

The benefits of follicular flushing on the number of oocyte retrieved in poor responder IVF/ICSI Cycles: Experience at King Hussein Medical Center

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ABSTRACT

Objectives: To evaluate whether follicular flushing would be beneficial in increasing the number of oocyte retrieved in poor responder patients.

Methods: This is a retrospective study at King Hussein Medical Center/Infertility unit over the years 2014-2015. The inclusion criteria was based on Bologna ESHRE criteria .All cases had -at day of human chorionic gonadotrophin trigger- follicles number less than 7 and more than 2 were included. The follicles mean diameter was more than 14mm. Patients were divided into two groups. The first group who underwent follicular flushing and the second group who had direct follicular aspiration without flushing. The parameters which were followed were age, FSH level, number of follicles, number of oocytes retrieved, metaphase I and II oocytes, fertilization rate, number of embryo transferred, biochemical pregnancy and clinical pregnancy rate. The data obtained were statistically analyzed.

Result: The total number of In vitro fertilization/Intracytoplasmic sperm injection cycles in the years 2014-2015 was 1238 and number of cases which were included according to our criteria was 52. Group one who underwent follicular flushing were 24 cases and the second group were 28 cases. The analyzed data showed no statistically significant difference in age, FSH level, number of oocytes retrieved, metaphase I and II oocytes, fertilization rate, number of embryo transferred, biochemical pregnancy and clinical pregnancy rate between the two groups.

Conclusion: Our results showed that follicular flushing is not beneficial practice and we do not recommend it as a routine procedure in poor responders IVF cycles. Despite our conclusion we suggest to expand this study for larger number of patients and in randomized controlled study.

Keywords: Poor responder, Follicular flushing, Ultrasound-guided oocyte retrieval/Aspiration, In-Vitro Fertilization.

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Introduction aspiration (TV-UDFA) has been introduced to
Transvaginal ultrasound-directed follicular the assisted reproductive techniques (ART)

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since 1985. ⁽¹⁾ It has substituted the previous laparoscopic procedure which was used for oocyte retrieval (OR) as proven that TV-UDFA is simpler, less costly, more efficient and safer ⁽²⁻⁵⁾ and it is considered now as the gold standard method for OR. Meanwhile, advances in modern technology developed the double-lumen needles for OR. The justification behind this development is to improve the number of oocytes retrieved. Double-lumen needles are designed to flush the ovarian follicles and consequently to overcome the potential retention of the oocytes either inside the follicles or within the follicular aspiration system. ^(3,5,6)

Where high level of evidence is lacking to support the advantage of using follicular flushing in normal responder IVF patients, but this procedure is still used worldwide in ART units. ⁽³⁾ Many studies earlier supported the advantage of using follicular flushing to increase the number of oocytes retrieved ^(3,7,8) but recently studies are demonstrating the opposite. ⁽³⁻⁵⁾ Most infertility units are now using follicular flushing for poor responders only and in natural cycle-IVF or monofollicular -IVF cycles. ⁽³⁻¹¹⁾

The aim of our study is to evaluate whether follicular flushing would be beneficial in increasing the number of oocyte retrieved in poor responder patients.

Methods

This is a retrospective study at King Hussein Medical Center/Infertility unit over the years 2014-2015. It was approved by Human Research Ethical Committee of Jordanian Royal Medical Services. The total number of IVF/ICSI cycles was 1238 and the mean age was 29 year and two months. We have selected our cases according to an inclusion criterion which include all cases that had -at day of HCG trigger- follicles number less than 7 and more than 2 and met the Bologna ESHRE criteria. ⁽¹²⁾ These inclusion criteria should include two out of the following. First, patient's age of 40 years

or more and/or has a history suggestive of poor ovarian response like previous history of chemotherapy, radiotherapy or ovarian surgery and others. Secondly, personal history of poor ovarian response (three oocytes or less retrieved despite adequate medication) and lastly poor ovarian reserve. The follicles mean diameter which was more than 14mm were aspirated. Dip-stick pregnancy test for follicular fluid was positive in all cases. The usual practice in our unit for normal IVF responder is administration of the HCG trigger when we have three or more leading follicles with average diameter of 16mm or more. Additionally, we perform dip-stick follicular fluid pregnancy test for all poor responders to ensure that patients had received their proper dose of HCG.

On the other hand, we have excluded all cases that have previous history of suspected empty follicular syndrome or proved to have negative pregnancy test in follicular fluid.

The follicular flushing was used once with 2ml media through double lumen needle. The retrieved oocytes were later managed by intracytoplasmic sperm injection (ICSI) as conventional IVF is not used in our unit and ICSI is the standard procedure for all cases. Fertilization was checked accordingly and all embryos were transferred on day 3. The number of embryos which were transferred was according to the number of fertilized eggs and grades of embryos. One case in the non-flushing group had 4 embryos transferred and the all other cases in the two groups had embryos transferred between 1 and 3. All patients were prescribed luteal phase support(natural progesterone vaginally), 75mg Aspirin orally and folic acid tablets.

Total of 52 cases were included in this study according to our selection criteria. Patients were divided into two groups. The first group 1(24 cases) who underwent follicular flushing by using double-lumen needle and media. Each follicle was flushed once with media. The second group 2 (28 cases) who had direct follicular aspiration without flushing (Fig. 1). The data were obtained from patients medical

records at our infertility unit. The parameters which were followed were age, FSH level, number of follicles, the number of oocytes retrieved, metaphase I and II oocytes, fertilization rate, number of embryo transferred, biochemical pregnancy and clinical pregnancy rate

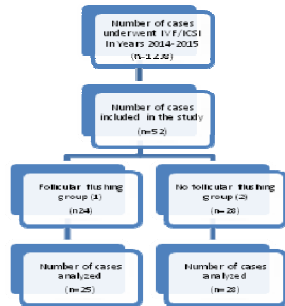


Fig. 1: Flow diagram of cases included in the study

The data were normally distributed based upon the value of Kolmogorov-Smirnov test of normality (P value= 0.2), therefore, parametric statistical analysis was used. Clinical parameters between 2 groups were compared using a chi-square test (χ^2) for the categorical data, and the Student's *t*-test for the numerical data to compare means. Data analysis was

performed by using the software Statistical Package for Social Sciences (SPSS) Version 16 (SPSS Inc 2008).The level of significance was taken at $P<0.05$.

Follicular flushing is not considered as a routine practice in our unit and it is dependent on the clinical judgment of the operator himself at day of OR. This procedure is only preserved for poor responders or when found to have no oocytes retrieved from the first ovary regardless of number of follicles. All the operators were expert infertility physicians.

Results

In our study, there was no demonstrable benefit between the flushing and no-flushing group. Both groups 1 and 2 basic characteristics were similar including the mean and the standard deviation of age(29.17 and 31.32 respectively) and FSH level (7.3279 and 7.9450 respectively)as can be seen in table I. All other parameters were similarly with no statistical difference including number of follicles, number of oocytes, M1, M2, fertilization rate and number of embryos transferred (Table I).

Table I: Mean and Standard deviation for all 8 variables for both groups (1,2)

		Flushing Group vs. Non-Flushing Group	N	Mean	Std. Deviation	Std. Error Mean
1.	Age	1	24	29.17	5.738	1.171
		2	28	31.32	5.004	.947
2.	FSH	1	24	7.3279	2.90537	.59306
		2	28	7.9450	3.44665	.65136
3.	# of follicles	1	24	3.88	1.777	.363
		2	28	4.25	1.647	.311
4.	# of oocytes	1	24	2.79	1.318	.269
		2	28	3.07	1.464	.277
5.	M1	1	24	.58	.881	.180
		2	28	.89	.685	.130
6.	M2	1	24	2.21	1.141	.233
		2	28	2.32	1.416	.268
7.	Fertilized	1	24	1.62	1.408	.287
		2	28	1.79	1.343	.254
8.	# of ET	1	24	1.42	1.100	.225
		2	28	1.50	1.171	.221

1=Flushing Group

2=Non-Flushing

As shown in table II all variables are not statistically significant; which means that both groups (Flushing Group and Non-Flushing Group) were comparable in term of FSH, number of follicles, number of oocytes, M1, M2, fertilized

oocytes and number of ET, with p-value >0.05. However, the mean in Non-Flushing Group was higher than the mean in Flushing Group, but this difference was not significant.

Table II: Independent Samples Test for Flushing Group vs. Non-Flushing Group

	t	df	Sig. (2-tailed) p-value	t-test for Equality of Means		95% Confidence Interval of the Difference	
				Mean Difference	Std. Error Difference	Lower	Upper
				1. FSH	-.691-	50	.493
2. No. of follicles	-.789-	50	.434	-.375-	.475	-1.329-	.579
3. No. of oocytes	-.719-	50	.475	-.280-	.389	-1.061-	.502
4. M1	-1.424-	50	.161	-.310-	.217	-.746-	.127
5. M2	-.314-	50	.755	-.113-	.361	-.838-	.611
6. Fertilized	-.421-	50	.676	-.161-	.382	-.928-	.607
7. No. of ET	-.263-	50	.794	-.083-	.317	-.720-	.553

Table III displays that there was no significant association between the occurrence of biochemical pregnancy and the used method (Flushing Group vs. Non-Flushing Group), although the rate of biochemical pregnancy (n,

%) 7 (29.2) was higher among the Flushing Group compared to the Non-Flushing Group 8 (28.6). However this difference was not statistically significant, with p-value =0.962, chi-square=0.002.

Table III: Flushing Group vs. Non-Flushing Group * Biochemical Pregnancy

		Flushing Group n(%)	Non-Flushing Group n(%)	Total	χ ² (p-value)
Biochemical Pregnancy	NO	17(70.8)	20(71.4)	37(71.2)	0.002(0.962)
	YES	7(29.2)	8(28.6)	15(28.8)	

Table IV shows that there was no significant association between the occurrence of clinical pregnancy and the used method (Flushing Group vs. Non-Flushing Group), although the

rate of clinical pregnancy (n, %) 6 (25.0) was higher among the Flushing Group compared to the Non-Flushing Group 6 (21.4) with p-value =0.761, chi-square=0.093.

Table IV: Flushing Group vs. Non-Flushing Group * Clinical Pregnancy

		Flushing Group n(%)	Non-Flushing Group n(%)	Total	χ ² (p-value)
Clinical Pregnancy	NO	18(75)	22(78.6)	40(76.9)	0.093 (0.761)
	YES	6(25)	6(21.4)	12(23.1)	

Discussion

There is lack of consensus among different studies regarding the best standardized method of follicular flushing. Meanwhile, Levy *et al* ⁽³⁾ had found in their meta-analysis that the best methodology is “Randomizing patients to flushing versus non-flushing is the best way to compare these approaches...” and consequently we had divided our study into two groups; the flushing and no flushing group.

Despite the term “poor responder” has been introduced to the terminology of infertility for more than 30 years now, but internationally accepted criteria was not clear until Bologna ESHRE criteria has been proposed in 2011. ⁽¹²⁾ This definition should include two out of the following. First, patient’s age of 40 years or more and/or has a history suggestive of poor ovarian response like chemotherapy, radiotherapy or ovarian surgery and others. Secondly, personal history of poor ovarian response of three oocytes collected or less despite proper dosing of ovulation induction medication and lastly poor ovarian reserve. The poor ovarian reserve is considered when the antral follicle count is less than 7 and/or anti-mullerian hormone level is less than 1.1 ng/mL. ⁽¹²⁾ Our selection criterion was based on the Bologna ESHRE criteria. Although, we did not have any case to be excluded at our study but our exclusion criteria was to assure that the patients had their proper dose of human chorionic gonadotrophin (HCG) and as empty follicular syndrome is a retrospective diagnosis, cases should be excluded when the follicular growth and steroidogenesis are normal but oocytes are not found ⁽¹³⁾.

The clinical significance of number of oocytes retrieved is its positive correlation with the cycle outcome. It is a common sense that when the number of oocyte retrieved is adequate, so the chances for fertilization are better with consequent better embryos in term of quality and quantity. This is demonstrated significantly in some studies when the number of oocytes is between 1 and 10 ^(3,9,10,14).

We also had performed one flushing with 2ml media because previous studies found that two or more flushes will add more time for the procedure and consequently more pain, longer anesthesia/analgesia time and possibly more complications. Additionally, the number of eggs collected were not proved to be larger when using multiple flushes and it is not cost effective ^(2-5,8,15).

The parameters which were followed in our study were age, FSH level, number of follicles, the number of oocytes retrieved, metaphase I and II oocytes, fertilization rate, number of embryo transferred, biochemical pregnancy and clinical pregnancy rate. We chose these parameters according to availability of data in our unit records. These parameters were similarly targeted by different studies ^(4-7,9,10). Haydardedeoglu *et al* ⁽⁴⁾ conducted their RCT study on normal responders and they added extra parameters on their study like body mass index, duration of infertility, E2 level at day of HCG, implantation rate, live birth rate and many other parameters. They found that follicular flushing did not add positive value for IVF cycles outcome and Santos-Haliscak *et al* ⁽⁷⁾ had the same conclusion for normal responders.

On the other hand, poor responders were chosen in our study as most infertility units are now using follicular flushing for poor responders only and in natural cycle-IVF or monofollicular-IVF cycles. ^(3-7,9,10,11) Mok-Lin *et al*. ⁽⁶⁾ concluded in their RCT that no statistical difference in the number of oocytes retrieved between the flushing and the non-flushing group and this is the result we found in our study. Interestingly, they found that follicular flushing group was with less reproductive outcome in terms of number of embryos transferred, implantation rate, and clinical pregnancy rate. While in our study there was no statistical difference in number of embryos and clinical pregnancy rate between the two groups.

Conclusion

Our results showed that follicular flushing is not beneficial practice and there is no statistical difference between the two groups in term of number of oocytes, fertilization rate, number of embryos transferred, chemical and clinical pregnancy rate. Though our study does not recommend follicular flushing as a routine procedure in poor responders IVF cycles but still our study is a retrospective one and cases were not randomized and controlled before the procedure. Also, the choice of flushing versus non-flushing was dependent on the surgeon doing the TV-UDFA and his clinical judgment which might add some bias between different cases. So, we suggest for the future research to include randomized controlled studies and the flushing being controlled according to established and agreed unit protocol for cases of poor responders only and in natural cycle-IVF or monofollicular-IVF cycles.

References

1. **A.K.Ludwig, M.Glawatz, G.Griesinger, K.Diedrich and M.Ludwig.** Perioperative and post-operative complications of transvaginal ultrasound-guided oocyte retrieval: prospective study of >1000 oocyte retrievals. *Human Reproduction* Vol.21, No.12 pp. 3235–3240, 2006.
2. **Marinko M. Biljan, Francois Bissonnette, Nicola Dean, Robert Hemmings, Seang.** Prospective randomized trial of the effect of two flushing; media on oocyte collection and fertilization rates after in vitro fertilization. *Fertility And Sterility*, Vol. 68, No. 6, December 1997.
3. **Levy G, Hill MJ, Ramirez CI, et al.** The use of follicle flushing during oocyte retrieval in assisted reproductive technologies: a systematic review and meta-analysis. *Hum Reprod* 2012;27:2373–2379.
4. **Haydardedeoglu B, Cok T, Kilicdag EB, Parlakgumus AH, Simsek E, Bagis T.** In vitro fertilization -intracytoplasmic sperm injection outcomes in single versus double-lumen oocyte retrieval needles in normally

responding patients: a randomized trial. *Fertil Steril* 2011;95:812–814.

5. **Levens ED, Whitcomb BW, Payson MD, Larsen FW.** Ovarian follicular flushing among low-responding patients undergoing assisted reproductive technology. *Fertil Steril* 2009;91:1381–4.
6. **Evelyn Mok-Lin, Anate Aelion Brauer, Glenn Schattman, et al.** Follicular flushing and in vitro fertilization outcomes in the poorest responders: a randomized controlled Trial. *Human Reproduction*, Vol.28, No.11 pp. 2990–2995, 2013.
7. **A. Avila P, Diaz-Spndola R Santos-Haliscak I, et al.** Garcia-Villafana. FOLLICLE FLUSHING DOES NOT Improve Reproductive Outcomes In Art Program. *Fertility Sterility*, Vol 100, No. 3, pp 1221-1222, September 2013.
8. **Bagtharia S, Haloob AR.** Is there a benefit from routine follicular flushing for oocyte retrieval? *J Obstet Gynaecol* 2005;25:374–6.
9. **Michael Wolff, Yu-Zhen Hua, Alessandro Santi, Erika Ocon, Benedicte Weiss.** Follicle flushing in monofollicular in vitro fertilization almost doubles the number of transferable embryos. *Acta Obstet Gynecol Scand* 2013 Mar; 92(3): 346–348.
10. **Bruce, Rose.** Follicle flushing for oocyte retrieval: Targeted analysis for patients with few follicles. *Journal of minimal stimulation IVF* 2014 Sep; Vol 1, Issue 2 ,pp : 75-80.
11. **Rohner S, Fäh M, Otti G, Kohl Schwartz A, Stute P. ; R. Schürch; von Wolff, M.** Follicular flushing in Natural Cycle IVF neither affects the length of the luteal phase nor the luteal body hormone production –a prospective controlled study http://ivf-naturelle.de/admin/upload/pdf/eshre_2016_rohner.pdf
12. **Filippo Ubaldi, Alberto Vaiarelli, Rosario D'Anna, and Laura Rienzi.** Management of poor responders in IVF: Is there anything new?, *BioMed Research International*, vol. 2014, 2014.
13. **K. Deepika, Suvarna Rathore, Nupur Garg, Kamini Rao.** Empty follicle syndrome: Successful pregnancy following dual trigger. *J Hum Reprod Sci* 2015 Jul-Sep; 8(3): 170–174.

14. **Sesh Kamal Sunkara, Vivian Rittenberg, Nick Raine-Fenning, Siladitya Bhattacharya, Javier Zamora, ArriCoomarasamy.** Association between the number of eggs and live birth in IVF treatment: an analysis of 400 135 treatment cycles. *Human Reproduction* Vol.26, No.7 pp. 1768–1774, 2011.
15. **Tan SL, Waterstone J, Wren M, Parsons J.** A prospective randomized study comparing aspiration only with aspiration and flushing for transvaginal ultrasound–directed oocyte recovery. *FertilSteril* 1992;58:356–60.