

Double-Lumen Needle Follicular Flushing System versus Single-Lumen Aspiration Needle in IVF/ICSI Patients with Poor Ovarian Response: A Meta-Analysis

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How to cite this paper: Xing, W.J., Ou, J.P., Cai, L.H. and Tao, X. (2017) Double-Lumen Needle Follicular Flushing System versus Single-Lumen Aspiration Needle in IVF/ICSI Patients with Poor Ovarian Response: A Meta-Analysis. *Advances in Sexual Medicine*, **7**, 167-178. https://doi.org/10.4236/asm.2017.74014

Received: September 11, 2017 Accepted: October 15, 2017 Published: October 18, 2017

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Abstract

Background: The present study performed a meta-analysis to comprehensively analyze existing randomized controlled trials (RCT) involving the use of double-lumen needle in patients with poor ovarian response to explore whether double-lumen needle was good for specific patients. Methods: The PubMed, EMBASE, Cochrane Library databases and two randomized controlled trials registration centers were thoroughly searched until April 2017. The clinical outcomes of IVF/ICSI cycles were compared between two groups with double-lumen needle and single-lumen needle. Results: Four RCT studies were included in this present meta-analysis. The oocytes yield was similar in two groups (OR 0.88, 95%CI 0.66 - 1.16; $I^2 = 4\%$). The procedure time with double-lumen needle was significantly longer than that with single-lumen needle (IV = 1.98, 95%CI 0.95 - 3.00; I^2 = 86%). The fertilization rate with double-lumen needle was lower than that with single-lumen needle (OR 0.66, 95%CI 0.44 - 0.97; $I^2 = 0$ %). There was no significant difference of live birth rate in two groups (OR 0.76, 95%CI 0.32 - 1.76; $I^2 = 41\%$). Conclusion: Double-lumen needle could not benefit patients with a POR in terms of the number of oocytes retrieved, oocyte recovery rate, normal fertilization rate, clinical pregnancy rate, and live birth rate, compared with single-lumen needle.

Keywords

Double-Lumen Needle, Single-Lumen Needle, Poor Ovarian Response

1. Background

In the past 20 years, ultrasound-guided transvaginal oocyte retrieval (TVOR) has

become the standard method of collecting oocytes in assisted reproductive technology (ART). This method is safer and more effective than the earlier laparoscopically assisted oocyte retrieval [1] [2]. The conventional application of TVOR involves a single-lumen oocyte retrieval needle. However, this could lead to oocyte retention within the follicles, especially in patients with poor ovarian response (POR) who have a lower number of follicles, which may result in the failure to collect oocytes. Therefore, a double-lumen oocyte retrieval needle was introduced to resolve this problem. When a double-lumen needle (DLN) is applied in oocyte retrieval, fluid is repeatedly injected into the follicle for flushing. Theoretically, follicular flushing should facilitate oocyte retrieval and should maximize the collection of oocytes [3] [4] [5] [6]. Nevertheless, with the development of oocyte retrieval technology, an increasing number of studies have found that skilled oocyte retrieval by direct aspiration can be used to obtain the vast majority of oocytes. Conversely, the use of a DLN for follicular flushing would prolong the operative time, affect the fertilization of oocytes, and confer no benefit compared with the use of a single-lumen needle (SLN) [6] [7]. A meta-analysis published by Roque et al. in 2012 concluded that in an unselected group of patients, there was no advantage in using a DLN in oocyte retrieval [8]. Another meta-analysis also reported that for patients with a normal ovarian function, the use of a DLN in oocyte retrieval did not improve the patients' ART outcomes [9].

Is there a need to perform follicular flushing using a DLN in oocyte retrieval for patients with a POR with fewer follicles? What is the developmental potential of oocytes obtained via follicular flushing? These questions await further investigation. The present study performed a meta-analysis to comprehensively analyze existing randomized controlled trials (RCTs) involving the use of DLNs in patients with a POR to explore whether DLNs are advantageous to specific patients.

2. Method

Literature Search

A comprehensive search was performed on the PubMed database, EMBASE database, Cochrane Library, and clinical trial registries (WHO International Clinical Trials Registry Platform and Clinical Trials.gov). The search was performed using the following subject headings or keywords: "double-lumen needle," "follicular flushing," "IVF," "ICSI," and "ART." The searching time span was 1916-2017, and the end date of the search was April 2017. The search strategy followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses statement.

Outcome Measures

The main outcome measures included the following: number of oocytes retrieved, operative time of oocyte retrieval, fertilization rate, clinical pregnancy rate, and live birth rate.

Study Selection

The inclusion criteria for the studies were as follows: 1) English studies; 2) study type: RCTs; and 3) study population: patients with a POR receiving IVF/ICSI treatments. The exclusion criteria were as follows: 1) reviews, conference abstracts, or meeting speeches; 2) studies with duplicate data; and 3) all studies with incomplete data.

Data Extraction and Quality Assessment

Two evaluators screened the studies independently based on the inclusion and exclusion criteria, which involved the following three steps: 1) Removal of duplicate studies using the End Note software; 2) Exclusion of studies that did not meet the inclusion criteria after reading the title and abstract of each study; 3) Screening of the entire study on the basis of the inclusion and exclusion criteria. Discrepancies in opinion were resolved by discussion or consulting with relevant experts.

The two evaluators extracted the study information independently. The extracted information included the following: first author, year of publication, study duration, experimental design, intervention measures, control measures, sample size, and outcome measures. They also crosschecked the information extracted from the other party, and discrepancies were resolved by joint discussion or by consulting with relevant experts.

The two evaluators evaluated the studies according to the Cochrane Handbook for Systematic Reviews of Interventions [10]. Six related domains were assessed in each included trial: 1) random sequence generation; 2) allocation concealment; 3) blinding of participants and personnel; 4) incomplete outcome data; 5) selective reporting; 6) other bias. Each item was judged as a rating of "low risk", "unclear risk" and "high risk" of bias.

3. Statistical Analysis

The meta-analysis was performed using the Review Manager (RevMan) 5.3 software. $I^2 > 50\%$ indicates a high heterogeneity, and a random effects model was used for the analysis. $I^2 \le 50\%$ indicates a low to moderate heterogeneity, and a fixed effects model was used for the analysis. P < 0.05 indicates that the difference was statistically significant.

Sensitivity analyses were performed to explore possible explanations for heterogeneity and to examine the influence of various exclusion criteria on the overall risk estimate. We investigated the influence of a single study on the overall risk estimate by omitting one study each time. Publication bias was assessed by funnel plots [11].

4. Results

Literature Search

A total of 41 articles were extracted. After screening by titles and abstracts, we got 20 articles. 16 articles were excluded by assessment for eligibility (Not in

POR: 10; Not a RCT: 2). Finally, 4 RCT studies were included in this present meta-analysis. The process for literature search was shown in **Figure 1**.

Study Characteristics

Characteristics of all studies from 2009-2017 included in the meta-analysis were summarized in Table 1. The fertility method was intracytoplasmic sperm



Figure 1. The process for literature search.

Гal	ole	1.	Characteristics	of al	l studi	es inc	luded	l in †	the s	ystematic	review.
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Study ID	Journal	Location	Duration of trial	Samples size	Interventions	Outcomes	conclusion
Haydardedeoglu 2017	BJOC	Turkey	2015.01-2015.06	SLN-Group:40 DLN-Group:40	SLN-Group: 17-G needle with single lumen (Cook) DLN-Group: 17-G needle with double lumen (Cook); follicular flushing with 10 mL culture medium for three times	No. of oocytes retrieved/No. of follicles aspirated, No. of MII oocyte, procedure time, FR, IR, PR, LBR	DLN is time consuming and has similar results comparing to SLN.
Horn 2017	Hum Reprod	Germany	2015.02-2016.03	SLN-Group:39 DLN-Group:39	SLN-Group: 17-G needle with single lumen (Gynetics [®]) DLN-Group: 17-G Steiner-Tan Needle [®] system with follicular flushing for three times	No. of oocytes retrieved/No. of follicles aspirated, procedure time, OPR/LBR,	The use of DLN is unlikely to benefit the prognosis of patients with poor ovarian response.
Levens 2009	Fertil Steril	USA	2007.04-2007.10	SLN-Group:15 DLN-Group:15	SLN-Group: 16-G Echotip [®] ovum aspiration needle with single lumen (Cook, K-J-ANC-16R-35) DLN-Group: 16-G double lumen needles (Cook, K-OPSD-1635-B-S); follicular flushing with 2 mL sterile PBS once	No. of oocytes retrieved/No. of follicles aspirated, No. of MII oocyte, procedure time, FR, OPR	The results did not demonstrate improved oocyte recovery with DLN even among those most likely to benefit from its application.
Mok-Lin 2013	Hum Reprod	USA	12-month period	SLN-Group:25 DLN-Group:25	SLN-Group: 16-G Echotip [®] ovum aspiration needle with single lumen (Cook) DLN-Group: 16-G Echotip [®] ovum aspiration needle with double lumen (Cook); follicular flushing with 5 mL culture medium for four times	No. of oocytes retrieved/No. of follicles aspirated, No. of MII oocyte, procedure time, FR, IR, PR, LBR	DLN in the poorest responders does not increase the number of oocytes retrieved and may result in lower implantation and clinical pregnancy rates.

injection (ICSI) in two studies and IVF/ICSI combination in two studies [5] [12]. There were 2 studies with embryo transferred on day 3 [12] [13], 2 studies without mentioned it. The main outcomes were oocytes yield (No. of oocytes re-trieved/No. of follicles aspirated), procedure time. The fertilization rate (FR) were compared in 3 studies [5] [12] [13]. The live birth rate (LBR) were compared in 3 studies [12] [13] [14].

Systematic risk assessment of methodological bias of included RCTs revealed two RCTs [5] [14] did not clearly describe "blinding of participants and personnel (performance bias)" and two RCTs [13] [14] did not clearly describe "blinding of outcome assessment (detection bias), therefore, we rated them at unclear risk of bias. We rated two studies [5] [14] at unclear risk in attribution bias, because we were unable to determine the integrity of data. We assessed one study [13] at an unclear risk of other bias, for an insufficient information about basic characters (**Figure 2** and **Figure 3**).

Meta-analysis

Oocytes yield

All of the four RCT studies reported the data about oocytes yield (No. of oocytes retrieved/No. of follicles aspirated) [5] [12] [13] [14]. As shown in **Figure** 4, the oocytes yield was similar in two groups (OR 0.88, 95%CI 0.66 - 1.16; $I^2 = 4\%$).

Procedure time

All of the four RCT studies reported the data about procedure time [5] [12] [13] [14]. From **Figure 5**, we found that the procedure time with double-lumen needle was significantly longer than that with single-lumen needle (IV = 1.98, 95%CI 0.95 - 3.00; $I^2 = 86\%$).

Fertilization rate

Only three RCT studies reported the data of fertilization rate [5] [12] [13]. In all of the three RCT studies, the fertilization rates with double-lumen needle were all lower than those with single-lumen needle. After meta-analysis, we found that the fertilization rate with double-lumen needle were all lower than that with single-lumen needle (OR 0.66, 95%CI 0.44 - 0.97; $I^2 = 0\%$; Figure 6).







Figure 3. Risk of bias summary. "+" means "low risk of bias"; "?" means "unclear risk of bias".

	Double-lumen	needle	Single-lumen	needle		Odds Ratio	Odds Ratio
Study or Subgroup	Events	Total	Events	Total	Weight	M-H, Fixed, 95% Cl	M-H, Fixed, 95% CI
Haydardedeoglu 2017	94	123	93	124	21.0%	1.08 [0.60, 1.93]	_ _
Horn 2017	94	156	121	173	43.9%	0.65 [0.41, 1.03]	
Levens 2009	108	130	97	121	16.4%	1.21 [0.64, 2.30]	- -
Mok-Lin 2013	96	118	112	135	18.7%	0.90 [0.47, 1.71]	
Total (95% CI)		527		553	100.0%	0.88 [0.66, 1.16]	
Total events	392		423				
Heterogeneity: Chi ² = 3.	12, df = 3 (P = 0.3	(7); l ² = 49	6				
Test for overall effect: Z					Double-lumen needle Single-lumen needle		

Figure 4. Forest plot of oocytes yield with DLNs versus SLNs.

Double-lumen needle			Single-I	umen ne	edle		Mean Difference		Mean Difference				
Study or Subgroup	Mean	SD	Total	Mean	SD	Total	Weight	IV, Random, 95% C		IV, Rand	<u>om, 95% (</u>		
Haydardedeoglu 2017	3.94	0.4	40	2.97	0.22	40	30.5%	0.97 [0.83, 1.11]			•		
Horn 2017	3.9	2.2	39	1.9	1.6	39	25.3%	2.00 [1.15, 2.85]					
Levens 2009	6.1	2.08	15	3.1	0.68	15	22.6%	3.00 [1.89, 4.11]			•		
Mok-Lin 2013	7	2.5	25	4.7	1.7	24	21.7%	2.30 [1.11, 3.49]			•		
Total (95% CI)			119			118	100.0%	1.98 [0.95, 3.00]			¥		
Heterogeneity: Tau ² = 0.89; Chi ² = 22.11, df = 3 (P < 0.0001); l ² = 86% Test for overall effect: Z = 3.78 (P = 0.0002)									-100	-50 Double-lumen needle	0 Single-lu	50 50	100

Figure 5. Forest plot of procedure time with DLNs versus SLNs.



Figure 6. Forest plot of fertilization rate with DLNs versus SLNs.

	Double-lumen	needle	Single-lumer	n needle		Odds Ratio	Odds Ratio
Study or Subgroup	Events	Total	Events	Total	Weight	M-H, Fixed, 95% CI	M-H, Fixed, 95% CI
Haydardedeoglu 2017	9	27	10	27	54.0%	0.85 [0.28, 2.60]	_
Horn 2017	3	39	1	39	7.5%	3.17 [0.31, 31.86]	
Mok-Lin 2013	1	20	5	20	38.5%	0.16 [0.02, 1.50]	
Total (95% CI)		86		86	100.0%	0.76 [0.32, 1.76]	-
Total events	13		16				
Heterogeneity: Chi ² = 3.3	38, df = 2 (P = 0.1	8); l ² = 41	1%				
Test for overall effect: $Z = 0.64$ (P = 0.52)							Double-lumen needle Single-lumen needle

Figure 7. Forest plot of live birth rate with DLNs versus SLNs.



Figure 8. Funnel plot illustrating publication bias. All studies lie inside the 95% CIs, with an even distribution around the vertical, indicating no obvious publication bias.

Live birth rate

Three out of four RCT studies reported the live birth rate (LBR) [12] [13] [14]. The LBR were lower in two studies with double-lumen needle than that with single-lumen needle [12] [13]. Only one study showed that the LBR was higher with double-lumen needle [14]. After meta-analysis, there were no significant difference of LBR in two groups (OR 0.76, 95%CI 0.32 - 1.76; $I^2 = 41\%$; Figure 7).

Sensitivity Analysis and Publication Bias

We investigated the influence of a single study on the overall risk estimate by omitting one study each time. In the sensitivity analysis, the exclusion of any one study did not materially alter the overall P value or heterogeneity (I^2) .

Figure 8 shows a funnel plot of the studies included in this meta-analysis. All studies lie inside the 95% CIs, with an even distribution around the vertical, indicating no obvious publication bias.

5. Discussion

The aim of *in vitro* fertilization-embryo transfer (IVF-ET) is to collect highquality oocytes at the minimum cost, thus obtaining high-quality embryos to improve clinical pregnancy rates. The majority of fertility centers in China and abroad have stopped using follicular flushing in patients with a normal ovarian response. However, follicular flushing is still applied to patients with a POR with the aim of increasing the number of oocytes retrieved. The reduced ovarian function in patients with a POR often leads to the increased dosage of gonadotropins (Gn), long duration of Gn stimulation, and fewer mature follicles. In ART, difficulty in oocyte retrieval is the most common problem that occurs during oocyte collection in these patients. The number of oocytes retrieved can directly affect their pregnancy outcomes, and surgeons often hope that mature ova can be obtained by repeated follicular flushing.

The results of the meta-analysis performed in this study indicate that compared with patients with a POR who used SLNs for oocyte retrieval, patients who used DLNs did not show a higher total number of oocytes retrieved or a significant increase in oocyte retrieval rates, but had a significantly longer operative time. A longer operative time would surely increase the patients' suffering and perioperative risk [9].

Furthermore, further analyses on the quality and developmental potential of the oocytes indicated that oocytes retrieved using DLNs showed significantly reduced normal fertilization rates and clinical pregnancy rates compared with those retrieved using SLNs; the live birth rate showed different extents of reduction. The possible reasons for these observations include the following:

1) Influence of the number of flushing. The follicular flushing fluid comes into direct contact with the ovum. Moreover, the ovum is extremely sensitive to the temperature, pH, osmotic pressure, and other factors in the external environment, whereby minor variations could lead to irreversible changes. Therefore, attention should be paid to the temperature of the flushing fluid and the sterile condition of the operation during follicular flushing. Repeated follicular flushing will not only increase the duration of transvaginal puncture, but also prolong the duration of oocyte immersion and exposure to the flushing fluid and increase the rate of oocyte morphological abnormalities. Studies have shown that approximately 40% of oocytes can be retrieved via direct aspiration; 82% can be retrieved after two times of follicular flushing; 97% can be retrieved after three times of follicular flushing; the oocyte retrieval rate decreases after more than three times of flushing [15] [16]. Nevens et al. [17] showed that the cumulusoocyte complex could be observed in approximately 90% of oocytes obtained by follicular flushing of up to two times, which did not affect the fertilization rate, pregnancy rate, and embryo quality. However, excessive follicular flushing significantly increased the rate of oocyte morphological abnormalities. Hussein compared the oocyte viability, cleavage rate after fertilization, and other outcome measures between oocytes recovered under transvaginal ultrasoundguided follicular puncture aspiration and in subsequent flushing after follicular aspiration. They found that the recovery rate and quality of oocytes retrieved under transvaginal ultrasound-guided follicular puncture and up to 4 mL of flushes were suitable for IVF. There was no need for repeated follicular flushing. During the process of follicular growth, the follicular cavity will continue to

expand; the follicular fluid will continue to increase, while the oocyte and granulosa cells will be pushed to one side to form the cumulus. Under natural conditions, the pre-ovulatory oocyte will eventually detach itself from the follicular wall and be suspended in the follicular cavity. We can infer from this that less mature oocytes will be more tightly bound to the follicular wall. Therefore, in theoretical terms, flushing via DLNs can be used to obtain a proportion of immature oocytes that are bound more tightly, thus increasing the oocyte retrieval rate. However, this would reduce the ratio of MII oocytes and lower the normal fertilization rate [16].

2) Flushing pressure: It is generally believed that the higher negative pressure during the oocyte retrieval process will damage the oocyte quality [16] [18].

3) Excessive flushing will also increase the probability of infection. This not only causes harm to the patients, but may also lead to pathogen contamination of the oocytes and embryos, thus causing the patients to have no viable embryos.

4) Patients with a POR themselves have fewer oocyte and embryo qualities; hence, follicular flushing is unable to change the essence of their poor-quality oocytes. Conversely, it may lead to the retrieval of oocytes with lower levels of maturity, thus leading to poorer IVF laboratory outcomes.

5) Lastly, follicular flushing would also aspirate more granulosa cells, leading to the excessive depletion of granulosa cells in the follicle. This could affect the functioning of the corpus luteum, thus influencing the pregnancy rates.

The Revman 5.3 software was used in this study to analyze the operative time, which found that the studies had a high heterogeneity ($I^2 = 86\%$). Further analysis of the included studies revealed that the type and model of the DLNs, number of flushes, and volume of flushing fluid used in each study were different. These factors may influence the operative time, thus causing the studies to have a slightly high level of heterogeneity. The meta-analysis of the other outcome measures, such as the number of oocytes retrieved, oocyte recovery rate, fertilization rate, clinical pregnancy rate, and live birth rate, did not indicate the presence of significant heterogeneity. The sensitivity analysis was performed by removing each study individually, and there was no significant decrease in heterogeneity when any study was removed.

This study has the following limitations: 1) The time span of the studies included was relatively long (from 2009 to 2017). There have been rapid developments in ART during this period, with major advancements in controlled ovarian hyper-stimulation protocols, embryo culture techniques, embryo cryopreservation techniques, transfer techniques, and other areas. Therefore, the results of the studies will be biased. 2) The included studies originated from different countries and fertility centers. 3) The sample sizes of the included RCTs were relatively small. 4) Lastly, the literature search may have been incomplete.

6. Conclusion

In summary, a meta-analysis was performed in this study to examine whether

DLNs could not benefit patients with a POR in terms of the number of oocytes retrieved, oocyte recovery rate, normal fertilization rate, clinical pregnancy rate, and live birth rate, compared with SLNs.

Competing Interests

The authors declare that they have no competing interests.

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Abbreviation Note List

TVOR	ultrasound-guided trans-vaginal oocyte retrieval
ART	assisted reproductive technology
POR	poor ovarian response
DLN	double-lumen needle
SLN	single-lumen needle
RCT	randomized controlled trials
IVF-ET	In vitro fertilization-embryo transfer
ICSI	intracytoplasmic sperm injection
FR	fertilization rate
IR	Implantation rate
PR	Pregnancy rate
OPR	Ongoing pregnancy rate
LBR	live birth rate
Gn	gonadotropins