

Flushing of the Follicles in Ovum Pick-Up Procedures Impacts the Chance for Pregnancy, But Not Birth Rates in Low Ovarian Reserve Patients

Yumurta Toplama İşlemi Sırasında Foliküllerin Yıkılması Düşük Over Rezervli Hastalarda Gebelik Şansını Etkiler, Ancak Canlı Doğum Oranlarını Etkilemez

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ABSTRACT

Objective: This study aims to compare the pregnancy and live birth rates between the oocytes retrieved without follicular flushing and those retrieved by follicular flushing in the oocyte pick-up procedure performed in women with diminished ovarian reserve. **Materials and Methods:** The study was conducted among patients diagnosed with diminished ovarian reserve according to Bologna criteria who applied to the clinic for IVF between 2017-2020. A total of 358 infertile women with follicles three and below on the hCG day were included, and each follicle was aspirated once in the OPU procedure, and if an egg was retrieved, the physician moved to the next follicle. If the egg could not be retrieved, the oocyte was tried to be retrieved by flushing the follicle a maximum of three times. The number of oocytes retrieved, clinical pregnancy, and live birth rates were compared. **Results:** The mean number of MII oocytes, rates of live births and the miscarriage rates between the two groups did not show any statistical difference. Pregnancy rates were higher in no follicular flushing group. **Conclusion:** Flushing of the follicles did not give the same pregnancy result as the oocytes aspirated without flushing, 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 these patients

Keywords: Oocyte retrieval; follicular flushing; diminished ovarian reserve; pregnancy rate

ÖZET

Amaç: Bu çalışma, azalmış yumurtalık rezervi olan kadınlarda yapılan oosit toplama işleminde foliküler yıkama yapılmadan elde edilen oositler ile foliküler yıkama ile elde edilen oositler arasındaki gebelik ve canlı doğum oranlarını karşılaştırmayı amaçlamaktadır. **Gereç ve Yöntemler:** Çalışma 2017-2020 yılları arasında Bologna kriterlerine göre azalmış yumurtalık rezervi tanısı alan ve tüp bebek için kliniğe başvuran hastalar arasında yapılmıştır. Çalışmaya 21-42 yaş arası, 358 infertil kadın dahil edildi. Oosit toplama işleminde her bir folikül bir kez aspire edildi ve yumurta alındıysa diğer foliküle geçildi. Yumurta çıkmayan foliküller ise en fazla 3 defa yıkama yapılarak oosit alınmaya çalışıldı. Hem yıkamasız hem de yıkamalı oosit elde edilen hastalar çalışmadan çıkartıldı. Elde edilen oosit sayısı, klinik gebelik oranı ve canlı doğum oranı karşılaştırıldı. **Bulgular:** İki grup arasında ortalama MII oosit sayısı, canlı doğum oranları ve abortus oranları istatistiksel olarak anlamlı farklılık göstermedi. Foliküler yıkama yapılmayan grupta gebelik oranı daha yüksek bulundu. **Sonuç:** Azalmış overyan rezervi olan hastalarda oosit toplama sırasında uygulanan foliküler yıkama, elde edilen oosit sayısını, klinik gebeliği ve canlı doğum oranlarını olumlu yönde etkilemedi. Hatta bunu yapmak olası düşük kaliteli yumurta nedeniyle gebelik oranını düşürebilir, ancak eğer ilk başta yumurta elde edilmemiş hastalarda folikül yıkamasaydı bu hastalarda hiçbir şekilde gebelik elde edilemeyecekti.

Anahtar Kelimeler: Yumurta toplama; folikül yıkama; azalmış yumurtalık rezervi; gebelik oranı

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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 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 allowing the follicles to be “washed” with the 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 trials (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 to follicular flushing in normal responding patients.

Studies evaluating the effect of FF in patients with poor response found that this group showed low pregnancy rates since FF also allowed the collection of poor-quality oocytes.^{9,10} These studies have also 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).

MATERIALS AND METHODS

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. This study was planned and conducted according to the ethical and patient protective criteria in the Helsinki Declaration of Human Rights. 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 three 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. This study did not evaluate patients with Repeated Implantation Failure (RIF) and Recurrent Pregnancy Loss (RPL).

Three hundred fifty-eight cases were processed under anesthesia using a 17G double-lumen Wallace (Cooper Surgical, US) needle during OPU. The OPU was assigned when at least there was one follicle bigger than 18 mm. Final triggering was done with Ovitrelle 250 micrograms, Merk Serono, Modugno (BA)/Italy) 35-36 hours before the ovum pick-up procedure. 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. We transferred one embryo when just one embryo was available for transfer, patients with female age less than 35 or if the IVF trial was their first or second embryo transfer procedure. In patients with female age over 35, we transferred two embryos if available.

After frozen embryo transfer (FET) procedures, patients' pregnancy and live birth rates were compared in both groups. The number of embryos at the blastocyst level where 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 reached 12-13 mm, medroxyprogesterone acetate tablet 10 mg PO (Tarlusal 5 mg, 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 strategy and the frozen embryo transfer protocol was applied to all of the cases.

ICSI PROCEDURE

Oocyte-cumulus complexes (OCC) retrieved after the OPU procedure were stripped, and intracytoplasmic sperm injection was performed after incubation. All embryos were cryopreserved by vitrification using the Cryotop method when they reached the blastocyst stage on the fifth or sixth day. Due to our policies, we transfer only good embryos who reached the blastocyst stage; according to this rule, the embryos transferred to all patients were 5AA, 4AA or 4AB

according to the Gardner & Schoolcraft Grading System.^{13,14}

FROZEN EMBRYO TRANSFER

All patients started taking PO estradiol tablets 4 mg (Estraferm, Novo Nordisk, Denmark) on 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; and for this, dydrogesterone tablet (Duphaston 10 mg, Abbott, Switzerland) was used PO: 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 PO and 8 mg estradiol tablet PO 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's 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 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%. The demographic characteristics of the patients are presented in [Table 1](#).

The mean ages of the patients in the FF (+) group and the FF(-) group were similar. Body mass indexes, AMH levels, collected oocyte number, number of transferred embryos and infertility dura-

TABLE 1: Demographic characteristics of the patients.

Age, years (mean±SD)	31(±3,61)
Body mass index kg/m ² (mean±SD)	28(±1.75)
Infertility duration, years (mean±SD)	9(±0.73)
Serum AMH, ng/mL (mean±SD)	0.61(±0.30)
Number of oocytes collected, n(mean±SD)	2(±0.23)
MII oocytes, n (mean±SD)	2.27(±0.63)
Frozen embryos, n (mean±SD)	1.61(±0.64)
Embryos transferred per patient, n (mean±SD)	1.49(±0.50)
Cycles with supernumerary embryos frozen, (%)	90.2 %
Pregnancy rate, (%)	55.2 %
Live birth rate, (%)	35.6 %

tions between the two groups were not significantly different. ($p > 0.05$) In both groups, when the cryopreserved embryos were thawed in some patients, they were not found suitable for transfer, and they 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 ($p < 0.001$). The MII oocytes after OPU were more in the FF(-) group (Table 2). This significant level does not change the main goal of our study; even in light of this finding, we can say that we collect better and mature oocytes when we get the oocyte without flushing the follicle.

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 pregnancy rates in transferred cases in both groups were compared, there was a significant differences in favor of 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 (Table 2). Miscarriage rates were higher in the FF(+) group, but this difference did not reach a significant level with this number of patients (38.1 vs. 35%, $p= 0.735$).

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 higher ($p=0.03$). 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 have a impact on 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 lower in the group that needed FF compared to the group where oocytes

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)	
Body Mass Index mean ±SD	28 (±0.76)	28 (±0.54)	$p>0.05$
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, mean ±SD	2,7±0.324	3,1±0.681	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, mean ±SD	1,9±0.244	1.6±0.712	0.160
Miscarriage rate (%)	38,1	35	0.735
Frozen embryo cycles (%)	%89.3	%90.9	0.679
Pregnancy, (%)	%45.7	62%	0.016
Live birth (%)	28,3	40	0.065

were collected without flushing, and it reached a significant level. Maybe in a larger study, this difference would be more prominent since the eggs could be obtained only by flushing in these patients; there will still be a chance of pregnancy and a better result than not having any eggs if flushing was not performed. There were no any complications that can be associated to the increase in the duration of the OPU due to the flushing of the follicles. When the factors affecting pregnancy were examined, it was found that oocyte retrieval directly without FF increased pregnancy 1.6 times, reminding us that oocytes may be more capable of obtaining the pregnancy than those 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 oocytes. So it seems that by flushing the follicles in DOR patients, we give them a chance for pregnancy. We are confident about this finding because when we do not make flushing after we cannot take the oocyte from the follicle, there would be no chance for this patient at all. With flushing of the follicle, although we get worse oocytes than the ones taken without flushing, we give the patient chance for embryo transfer. These oocytes with less probability can give pregnancy, and live birth rates are the same compared to the patients with oocytes taken without flushing.

Due to the increase in the planned age of maternity, the age of women giving birth is increasing, and the demand for assisted reproductive technologies is increasing yearly.¹⁵ Advanced maternal age is a risk factor for poor response to IVF, and various methods are used to reduce this risk factor.¹² With direct puncture to the follicle, only 60-80% of the oocytes 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 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. Significant results

cannot be obtained with FF in cases that are thought to be related to an intrinsic gametogenesis problem, and 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} In randomized controlled studies with a strong methodology, it was found that FF did not affect oocyte retrieval, clinical pregnancy, or live birth.^{9,19-21}

The literature does not evaluate the effect of oocytes retrieved by FF on pregnancy and live birth in women with DOR. A recently published meta-analysis reported that FF did not significantly affect live birth, pregnancy and 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, 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 diagnosed with DOR, and the presence of poor-quality oocytes is possible. In the study of Mok-Lin et al., 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.¹⁰ 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 lower with no significant difference than the group that did not require FF. Even with this number of patients without any significance, higher miscarriage rates favor the finding that oocytes taken without FF are better than oocytes taken with FF.

In 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, it was determined that oocyte retrieval without the need for FF showed a higher pregnancy rate.

The study has some limitations. The first is that it was a non-randomized controlled study, and 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. FF applied during oocyte retrieval in DOR cases does not give a chance for a pregnancy similar to those eggs collected without flushing, but we know that without flushing procedure in these patients, there will be no 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 clinical pregnancy rates even oocytes taken by flushing 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.

Source of Finance

During this study, no financial or spiritual support was received neither from any pharmaceutical company that has a direct connection with the research subject, nor from a company that provides or produces medical instruments and materials which may negatively affect the evaluation process of this study.

Conflict of Interest

No conflicts of interest between the authors and / or family members of the scientific and medical committee members or members of the potential conflicts of interest, counseling, expertise, working conditions, share holding and similar situations in any firm.

Authorship Contributions

Idea/Concept: Serkan Oral, Alper Şişmanoğlu; **Design:** Alper Şişmanoğlu; **Control/Supervision:** Serkan Oral, Alper Şişmanoğlu; **Data Collection and/or Processing:** Serkan Oral; **Analysis and/or Interpretation:** Alper Şişmanoğlu; **Literature Review:** Serkan Oral, Alper Şişmanoğlu; **Writing the Article:** Alper Şişmanoğlu; **Critical Review:** Alper Şişmanoğlu; **References and Fundings:** Serkan Oral; **Materials:** Serkan Oral.

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