flushing. Without flushing procedure in this patients there will be no any oocytes collected if flushing was not tried and by the way there would be 0 % pregnancy rate and this is why we advise follicular flushing especially in DOR patients. FF applied during oocyte retrieval in DOR did not positively affect the number of retrieved oocytes, clinical pregnancy, and live birth rates even doing this may decrease the pregnancy rate but we should not forget that if we did not do flushing after once we aspirated the follicle we would not be able to obtain any pregnancy at all in this patients. Large-scale studies are needed to evaluate the quality of oocytes retrieved with FF in various patient groups and monitor their effects on live birth rates.

Conflict of Interest

None

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