



Published in final edited form as:

Fertil Steril. 2009 April ; 91(4 Suppl): 1381–1384. doi:10.1016/j.fertnstert.2008.04.034.

Ovarian follicular flushing among low responding ART patients

Eric D. Levens, M.D.^{a,b,c}, Brian W. Whitcomb, Ph.D.^d, Mark D. Payson, M.D.^{a,b}, and Frederick W. Larsen, M.D.^{a,b}

^a Walter Reed Army Medical Center ART Program, Washington, DC

^b Combined Federal Fellowship in Reproductive Endocrinology and Infertility at the National Institutes of Health, Walter Reed Army Medical Center, National Naval Medical Center, and Uniformed Services University of the Health Sciences

^c Reproductive Biology and Medicine Branch, National Institute of Child Health and Human Development, NIH, Bethesda, MD

^d Epidemiology Branch, National Institute of Child Health and Human Development, NIH

Abstract

Follicular re-aspiration yielded similar oocyte recovery compared to direct aspiration (RR: 0.7; 95% CI: -0.9, 2.4; $p=0.38$), but entailed a longer procedure time (180 seconds; $p<0.001$) among low responding patients.

A randomized comparison trial was performed to evaluate whether follicular re-aspiration using a double-lumen retrieval needle improves oocyte recovery when compared to direct follicular aspiration among low responding ART patients. There were no differences observed in the number of oocytes retrieved (single-lumen: 6.5 ± 2.2 oocytes, double-lumen: 7.2 ± 2.3 oocytes; $p=0.38$); while follicular re-aspiration with the double-lumen retrieval needle resulted in a 2-fold increase in procedure time ($p<0.001$).

Keywords

assisted reproductive technology; oocyte recovery; retrieval needle; follicular flushing; randomized trial

Utilized for more than two decades, ultrasound-guided transvaginal retrieval is the standard of care for oocyte recovery (1–3). During this time, double-lumen retrieval needles, capable of

Correspondence: Eric D. Levens, M.D., Reproductive Biology and Medicine Branch, National Institute of Child Health and Human Development, Building 10, CRC, Room E1-3140, 10 Center Drive, Bethesda, MD 20892 (FAX: 301-402-0884; e-mail: levense@mail.nih.gov).

Where the work was done: Walter Reed Army Medical Center ART Program, Washington, DC; the Reproductive Biology and Medicine Branch and the Epidemiology Branch, National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, MD.

Conflict of interest: None

Disclosure: The opinions or assertions contained herein are the private views of the authors and are not to be construed as official or as reflecting the views of the Department of Health and Human Services, the Department of the Army or the Department of Defense.

Presented at a meeting: Presented in part at the 63rd Annual Meeting of the American Society for Reproductive Medicine, Washington, DC, October 13-17, 2007

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

flushing ovarian follicles, were developed to overcome the potential for oocyte retention within ovarian follicles and the retrieval collection system. No data exists as to the proportion of American ART practices that perform follicular flushing in addition to direct aspiration during oocyte retrieval; however in a recent Australian survey more than 50% of ART practices reported using this retrieval method (4). While generally utilizing direct follicular aspiration among normal responding patients, at our institution follicle flushing remains the technique of choice among low responding patients, in whom the optimization of oocyte recovery may be critical to pregnancy outcomes. To date, no studies have evaluated whether follicular flushing in this population improves oocyte recovery. Therefore, we performed a pilot, randomized trial of follicular fluid re-aspiration using a double-lumen needle to direct aspiration using a single-lumen needle.

Institutional Review Board (IRB) approval was obtained and all patients underwent oocyte retrieval as part of standard ART care. Pituitary suppression protocols and initial gonadotropins dosages were selected by the treating physician (F.W.L) prior to commencing oral contraceptive pills (OCPs) based on pre-cycle screening characteristics: diagnosis, day 3 FSH, antral follicle count, and ovarian volumes. Long-luteal leuprolide acetate (LL) or microdose follicular flare (MDF) protocols were employed as previously described (5). Briefly, for scheduling purposes all patients were pre-treated with OCPs (Lo-ovral; Wyeth Pharmaceuticals, Collegeville, PA) during the cycle preceding ovarian stimulation. Ovarian hyperstimulation was accomplished using a combination of recombinant follicular stimulating hormone (Gonal-F; EMD Serono, Rockland, MA) and human menopausal gonadotropins (Repronex; Ferring Pharmaceuticals, Suffern, NY) given twice daily. Adequate follicular development was assessed by serial serum estradiol concentration and follicular ultrasound (Acuson Sequoia 512; Siemens Medical Solutions, Malvern, PA) using an 8 MHz transvaginal probe.

Low responding patients, determined by a cumulative ovarian follicle count of 4–8 follicles ≥ 12 mm (with at least 2 follicles achieving ≥ 16 mm) on the day of hCG administration (Novarel; Ferring Pharmaceuticals, Suffern, NY), were eligible for this study. As per our clinic standard, patients received hCG 10,000 IU I.M. and transvaginal follicular aspiration followed 34–36 hours later. Patients deemed ineligible for transvaginal oocyte retrieval secondary to hyporesponse were excluded. The timing of and eligibility for follicular aspiration were not altered by the potential for study participation. Eligible patients were formally offered enrollment and randomized on the day of oocyte retrieval, immediately prior to the procedure. Treatment allocation was through computerized randomization in blocks of 10 and 20 to ensure balanced group sizes. Allocation was performed by the WRAMC Department of Clinical Investigation and concealed using sequentially numbered, opaque envelopes that were opened in the operating theater after anesthesia was administered. Randomization was to one of two groups: 1) a 35 cm 16-gauge single-lumen transvaginal oocyte retrieval needle (Echotip® ovum aspiration needle; K-J-ANC-16R-35, Cook Medical; Spencer, IN); 2) a 35 cm 16-gauge double-lumen transvaginal oocyte retrieval needle (Echotip® Double Lumen Aspiration Needle; K-OPSD-1635-B-S, Cook Medical). At oocyte retrieval, a single puncture of each ovary was performed and all the follicular fluid was aspirated using the Pioneer Pro-pump at 150–200 mmHg (GenX International; Guilford, CT) under direct transvaginal ultrasound guidance (Acuson Sequoia 512 with an 8 MHz transvaginal probe). Those in the single-lumen needle group did not undergo saline follicular flushing (direct aspiration); while those in the double-lumen group had each aspirated follicle flushed once with 2 mL of sterile phosphate buffered saline (PBS) and subsequently re-aspirated (follicular flushing). Previous studies have observed little benefit to a second follicular flush on the overall number of oocytes retrieved (4.7%) (6); multiple flushes have been shown not to result in a difference in the number of oocytes retrieved when compared to those undergoing direct aspiration (8–10). Where additional flushes were observed to increase the number of oocytes retrieved, a variable number

of follicle flushes were used and improvements occurred with every other flushing event (7). Regardless, additional flushes prolong retrieval time (9,10). As a result, we elected to employ a single follicular flushing for this protocol.

Subjects were enrolled in the order they gave consent. By necessity, the providers (F.W.L. or M.D.P) were aware of treatment allocation; however, the embryologist identifying and collecting the oocytes remained blinded to the group assignments. The providers performing the oocyte retrieval remained blinded to the number of oocytes retrieved until the completion of the procedure. Nursing staff recorded the total procedure time from the insertion of the retrieval needle into the first ovary to the removal of the needle upon completion of the second ovary. During retrieval, the providers remained blinded to this outcome measure. The primary end-point was the total number of oocytes retrieved. An *a priori* sample size calculation determined that 9 subjects in each group would be required to detect a 2 oocyte difference between groups ($\alpha=0.05$) with 80% power ($\beta=0.2$), with standard deviation of 1.3 oocytes (8). Because of the lack of evidence regarding oocyte recovery in low responders, we increased the sample size to 15 patients in each arm. Secondary end-points included recovery rate, total number of mature oocytes, maturity rate, fertilization rate, number of embryos transferred, implantation rate, on-going pregnancy rate, and retrieval time. Cycle characteristics and outcomes were evaluated by t-test, χ^2 or generalized linear models as appropriate with $p \leq 0.05$ considered statistically significant (SAS 9.1; SAS Institute, Cary, NC).

From April 2007 to October 2007, 45 patients meeting study requirements were offered enrollment; 30 patients consented and were randomized. Baseline characteristics and cycle outcomes are presented in the Table. There were no differences in age, total gonadotropins received, duration of stimulation, the proportion of ICSI, and the diagnosis between groups (data not shown). By chance, the day 3 FSH differed slightly between groups (single: 6.7 ± 1.7 mIU/mL; double: 8.2 ± 2.1 mIU/mL; $p=0.03$). The MDF protocol was used in all but one patient (double-lumen group). There were no significant differences between the groups regarding the mean number of ovarian follicles ≥ 12 mm and mature oocytes retrieved (Table). Similarly, the total oocytes retrieved did not differ between groups (single: 6.5 ± 2.2 , double: 7.2 ± 2.3 ; mean difference: 0.7; 95% CI: -0.9, 2.4; $p=0.38$). Furthermore, the proportion of mature oocytes recovered from follicles ≥ 12 mm was 83% in the single-lumen group and 85% in the double-lumen group ($p=0.70$). There were no differences in oocyte recovery between providers (data not shown). The retrieval time was 2-fold higher among those undergoing follicular flushing using the double-lumen needle, with an estimated increase of 180 seconds (95% CI: 108, 253; $p < 0.001$). There were no significant differences between the groups in terms of fertilization (RR: 0.84; 95% CI: 0.62, 1.14; $p=0.27$), implantation (RR: 0.43; 95% CI: 0.13, 1.40; $p=0.19$), or ongoing pregnancy (RR: 0.58; 95% CI: 0.18, 1.86; $p=0.43$).

In this pilot study, we compared the oocyte recovery efficiency of follicular re-aspiration to direct follicular aspiration in a sub-set of low responding ART patients. Unlike previous studies, we standardized the length and diameter of retrieval needles between the groups, which has been proposed to affect flow dynamics within the needle and affect oocyte recovery (11, 12). Our results did not demonstrate an improvement in oocyte recovery with ovarian follicle re-aspiration using the double-lumen retrieval needle compared to the single-lumen needle. We evaluated the retrieval efficiency of single, direct follicular aspiration compared to that attained with follicular flushing by assessing total oocytes retrieved—the most direct measure of the retrieval technique. If no differences in the number of oocytes retrieved were observed, one would not expect to see a difference in clinical outcomes. Other outcomes, including fertilization, implantation and clinical pregnancy rates, depend upon factors unrelated to the retrieval protocol under study (e.g. male or uterine factors) and are dependent upon post-randomization events. Results for these outcomes are shown as those of secondary end-points in this study. Of note, the per group sample size necessary to detect the observed difference in

clinical pregnancy with statistical significance ($\alpha=0.05$; $\beta=0.20$) would be about 90—beyond the scope of this pilot study.

Previous studies among reportedly randomized, unselected patients have likewise not demonstrated an improvement in oocyte recovery with follicular re-aspiration (8–10,13). Moreover, those studies reporting benefit from re-aspiration techniques lacked comparison groups and were not randomized (6,7,14). Omissions such as a lack of randomization or allocation concealment may lead to selection and confounding biases. While frequently employed, follicular flushing has not been demonstrated to be superior to direct follicle aspiration in randomized studies, though it has been associated with an increase in procedure time. Although the utility of follicular re-aspiration remains to be fully determined, estimates from this small randomized controlled trial did not demonstrate improved oocyte recovery with ovarian follicular flushing even among those most likely to benefit from its application.

Acknowledgments

Financial Support: This research was supported, in part, by the Reproductive Biology and Medicine Branch and the Epidemiology Branch, NICHD, NIH, Bethesda, MD.

References

- Gembruch U, Diedrich K, Welker B, Wahode J, van der Ven H, Al-Hasani S, et al. Transvaginal sonographically guided oocyte retrieval for in-vitro fertilization. *Hum Reprod* 1988;3 (Suppl 2):59–63. [PubMed: 3230121]
- Wikland M, Enk L, Hamberger L. Transvesical and transvaginal approaches for the aspiration of follicles by use of ultrasound. *Ann N Y Acad Sci* 1985;442:182–94. [PubMed: 3893266]
- Ludwig AK, Glawatz M, Griesinger G, Diedrich K, Ludwig M. Perioperative and post-operative complications of transvaginal ultrasound-guided oocyte retrieval: prospective study of >1000 oocyte retrievals. *Hum Reprod* 2006;21:3235–40. [PubMed: 16877373]
- Knight DC, Tyler JP, Driscoll GL. Follicular flushing at oocyte retrieval: a reappraisal. *Aust N Z J Obstet Gynaecol* 2001;41:210–3. [PubMed: 11453275]
- Levens ED, Whitcomb BW, Kort JD, Materia-Hoover D, Larsen FW. Microdose follicular flare: A viable alternative for normal-responding patients undergoing in vitro fertilization? *Fertil Steril*. 2008
- el Hussein E, Balen AH, Tan SL. A prospective study comparing the outcome of oocytes retrieved in the aspirate with those retrieved in the flush during transvaginal ultrasound directed oocyte recovery for in-vitro fertilization. *Br J Obstet Gynaecol* 1992;99:841–4. [PubMed: 1419996]
- Bagtharia S, Haloob AR. Is there a benefit from routine follicular flushing for oocyte retrieval? *J Obstet Gynaecol* 2005;25:374–6. [PubMed: 16091324]
- Scott RT, Hofmann GE, Muasher SJ, Acosta AA, Kreiner DK, Rosenwaks Z. A prospective randomized comparison of single- and double-lumen needles for transvaginal follicular aspiration. *J In Vitro Fert Embryo Transf* 1989;6:98–100.
- Kingsland CR, Taylor CT, Aziz N, Bickerton N. Is follicular flushing necessary for oocyte retrieval? A randomized trial *Hum Reprod* 1991;6:382–3.
- 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. *Fertil Steril* 1992;58:356–60. [PubMed: 1633902]
- Confino E, Gleicher N, Keskey TS, Friberg J, Dudkiewicz A. Comparison between 14" and 16" gauge needles for aspiration of human oocytes. *Mt Sinai J Med* 1986;53:250–3. [PubMed: 3487719]
- Reeves G. Single- and double-lumen needles for oocyte harvesting. *J In Vitro Fert Embryo Transf* 1989;6:353–5.
- Haines CJ, Emes AL, O'Shea RT, Weiss TJ. Choice of needle for ovum pickup. *J In Vitro Fert Embryo Transf* 1989;6:111–2.
- Waterstone JJ, Parsons JH. A prospective study to investigate the value of flushing follicles during transvaginal ultrasound-directed follicle aspiration. *Fertil Steril* 1992;57:221–3. [PubMed: 1730321]

Table
The baseline characteristics and outcomes of study participants by treatment assignment

	Single-lumen (Direct aspiration)	Double-lumen (Follicular re-aspiration)	<i>p</i> -value
	(n=15)	(n=15)	
<i>Baseline characteristics</i>			
Age (years)	37.1 ± 3.2	36.2 ± 3.4	0.48
Day 3 FSH (mIU/mL)	6.7 ± 1.7	8.2 ± 2.1	0.03
Total gonadotropins (amps)	63.8 ± 9.5	58.1 ± 10.2	0.12
Duration of stimulation (days) *	11.3 ± 1.5	10.5 ± 1.1	0.13
ICSI (n, %)	10 (67%)	12 (80%)	0.68
Total ovarian follicles (all sizes)	8.1 ± 1.8	8.7 ± 2.0	0.45
Total ovarian follicles (≥12 mm)	5.9 ± 1.3	6.5 ± 1.1	0.25
<i>Cycle outcomes</i>			
Total oocytes retrieved (n)	6.5 ± 2.2	7.2 ± 2.3	0.38
Recovery (%)	83%	85%	0.70
Total oocytes mature (n)	4.9 ± 1.9	5.5 ± 2.6	0.43
Maturity (%)	76%	76%	0.96
Fertilization (%)	67%	57%	0.27
Number of embryos transferred	2.1 ± 1.0	1.8 ± 1.0	0.48
Implantation (%)	26%	11%	0.19
On-going pregnancy (n, %)	6 (40%)	3 (20%)	0.43
Retrieval time (sec)	186 ± 41	366 ± 125	<0.001

mean ± SD

* calculated from the first day of gonadotropin administration in each group