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Original Article

IVF/ICSI outcomes in single-versus double-lumen oocyte retrieval needles in patients with unexplained infertility

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Abstract

Aim: To compare IVF-ICSI outcomes in single-versus-double lumen oocyte retrieval needles in patients diagnosed with unexplained infertility

Materials and Methods: A total of 188 patients aged 23-33 years who were diagnosed as unexplained infertility and underwent IVF/ICSI treatment were included and divided into two groups according to whether the catheter used in oocyte collection was single or double lumened: group 1 patients with single lumen (n: 59) and group 2 patients with double lumen (n:129). In addition, patients were divided into two subgroups according to the treatment protocol: antagonist protocol (n:134; 45 patient in group 1 and 89 patient in group 2) and long agonist protocol (n:54; 14 patient in group 1 and 40 patient in group 2).

Results: Implantation rate (32.2% vs. 48.1%, p:0.042) clinical pregnancy rate (25.4% vs. 41.9%, p:0.03) and live birth rate (17.2% vs. 28.6%, p: 0.011) were higher in patients to whom double-lumen was used. According to multivariate logistic regression analysis, double lumen use was found to be an independent predictor for clinical pregnancy (p = 0.041). According to the treatment protocol; clinical pregnancy rates (15 (25.4%) vs. 54 (41.9%), p: 0.011) and live birth rates (8 (13.6%) vs. 40 (31.0%), p: 0.030) of patients to whom double lumen was used during oocyte pick-up after the antagonist protocol were significantly higher.

Conclusion: Our study showed that the use of double lumen increased implantation rate, clinical pregnancy rate and live birth rate and it is evaluated as an independent factor that increases IVF/ICSI outcomes.

Keywords: Clinical pregnancy rate; double lumen needle; oocyte retrieval; unexplained infertility

INTRODUCTION

Transvaginal follicle aspiration is the standard procedure for oocyte retrieval during ART. This procedure is performed under USG guidance and safer and effective than previous laparoscopic methods (1). The goal of oocyte retrieval is to maximize the number of oocytes recovered. For this purpose, double lumen needles were develpoed to overcome the possibility of oocyte retention and flushing was perfomed after aspiration to pick up more oocytes in many patients groups. However, some studies (1-3) in unselected IVF patients showed that there was no significant difference in terms of the number of oocytes retrieved between single-lumen and double lumen needle.

Recent studies in patients with normo-responder (4) showed that double lumen aspiration did not increase the number of retrieved oocytes and clinical pregnancy rates, and studies on this subject started to increase. However, normo-responder patients were not analyzed according to their etiologies. Therefore, in this study we compared IVF-ICSI outcomes in single-versus-double lumen oocyte retrieval needles in patients diagnosed with unexplained infertility and we investigated whether double lumen use can benefit patients or not. In addition, we aimed to obtain more embryos bu collecting more oocytes by retrieved more oocytes with double lumen needles in this patient group.

MATERIALS and METHODS

This study was conducted as a retrospective, single-center cohort trial in the IVF clinic of the University of Health Sciences School of Medicine Etlik Zubeyde Hanım Research and Training Hospital between January 2016 and December 2018. The study protocol was approved

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by the hospital's Ethics Committee (No:90057706-799). Informed consent was obtained from all participants.

The women aged 23-33 years who were diagnosed as unexplained infertility and underwent IVF/ICSI treatment were included in the study. If all basic infertility assessment tests performed to couples who could not get pregnant within a year despite the unprotected regular sexual relationship were within normal limits, these patients were diagnosed as unexplained infertility. Patients with another etiology for infertility, patients with history of endometrial polyps, intrauterine adhesions, endometriosis, sub-mucosal myoma and hydrosalpinx, Patients undergoing freeze-all for OHSS and patients in whom oocyte pick-up (OPU) or embryo transfer was not possible were excluded. In addition, patients with retrieved oocyte count of 7 or less were defined as poor-responder and 15 or high were defined as high-responder (5) and these patients were also excluded.

In conventional protocols, recombinant FSH (Gonal-F, Merck Serono, Germany; Puregon, Organon, the Netherlands) with or without human menopausal gonadotropin (Menogon, Ferring Pharmaceuticals, Germany; Merional, IBSA, Switzerland) was used at doses ranging from 150 IU/day to 450 IU/day in accordance with body mass index, patient's age, and the number of antral follicles. Patients underwent pituitary down regulation using the luteal long protocol with a GnRH agonist (Lucrin, Abbott, France) or the GnRH antagonist protocol (Cetrotide, 0.25 mg/day, Serono, Germany). During follow-up, 0.25 micrograms of GnRH antagonist (Cetrotide, MerckSerono, Darmstadt, Germany) was started daily in patients with at least one follicle above 13-14 mm in the antagonist protocol. Ultrasound and E2 monitoring were continued until HCG criteria (at least 2 follicles 18 mm or more) were met. 250 micrograms of recHCG (Ovitrelle, Merck, Rome, Italy) were administered. With transvaginal ultrasound, oocyte retrieval was performed 35,5 hours after hCG injection. OPU was performed under sedation with 1% propofol (Fresenius Kabi, Homburg, Germany) by transvaginal ultrasound guided aspiration of the preovulatory follicle fluid with a 17 gauge single or double lumen catheter. 1 ml was injected into each follicle using a manually pressed syringe containing 10 ml of culture medium warmed to 37°C and re-aspirated and re-injected up to four times for each punctured follicle.

A total of 188 patients were included in the study and divided into two groups according to whether the catheter used in oocyte collection was single or double lumened: group 1 patients with single lumen (n: 59) and group 2 patients with double lumen (n:129). In addition, patients were divided into two subgroups according to the treatment protocol: antagonist protocol (n:134; 45 patient in group 1 and 89 patient in group 2) and long agonist protocol (n:54; 14 patient in group 1 and 40 patient in group 2). The total number of oocytes, mature oocyte counts, clinical pregnancy rates and live birth rates were also compared according to these catheters used for patients. A 17-gauge needle (VitrolifeSweden, Frölunda, Sweden) was used to aspirate follicles in both patient groups. After ICSI, the embryos were transferred on the 3rd or 5th day. P vaginal gel (90mg/d, Crinone 8% Vaginal Gel, Merck-Serono, Switzerland) was applied twice a day after oocyte collection and continued in pregnant patients until approximately 12 weeks of gestation for luteal phase support. Serum HCG test was performed 10 days following embryo transfer. The positive ones were evaluated as implantation and patients were called for B-hCG control after 48 hours and 10 days later for transvaginal ultrasound with the intauterin sac image.

The primer outcomes were clinical pregnancy rates (the presence of an intrauterine gestational sac confirmed by transvaginal ultrasonography), implantation rates (positive bhCG test (≥10 IU) 10 days after embryo transfer) miscarriage rate and live birth rate (the delivery of a viable infant after 24 weeks gestation). Sekonder outcome was the total number of retrieved oocytes and mature oocyte count.

Statistics

Statistical analysis was carried out through the use of SPSS program (version 20, SPSS, Chicago, IL). Data was expressed as average ± SD and in percentages. Continuous variables were investigated using visual (histograms, probability plots) and analytical methods (Kolmogrov-Simirnov / Shapiro-Wilk's test) to determine whether or not they are normally distributed. If the numerical data was non-parametric, Student's t test was conducted, if it was parametric, Mann Withney U test was carried. Categorical data was compared through the use of Chi-square test. Multivariate logistic regression analysis was used to determine independent predictors of clinical pregnancy. $p<0.05$ were accepted as statistically significant.

RESULTS

188 patients were analayzed. Single lumen was used in 59 (31.3%) and double lumen was used in 129 (68.7%) of these patients. In Table 1, demographic characteristics, oocyte collection procedures and embryology laboratory results were compared. BMI (28.4±5.6 vs. 26.7±5, p: 0.037), AFC (17.6±8.2 vs. 14.1±8.1, p:0.003), Number of follicles 15-17mm in diameter on HCG day (4.3±2.7 vs. 3.2±1.9, p:0.003), Number of follicles ≥ 17 mm in diameter on HCG day (4.3±2.8 vs. 2.8±1.9, p: <0.001) and E2 value on HCG day (3011±1817.7 vs. 1890.7±900, p<0.001) were all higher in patients in whom single-lumen catheter was used. Basal FSH (6.6 ± 1.8 vs. 7.4 ± 2.3, p: 0.004) and the total dose of gonadotropin given for induction of ovulation (1823.7 ± 599 vs. 2179.3 ± 754.9, p: 0.002) were higher in patients in whom double lumen catheters were used. Number of retrieved oocytes, number of mature oocytes, and number of 2PN, number of transferred embryos and grade of transferred embryos were similar in both groups.

Ovarian stimulation outcomes were shown in Table 2. Abortus rates were similar in both groups. Implantation rate (32.2% vs. 48.1%, p:0.042) clinical pregnancy rate (25.4% vs. 41.9%, p:0.03) and live birth rate (17.2% vs. 28.6%, p:

0.011) were higher in patients to whom double-lumen was used and it was statistically significant. Multivariate logistic regression analysis was performed to determine the factors affecting clinical pregnancy rate (Table 3). According to this analysis, double lumen use was found to be an independent predictor for clinical pregnancy with 2.760 OR (p = 0.041). In Figure 1 where patients were divided (Figure 2) into 2 subgroups according to the treatment protocol; clinical pregnancy rates (15 (25.4%) vs. 54 (41.9%), p: 0.011) and live birth rates (8 (13.6%) vs. 40 (31.0%), p: 0.030) of patients to whom double lumen was used during OPU after the antagonist protocol were significantly higher but there was no significant difference for number of retrieved oocytes (10.15±3.12 vs. 9.16±3.49, p: 0.063), number of mature oocytes (7.49±2.58 vs. 7.20±2.94, p: 0.359) according to the number of lumen of catheter used. Although the number of retrieved oocytes (10.50 ± 3.37 vs. 8.48 ± 3.24, p: 0.052) and the number of mature oocyte (8.00 ± 2.29 vs. 6.58 ± 3.37, p: 0.063) were higher in patients that received long-term agonist protocol in whom single lumes was used; clinical pregnancy rates (3 (21.4%) vs.21 (52.5%), p: 0.044) and live birth rates (2 (14.3%) vs.17 (42.5%), p: 0.057) were higher in patients to whom double-lumen was used.

Table 1. Baseline characteristics, oocyte retrived and embryology laboratory of participants

	Single-lumen (n:59)	Double-lumen (n: 129)	p Value
Maternal age(years)	30.8±4.5	31±4.6	0.684
BMI (kg/m²)	28.4±5.6	26.7±5	0.037
Duration of infertility(month)	83.3±46.9	84.6±52.1	0.986
Number of cycle	1.9±1.1	1.8±1.1	0.456
Basal FSH (IU/l)	6.6±1.8	7.4±2.3	0.004
Basal LH(IU/l)	4.6±2.8	4.9±2.5	0.220
Basal E2 (pmol/l)	53±34.2	48.9±22.4	0.497
AFC	17.6±8.2	14.1±8.1	0.003
AMH	3.9±2.9	3.1±2.4	0.153
Number of follicles 15-17mm in diameter on HCG day	4.3±2.7	3.2±1.9	0.003
Number of follicles ≥ 17 mm in diameter on HCG day	4.3±2.8	2.8±1.9	<0.001
E2 value on HCG day(pmol/l)	3011±1817.7	1890.7±900	<0.001
Total gonadotropin dose (IU)	1823.7±599	2179.3±754.9	0.002
No. of retrieved oocytes	10.2±3.1	9.2±3.5	0.065
No. of 2PN	4.4±2	4.4±2.3	0.703
No. of mature oocytes	7.5±2.6	7.2±2.9	0.359
Transferred embryos	1.3±0.4	1.3±0.5	0.277
Grade of transferred embryos	1.8±0.8	1.7±0.7	0.717

Values were presented as mean±SD. P value<0.05 was statistically significant. SD: standard deviation; BMI: body mass index; AFC: antral follicle count; AMH: anti-mullerian hormone; FSH: follicle stimulating hormone; LH: luteinizing hormone; E2: estradiol; HCG: human chorionic gonadotropin; PN:Pronucleus

Table 2. Ovarian stimulation outcomes

	Single-lumen (n:59)		Double-lumen (n:129)		p
	n	%	n	%	
Abortus rate	2	3.4%	12	9.3%	0.152
Live birth rate	20	17.2%	44	28.6%	0.011
İmplantation rate	19	32.2%	62	48.1%	0.042
Clinical pregnancy rate	15	25.4%	54	41.9%	0.030

Values were presented as numbers and percent (%). P value<0.05 was statistically significant. SD: standard deviation

Table 3. Logistic regression analysis for clinical features associated with clinical pregnancy rates

	B	S.E.	p	OR	95% C.I.	
					Lower	Upper
Basal FSH (IU/l)	-0.142	0.111	0.198	0.867	0.698	1.077
Double-lumen needle	1.015	0.496	**0.041**	2.760	1.044	7.296
AFC	-0.002	0.032	0.942	0.998	0.937	1.063
AMH	0.037	0.104	0.720	1.038	0.846	1.274
No. of retrieved oocytes	-0.023	0.068	0.734	0.977	0.855	1.117

B: Standardized regression coefficient SE: standard error. OR: odds ratio. CI: confidence interval. AFC: antral follicle count; AMH: anti-mullerian hormone; FSH: follicle stimulating hormone
p values with statistical significance (p < 0.05) are shown in bold

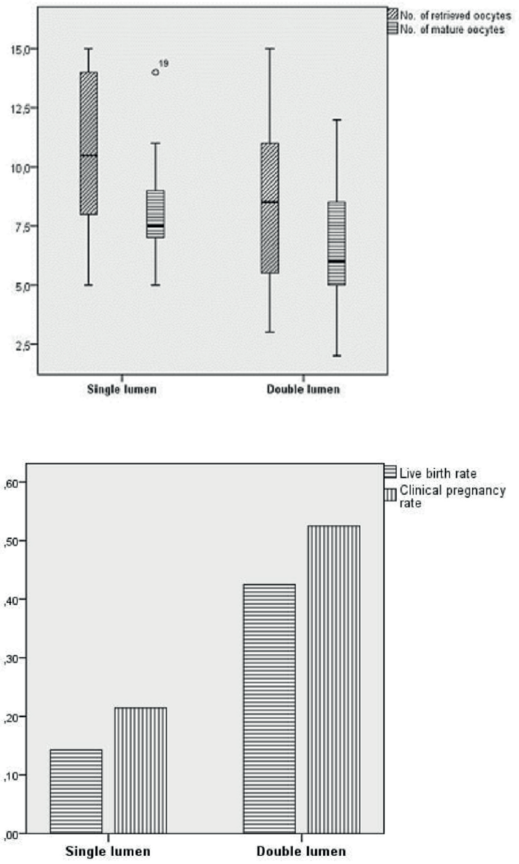

Figure 1. Comparison of total oocyte count, mature oocyte count, clinical pregnancy rate and live birth rate of patients applying single lumen and double lumens in long term luteal protocol

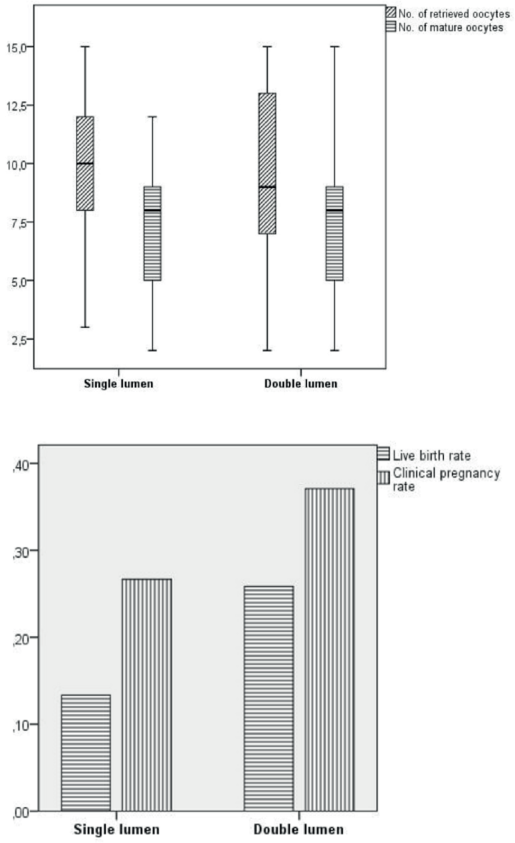

Figure 2. Comparison of total oocyte count, mature oocyte count, clinical pregnancy rate and live birth rate of patients applying single lumen and double lumens in antagonist protocol

DISCUSSION

Our study showed that in patients with unexplained infertility diagnosis from whom oocytes were collected using single lumen or double lumen; the lumen type of the catheter did not have an affect on the number of retrieved oocytes, number of mature oocytes and the grade of transferred embryos. But the use of double lumen increased implantation rate, clinical pregnancy rate and live birth rate and double lumen is evaluated as an independent factor that increases IVF/ICSI outcomes.

Today, it is aimed to increase the number of retrieved oocytes by flushing after follicle aspiration with double lumen catheter in many clinics. Patients with less follicles ≥ 14 mm in diameter on HCG day after treatment may produce an anxiety in terms of retrieved oocyte yield. Haydardedeoglu et al. showed that this anxiety is unnecessery (6). In their study with patients with low ovarian reserve, they found that total retrived oocyte counts were similar in single or double lumen use. In addition, in recent reviews (1,7,8); it was clearly emphasized that the double lumen catheter used does not contribute to the number of retrieved oocytes statistically. In our study, similar to the literature, we demonstrated that double lumen use had no positive effect on the number of retrieved oocytes, M2 and 2PN ratios; but unlike other studies with similar results (4,6,9); although the number of follicles over 15 mm on the HCG day was higher in the patient group who underwent OPU with a single lumen, the number of retrieved oocytes were similar in both groups. In previous RCTs (4,6), number of follicles ≥ 14 mm in diameter on HCG day were similar in both groups. Although double lumen contributes to this situation, the limited number of our study groups may have prevented statistical proof of this contribution.

The high flow rate in the catheter may strip the cumulus from the oocyte. When the aspiration pressure reached a 150 mmHg using a 17-gauge needle, it was found that all oocytes lost their cumulus mass (10). Therefore, it is recommended to keep the pressure below 120 mmHg. In addition turbulent non-laminar flow can also damage the oocyte, either stripping its cumulus mass or fracturing the zona (10). In order to avoid damaging the cumulus-oocyte mass during aspiration, the dead-space between the needle and the aspiration tube should be filled to prevent turbulent flow. Since the recommended dead space to be filled in the double lumen needle is less, the non-laminar flow within the collection tube, which is likely to damage the oocyte, is also less. This technical condition may affect the quality of the oocytes rather than the number. In our study unlike the literatüre and for the first time, implantation, clinical pregnancy and livebirth rates were significantly higher in the double lumen group. We associate this data with the technical information described above. Multivariate logistic regression analysis was performed to determine the factors affecting clinical pregnancy rate. According to this analysis, double lumen use was found to be an independent predictor for clinical pregnancy with 2.760 OR ($p = 0.041$). In contrast, we demonstrated that double lumen use has no reducing effect on abortion rates.

The relationship between the type of catheter used in the OPU and ovarian stimulation protocols is not clear, but Hill and Levens reported in their review (11) that more mature embryo are correlated with follicle washing in natural cycle or minimal stimulation protocols. In addition, another study (12) showed increased implantation rates in patients undergoing minimal stimulation, although follicle washing did not change with clinical pregnancy rate.

These publications suggest that the effects of the double lumen may vary according to the protocols applied to the patients (11,12). Although we could not find any data to support the relationship between the protocol applied and the lumen used, we did not want to ignore this incidental situation. Therefore, we subgrouped the patients included in our study according to the protocols applied and questioned the utility of double lumen use in antagonist and long-term agonist protocols. While there was no difference between the type of catheter and the number of retrieved oocytes and mature oocytes in both protocols, clinical pregnancy rates were higher in patients to whom double lumen was used. It was surprising that clinical pregnancy rates were significantly higher in patients to whom double lumen was used, although there were more retrieved oocytes and mature oocytes in patients

to whom single lumen was used in both protocols. The difference in clinical pregnancy rates may differs from the oocyte pick up process, such as male factor, endometrial factor or underlying reason of infertility. In addition, the significantly higher rates of live births in patients with antagonist protocol using double lumen could only be a coincidence or may be a condition associated with unknown etiological factors at unexplained infertility Although there is no clear information to explain this situation, this study showed that there are several factors that need to be clarified and evaluated in further studies on single / double lumen success according to different treatment protocols. Although the results reflect a certain patient population, it is valuable enough to draw attention to the relationship between the protocols used and the type of lumen used and to encourage discussion.

One of the important advantages of our study is miscarriage rates and subgrouping of patients according to protocols, which we could not find any data in the literatüre. Moreover, we worked with a more specific group of patients, which positively reflected our results. There are some limitations of our study. First it was a retrospective study. Second, the number of patients in this study is limited; an increased number of study participants would yield stronger results. Third, since anesthesia times could not be reached due to retrospective design, OPU durations could not be compared. However, the anaesthetic cannot reach a sufficiently high intrafollicular concentration during the approximately 5 minute operation.

CONCLUSION

In conclusion, the double lumens are not needed to retrieve more oocytes during OPU of the patients undergoing IVF / ICSI for unexplained infertility. However, the use of double lumens during OPU can be tried to increase live birth rates in this patient group. Randomized double-blind multicentre studies are needed to obtain more reliable data and confirm our results.

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