

# Oocyte retrieval at 140-mmHg negative aspiration pressure: A promising alternative to flushing and aspiration in assisted reproduction in women with low ovarian reserve

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Received: 17.01.2015  
Review completed: 27.02.2015  
Accepted: 04.05.2015

## ABSTRACT

**BACKGROUND:** Oocyte retrieval in women with low ovarian reserve is challenging and often results in poor pregnancy outcomes. **AIM:** The aim was to compare outcomes of oocyte retrieval using three aspiration methods in women with normal and low antral follicle counts (AFCs). **STUDY DESIGN:** Retrospective cohort study. **SETTING:** Tertiary care hospital. **MATERIALS AND METHODS:** Data from 172 women who underwent *in-vitro* fertilization/intracytoplasmic sperm injection over 3 years was studied after dividing into three groups: Group A (96) - normal AFC ( $\geq 10$ ), direct oocyte retrieval at 120 mmHg, Group B (41) - low AFC ( $< 10$ ), direct retrieval at 140-mmHg, Group C (35) - low AFC, follicular flushing, and aspiration at 120-mmHg. In each group, oocyte yield, embryo quality, and pregnancy outcomes were assessed. **RESULTS:** The oocyte and embryo yield in Groups A and B were comparable ( $P > 0.05$ ) but were significantly less in Group C ( $P < 0.05$ ). The pregnancy rates in Groups A and B were similar (35.4%, 39%) but were significantly less (17.1%) in Group C ( $P < 0.05$ ). The live birth rate was 76% in Group A, 69% in Group B, and 50% in Group C. The miscarriage rates in the three groups were 21%, 31%, and 50%, respectively. **CONCLUSION:** Direct oocyte retrieval with 140-mmHg showed significantly better oocyte yield and pregnancy outcomes compared to flushing and aspiration in women with low AFC.

**KEY WORDS:** Flushing and aspiration, oocyte aspiration, vacuum aspiration pressure

## INTRODUCTION

Oocyte retrieval is a key step in assisted reproduction, the transvaginal route being the current preferred method. The quality of the oocyte so collected is an important determinant of the overall embryo quality and post embryo-transfer outcomes. Apart from the inherent characteristics of the oocyte, its quality is affected by the actual process of oocyte retrieval; including the expertise of the aspirating doctor, the type of equipment and the techniques used. The aspirating pressure may affect the integrity of the oocytes aspirated. It is generally postulated that increasing the vacuum aspiration pressure might decrease the quality of oocytes retrieved.<sup>[1]</sup> In cases of anticipated difficult oocyte retrievals the practice is to flush and

aspirate 2–4 times maintaining the standard pressure.<sup>[1,2]</sup> This can cause loss of ovarian granulosa cells that may result in subsequent corpus luteal insufficiency.

The Cochrane review in 2010, followed by the meta-analysis in 2012<sup>[3,4]</sup> concluded that follicular flushing in unselected women undergoing *in-vitro* fertilization (IVF) did not affect the oocyte yield or pregnancy outcomes. It, in fact, increased the operating time marginally and increased the analgesic requirement. More recently, a small study<sup>[5]</sup> showed that even in poor responders the method of follicular flushing does not improve the oocyte yield.

There is a lack of studies looking at the effect of aspiration pressure on oocyte quality and

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10.4103/0974-1208.158617

pregnancy outcomes. A few studies have quoted using aspirating pressures ranging between 150 mmHg and 200 mmHg<sup>[6]</sup> or occasionally, as low as 80 mmHg.<sup>[5]</sup>

We routinely used aspirating pressures of 120 mmHg but like many others in this field found that oocyte yield was less in women with low antral follicle count (AFC) ( $\leq 10$ ). Hence, we decided to try oocyte retrieval at a slightly higher aspiration pressure of 140-mmHg in women with low follicular counts. We analyzed 3-year data from our center to compare the outcomes following the three methods of oocyte retrieval— aspirating at the standard negative pressure of 120-mmHg, higher negative aspiration pressure of 140-mmHg, and flushing and aspiration at 120-mmHg.

### Aim(s) and objectives

To compare oocyte retrieval outcomes using three methods of aspiration:

- Direct aspiration pressure of 120-mmHg in those with normal AFC
- Direct aspiration pressure at 140-mmHg in those with low AFC
- Aspiration pressure of 120-mmHg with flushing in those with low AFC.

The AFC in both the ovaries together of  $\geq 10$  was considered normal and anything less was low.

## MATERIALS AND METHODS

This retrospective study was conducted at the assisted reproduction center in our Tertiary Care Hospital. We included data from IVF records of 172 women who underwent oocyte retrieval and IVF/intracytoplasmic sperm injection in our center over a period of 3 years from May 2010 to June 2013. The data were divided into three groups as described previously. Group A consisted of 96 women with normal AFC who underwent oocyte retrieval with negative aspiration pressure of 120-mmHg, Group B consisted of 41 women with low AFC who had oocyte retrieval using negative pressure of 140-mmHg, and Group C consisted of 35 women with low AFC in whom oocyte retrieval was done after flushing if initial aspiration at 120-mmHg did not yield oocytes. Women who had oocyte retrieval with 120-mmHg from one ovary and 140-mmHg from the other ovary were excluded from the study. The groups were comparable with respect to semen parameters and other co-morbidities like endometriosis. They underwent stimulation with antagonist protocol. The number of oocytes retrieved, the oocyte yield (the number of oocytes retrieved divided by follicles aspirated), and the fertilization rate (the number of embryos developed divided by number of eggs retrieved) for each patient was calculated.<sup>[7]</sup> The oocyte and embryo quality were graded according to the Istanbul consensus criteria [Table 1].<sup>[8]</sup> Serum beta-human

chorionic gonadotropin (bHCG) level was done 2 weeks after embryo transfer. Those who had a bHCG level  $\geq 30$  mIU/mL but showed no evidence of pregnancy on ultrasound scan were termed biochemical pregnancies. The rest were clinical pregnancies. Pregnancy losses at  $< 24$  weeks were termed miscarriages. Those that delivered live after 24 weeks were termed live births. Ethical Committee Clearance was obtained prior to the study.

### Analysis

Statistical analysis was done by Statistical package for the social sciences version 16 (SPSS, Chicago, USA). Data was analyzed by applying Chi-square tests, and  $P < 0.05$  was considered significant.

## RESULTS

As shown in Table 2, a higher mean number of oocytes and embryos were noted in Group A, compared to Groups B or C ( $P < 0.001$ ). As Group A had a higher AFC to begin with, we analyzed the oocyte yield (number of oocytes divided by number of follicles tapped in each patient) and the embryo yield (number of embryos formed divided by the number of oocytes retrieved per patient) to enable comparison between the three groups [Table 3]. No statistically significant difference was noted between the oocyte yield in Groups A and B ( $P = 0.404$ ) while that in Group C was comparatively less. The mean embryo yield in all three groups was comparable, but the maximum yield was in Group B.

**Table 1: Consensus scoring system for cleavage-stage embryos<sup>[8]</sup>**

Grade	Rating	Description
1	Good	<10% fragmentation Stage-specific cell size No multinucleation
2	Fair	10-25% fragmentation Stage-specific cell size for majority of cells No evidence of multinucleation
3	Poor	Severe fragmentation (>25%) Cell size not stage-specific Evidence of multinucleation

Courtesy-Basak Balaban *et al.*, The Istanbul consensus workshop on embryo assessment: Proceedings of an expert meeting; Alpha Scientists in Reproductive Medicine and ESHRE Special Interest Group of Embryology Human Reproduction, 2011, 26, 6 1270-1283, 2011 Advanced Access Publication on April 18

**Table 2: Mean parameters from each group**

Parameter	Mean		
	Group A 120 mmHg n=96	Group B 140 mmHg n=41	Group C 120 mmHg with flushing n=35
Follicles tapped	18.72	7	6.8
Eggs retrieved	8.52	4	2.01
Fertilized eggs	5.11	2.73	1.01
Embryos obtained	3.91	2.25	1.00

**Table 3: Oocyte and embryo yield**

Parameter	Group A		Group B		Group C	
	120 mmHg n=96		140 mmHg n=41		120 mmHg with flushing n=35	
	Mean	SD	Mean	SD	Mean	SD
Oocyte yield	0.46	0.16	0.57	0.24	0.29	0.04
Embryo yield	0.46	0.23	0.56	0.27	0.50	0.06

SD: Standard deviation

The pregnancy rates and outcomes are depicted in Table 4 and Figure 1. The number of biochemical pregnancies in all the three groups was small and did not differ much. The clinical pregnancies in Group A (35.4%) and Group B (39%) were significantly higher than the 17.1% in Group C. Of these clinical pregnancies, 76% in Group A and 69% in Group B resulted in live births as against 50% in Group C. Considering the original number of women in each group, the live birth rates were comparable in Group A (27%) and B (26.8%) but significantly less in Group C (8.57%). The miscarriages in Group A and B were 21 and 31%, respectively as against the 50% in Group C.

## DISCUSSION

Aspiration of oocyte cumulus complex is the defining step in assisted reproduction.<sup>[5]</sup> Transvaginal oocyte recovery is the least invasive, least painful, most accurate, and simple method to collect oocytes.<sup>[9,10]</sup> Apart from the inherent characteristics of the oocyte, its quality is affected by the techniques used for oocyte retrieval. The aspirating pressure used for oocyte retrieval can affect the integrity of the oocytes.<sup>[11]</sup>

Initially, manual syringe aspiration was used for oocyte pick up. Maintaining a steady aspiration pressure below 120-mmHg was difficult with this technique. Manual syringing has consequently been replaced by electronic aspiration pumps, which can maintain a steady aspiration pressure.<sup>[10]</sup> Morphologically abnormal oocytes have been seen more at higher aspiration pressures particularly around 180-mmHg, which were frequently used during laparoscopic oocyte retrieval.<sup>[12-16]</sup> Aspiration pressures between 90 mmHg and 120 mmHg have been associated with good oocyte yield and minimal damage.<sup>[1,2,8]</sup> Few published studies that have assessed the effect of higher aspiration pressure for oocyte retrieval in humans.<sup>[10,11,17,18]</sup> Higher than standard pressures have been used for aspiration of immature human oocytes for *in-vitro* maturation. A study done in this field noted that aspiration pressures of more than 180-mmHg cause immature oocyte damage and poor embryogenesis.<sup>[10]</sup>

However, there is no literature citing the effects of slightly higher aspiration pressure of 140-mmHg on oocytes. Moreover, many recent studies have shown that most morphologic abnormalities especially the fractured zona pellucida that is attributed to higher than standard aspiration

pressure does not significantly affect favorable assisted reproduction technology (ART) outcomes.<sup>[19-20]</sup> A review published in 2011 also stated that the predictive potential of oocyte morphology in IVF outcome is not clear at present.<sup>[19]</sup>

In women with low ovarian reserve (AFC < 10), the standard aspirating pressure of 120-mmHg often yields very less oocytes.<sup>[1,2]</sup> In these women flushing the follicles with tubal fluid and aspiration was believed to improve the oocyte yield and thereby the ART outcome.<sup>[21,22]</sup> In fact, a study published in 2005 found that flushing up to four times was optimal in maximizing the oocyte recovery rate per follicle tapped.<sup>[22]</sup> However, the 2010 Cochrane review on flushing, found no added benefit in using flushing compared to direct aspiration. Flushing and aspiration lengthened the oocyte retrieval time.<sup>[4]</sup> Flushing was also believed to increase loss of granulosa cells causing luteal phase defects and miscarriages.<sup>[23,24]</sup>

This study showed that the oocyte yield, quality of oocytes retrieved, as well as pregnancy rate (39%) following direct oocyte aspiration at 140-mmHg, was comparable to that with the standard aspiration pressure of 120-mmHg (34.4%); whereas flushing and oocyte retrieval resulted in only 17.1% clinical pregnancies, significantly less compared to the other groups.

Oocyte quality was determined based on nuclear maturation. Morphological differences in oocytes retrieved were not seen among the groups on analysis by our embryologists. Cleavage stage embryo assessment was done either on day 2 or 3. These were done as per the Istanbul consensus criteria as mentioned earlier.<sup>[8]</sup> No significant difference in the morphology of oocytes retrieved was noted between the three groups. Nevertheless, the oocyte and embryo yields were more in the direct aspiration groups compared to the flushing group.

The clinical pregnancy rate in the flushing group was a dismal 17.1% compared to 39% in the 140 mmHg group [Table 1 and Figure 1]. The flushing group also had a high miscarriage rate (50%). On the other hand, pregnancy outcomes in groups undergoing oocyte aspiration at 120 and 140-mmHg were comparable, even though, the latter group included women with low AFC. The abortion rate of 31% was higher in the 140-mmHg compared to the 21% in the 120-mmHg group, but the difference was not statistically significant [Figure 1]. Loss of granulosa cells results in poor ovarian hormonal support in the luteal phase, as well as the first trimester of pregnancy, which might explain the poor pregnancy rate and increased abortions in the flushing group.<sup>[25]</sup>

The present study showed that increasing oocyte pickup pressure to 140-mmHg did not adversely affect the

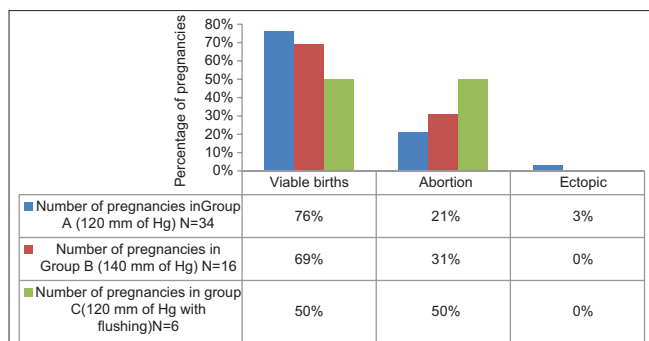


Figure 1: Pregnancy outcomes

Table 4: Pregnancy rates

	Oocyte retrieval technique		
	Group A Aspiration at 120 mmHg n=96 (%)	Group B Aspiration at 140 mmHg n=41 (%)	Group C Flushing and aspiration at 120 mmHg n=35 (%)
Number of biochemical pregnancies	4 (4.1)	1 (2.43)	3 (8.5%)
Number of clinical pregnancies	34 (35.4)	16 (39.0)	6 (17.1)

oocyte yield, embryo yield, and quality or pregnancy outcome compared to the standard aspiration pressure of 120-mmHg, even though it was used in women with low AFC. This increased pickup pressure seemed logical since it was practically feasible and gave hope to a segment of patients where the oocyte retrieval was difficult with the stipulated standard pressure of 120-mmHg. Moreover, oocyte aspiration pressure of 140-mmHg gave promising retrieval rates and pregnancy outcomes in women with poor AFC, where the alternative practice of flushing and aspiration showed dismal outcome.

Assisted reproduction technology outcome in women with low AFCs was expected to be poorer compared to those with the good follicular count. However, in our study the ART outcome using direct aspiration at 140-mmHg in these women was comparable to that in women with normal AFC. Thus direct oocyte aspiration at negative pressure of 140-mmHg was found to be a good alternative to flushing and aspiration in women with poor AFC as flushing and aspiration was associated with significantly lesser pregnancy rates and more early miscarriages. Our study is the only study to our knowledge comparing two specific aspiration pressures and comparing them with follicular flushing. The results open newer horizons for safe and productive oocyte aspiration for women with low AFC. The limitation of our study is that, it is retrospective and confined to one center only. Nevertheless, the study model may be used to conduct large multicentric prospective trials to arrive at a definitive recommendation creating a new trend for ART in women with low AFC.

## CONCLUSION

Direct oocyte retrieval using higher aspiration pressure of 140-mmHg resulted in better oocyte yield and pregnancy outcomes compared to flushing and aspiration in women with poor AFCs. This innovation also showed ART outcomes comparable to those in women with normal ovarian reserve. Hence, it offers a safe and promising alternative to flushing in women with low AFCs.

## ACKNOWLEDGMENTS

We would like to acknowledge Professors Dr. Lavanya Rai and Dr. Muralidhar V Pai Additional Professor Dr. Jyothi Shetty and Associate Professor Dr. Sapna Vinit Amin, Department of OBG, Kasturba Medical College, Manipal University, Manipal for providing generous academic insight for our study.

## REFERENCES

1. Jeffcoate N. Infertility and assisted reproductive technologies. In: Kumar P, Malhotra N, editors. *Jeffcoate's Principles of Gynaecology*. 7<sup>th</sup> ed. New Delhi: Jaypee; 2008. p. 721-3.
2. Steiner HP. Follicular aspiration and flushing, milder, mildest or back to nature –Textbook of Minimal Stimulation IVF. 1<sup>st</sup> ed. New Delhi: JP Medical Ltd.; 2011. p. 98-102.
3. Roque M, Sampaio M, Geber S. Follicular flushing during oocyte retrieval: A systematic review and meta-analysis. *J Assist Reprod Genet* 2012;29:1249-54.
4. Wongtra-Ngan S, Vutyavanich T, Brown J. Follicular flushing during oocyte retrieval in assisted reproductive techniques. *Cochrane Database Syst Rev* 2010;9:CD004634.
5. Mok-Lin E, Brauer AA, Schattman G, Zaninovic N, Rosenwaks Z, Spandorfer S. Follicular flushing and *in vitro* fertilization outcomes in the poorest responders: A randomized controlled trial. *Hum Reprod* 2013;28:2990-5.
6. 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.
7. Levy G, Hill MJ, Ramirez CI, Correa L, Ryan ME, DeCherney AH, *et al.* The use of follicle flushing during oocyte retrieval in assisted reproductive technologies: A systematic review and meta-analysis. *Hum Reprod* 2012;27:2373-9.
8. Alpha Scientists in Reproductive Medicine and ESHRE Special Interest Group of Embryology. The Istanbul consensus workshop on embryo assessment: Proceedings of an expert meeting. *Hum Reprod* 2011;26:1270-83.
9. Fritz MA, Speroff L. Assisted reproductive technologies. *Clinical Gynaecologic Endocrinology and Infertility*. 8<sup>th</sup> ed. New Delhi: Wolters Kluwer, Lippincott Williams and Wilkins; 2011. p. 1352-4.
10. Belaisch-Allart JC, Hazout A, Guillet-Rosso F, Glissant M, Testart J, Frydman R. Various techniques for oocyte recovery in an *in vitro* fertilization and embryo transfer program. *J In Vitro Fert Embryo Transf* 1985;2:99-104.
11. Horne R. Aspiration of oocytes for *in-vitro* fertilization. *Human reproduction update*. *Eur Soc Hum Reprod Embryol* 1996;2:77-85.
12. Hashimoto S, Fukuda A, Murata Y, Kikkawa M, Oku H, Kanaya H, *et al.* Effect of aspiration vacuum on the developmental competence of immature human oocytes retrieved using a 20-gauge needle. *Reprod Biomed Online* 2007;14:444-9.
13. Antosik P, Jaśkowski JM, Jeziorkowski M, Olechnowicz J. The



- influence of vacuum pressure on quality and number of recovered oocytes aspirated from ovarian follicles of swine and cow. Arch Tierz Dummerstorf 2007;3:260-6.
14. Bols PE, Van Soom A, Ysebaert MT, Vandenhede JM, de Kruif A. Effects of aspiration vacuum and needle diameter on cumulus oocyte complex morphology and developmental capacity of bovine oocytes. Theriogenology 1996;45:1001-14.
  15. Morton KM, Maxwell WM, Evans G. Effect of aspiration pressure during oocyte harvesting on oocyte recovery and *in vitro* development of ovine oocytes. Reprod Domest Anim 2008;43:106-10.
  16. Wikland M. Oocyte retrieval In: Gardner DK, editor. *In vitro* Fertilization a Practical Approach. New York: Informa Healthcare USA, Inc.; 2006. p. 120-8.
  17. Flood JT, Muasher SJ, Simonetti S, Kreiner D, Acosta AA, Rosenwaks Z. Comparison between laparoscopically and ultrasonographically guided transvaginal follicular aspiration methods in an *in vitro* fertilization program in the same patients using the same stimulation protocol. J *in vitro* Fert Embryo Transf 1989;6:180-5.
  18. Cohen J, Avery S, Campbell S, Mason BA, Riddle A, Sharma V. Follicular aspiration using a syringe suction system may damage the zona pellucida. J *In Vitro* Fert Embryo Transf 1986;3:224-6.
  19. Rienzi L, Vajta G, Ubaldi F. Predictive value of oocyte morphology in human IVF: A systematic review of the literature. Hum Reprod Update 2011;17:34-45.
  20. Esfandiari N, Ryan EA, Gotlieb L, Casper RF. Successful pregnancy following transfer of embryos from oocytes with abnormal zona pellucida and cytoplasm morphology. Reprod Biomed Online 2005;11:620-3.
  21. Kingsland CR, Taylor CT, Aziz N, Bickerton N. Is follicular flushing necessary for oocyte retrieval? A randomized trial. Hum Reprod 1991;6:382-3.
  22. Bagtharia S, Haloob AR. Is there a benefit from routine follicular flushing for oocyte retrieval? J Obstet Gynaecol 2005;25:374-6.
  23. 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.
  24. Nyboe Andersen A, Popovic-Todorovic B, Schmidt KT, Loft A, Lindhard A, Højgaard A, *et al.* Progesterone supplementation during early gestations after IVF or ICSI has no effect on the delivery rates: A randomized controlled trial. Hum Reprod 2002;17:357-61.
  25. Kerin JF, Broom TJ, Ralph MM, Edmonds DK, Warnes GM, Jeffrey R, *et al.* Human luteal phase function following oocyte aspiration from the immediately preovular graafian follicle of spontaneous ovular cycles. Br J Obstet Gynaecol 1981;88:1021-8.

**How to cite this article:** Kumaran A, Narayan PK, Pai PJ, Ramachandran A, Mathews B, Adiga SK. Oocyte retrieval at 140-mmHg negative aspiration pressure: A promising alternative to flushing and aspiration in assisted reproduction in women with low ovarian reserve. J Hum Reprod Sci 2015;8:98-102.

**Source of Support:** Nil, **Conflict of Interest:** The authors of this publication are affiliated with Department of OBG, Kasturba Medical College, Manipal University, Manipal. The terms of this arrangement have been reviewed and approved by the Manipal University, Manipal, in accordance with its policy on objectivity in research.

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