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Effectof Follicular Flushing during Oocyte Retrievalon Clinical Outcomeof Assisted Reproductive Technology

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ABSTRACT

Objective:is to determine whether follicular aspiration and flushing increase the number of oocytes yield and pregnancy outcome over aspiration alone in women undergoing ICSI.

Study design: prospective randomized controlled trial. One hundred eighty five infertile women who underwent ICSI were included in the study. They were randomized into two groups 92 cases in (aspiration and flushing group) and 93 cases in (aspiration only group),during the period from September 2011 to September 2013.

Intervention(S): Controlled ovarian hyperstimulation using long GnRH agonist was the standard protocol, hCGadministrated 10000 iu when three or more follicles were at least 18 mm in largest diameter, Trans-vaginal follicular aspiration performed 34-36 hours after hCG trigger.In the aspiration alone group, a 16 gauge single lumen needle used, with suction continue until a small amount of blood stained fluid appeared in the tubing or flow stop, When flushing accompany aspiration of follicular fluid in the study group, the same needle used with a double-way tap allowing flushing of (2 ml) of embty follicleby Earl's medium till oocyte retrieved or maximum two times.

Results: The study observed 60.5 % oocyte recovery rate with aspiration only compared with 80.9 % with follicular aspiration and flushing.Operative time (minutes) was significantly longer among flushing group,the retrieval time was 1.3 fold higher among those undergoing follicular flushing. Pregnancy was non-significantly more frequent among flushing. Implantation rates non-significantly more frequent among flushing group than non-flushing group (31.6% versus 26.3%, P= 0.424) and ongoing clinical pregnancy non-significantly more frequent among flushing group (27.4% versus 21.1% , P= 0.31).

Conclusion, flushing non-significantly increase implantation and clinical pregnancy outcome and associated with a significant increase in the procedure time for oocyte retrieval, so patient groups where a small number of oocytes are available for retrieval may represent patients most likely to benefit from follicle flushing as only one extra oocyte may affect the outcome.

Keywords: follicle flushing, oocyte retrieval, in-vitro fertilization, assisted reproductive technology**.**

INTRODUCTION

Assisted reproductive techniques are an accepted form of treatment for infertility and the procedures used are generally assumed to be relatively constant in the way they are performed between centers. Transvaginal ultrasound guided oocyte collection is now almost universal.[1]

In early stages of assisted reproductive technologies (ART), oocyte retrieval was performed via laparoscopy, a cumbersome and expensive process requiring general anesthesia. [2] Today, transvaginal oocyte retrieval for ART is a routine procedure performed under ultrasound guidance. Double lumen retrieval needles, which are capable of flushing ovarian follicles, were developed to overcome the possibility of oocyte retention within the ovarian follicles and retrieval collection system**.** [3,9]

The overall aim of ART is to increase the chances of conception, with the desired outcome a live baby, while not placing the woman at undue risk. Variations in accepted methodology are often attempted in order to improve the desired outcome. It is important to evaluate whether these variations do improve outcome. ultrasound guided transvaginal needle collection of oocytes has become the preferred method of oocyte

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retrieval for ART and has improved the collection rate per follicle from 19.8% and 46% when laparoscopic methods were used to between 52% and 64% and more recently 82.%**.** [4,11]

The aspiration of follicular fluid alone, or aspiration and flushing, both continue in different clinics suggests neither technique has overriding advantages or disadvantages when compared with the other. This may reflect historical methodology, inertia or the preference of the surgeons involved**.** [5,12]

Follicular flushing at oocyte retrieval is based on the premise that more oocyte would be collected than by aspiration of follicular fluid alone, and the subsequent inference that this will increase pregnancy rates. Waterstone and Parsons found that 14% of total numbers of oocytes were collected in first three flushes and a further 3% in the next three when flushing was performed continuously after aspiration. [6,13]

The place of follicular flushing during oocyte recovery in ART is still uncertain. The pros of flushing include the possibility of obtaining more oocytes, and subsequently more embryos. Whether this translates into a higher pregnancy rate and live births remains unknown. The cons of flushing are a longer operative time and larger amounts of required anaesthetics and analgesics. From a patient's perspective, it could also mean higher costs. [7]

PATIENT AND METHODS

The study was a prospective randomized controlled trial. One hundred ninety cases were chosen (complete outcome data were available in 185 (97%) infertile women who underwent IVF/ICSI were included in the study after verbal and written consent. They were recruited from Assisted Reproductive Technology unit, Ain Shams University maternity Hospital during the period from September 2011 to September 2013.

The total sample size was calculated as 190 cases, randomized using a block randomization method on the day of oocyte retrieval into two equal groups:

- Group 1(control group): this group consisted of 95 (93 completed the study) cases underwent aspiration alone during oocyte retrieval.
- Group 2 (study group): this group consisted of 95 (92 completed the study) cases underwent aspiration and flushing during oocyte retrieval.

Patients meeting study requirements were offered enrollment. Each patient in the study and control groups subjected to the following:

controlled ovarian hyperstimulation using down regulation with GnRH analogue in mid-luteal phase of pretreatment cycle as the standard protocol withTriptorelin acetate (Decapeptyl) 0.1 mg subcutaneous at day 14 to 16 of pre-induction cycle, followed by ovarian stimulation with HMG (human menopausal gonadotropin) at day 3, dosage of HMG depends on the BMI, age, FSH, and ovarian size by TVUS, At the first scan, the number, sizes of the follicles were written. If he size is 12-14 mm allowed two days before next scan, if 14 mm or more next scan was within 24 hours , human chorionic gonadotropin (choriomon 5000iu, IM injection) administrated 10000 iu when three or more follicles were at least 18 mm in largest diameter, Trans-vaginal follicular aspiration performed 34-36 hours after hCG trigger as the following:

During the trial period the following parameters kept constant, The manufacturer of the aspiration needles (Wallace Oocyte Recovery Systems; Oocyte Recovery Needle 33cm x 16), the pump (Pioneer Pro-pump, GenX International; Guilford, CT) and pressure used set to maximum of 120 mmHg at aspiration, and up to 200 mmHg at re-aspiration in flushing group, staff involved in all set procedures, the premises and equipment at oocyte collection and culture.

In the aspiration alone group, a 16 gauge single lumen needle used, with suction continue until a small amount of blood stained fluid appeared in the tubing or flow stop. The dead space within the needle and tubing suctioned upon removal from the vagina.

When flushing accompany aspiration of follicular fluid in the study group, the same needle used with a double-way tap allowing the same amount of culture medium as the follicle volume to be circulated through the empty follicle (2 ml) of Earl's medium till oocyte retrieved or maximum two times.

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.

The patient kept under observation for two hours to assess presence of adverse events, including complication rate for the surgical procedure and during the flushing procedure (post operative pain (need for diclophen 75 mg im vial, internal hemorrhage, infection, vomiting and hypotension). Secondary end-points included recovery rate, total number of mature oocytes, maturity rate, fertilization rate, number of embryos transferred, implantation rate, and on-going pregnancy rate.

Embryo transfer for both groups done in the morning of day 3 using (Labotect catheter, Germany) while the patient in Lithotomy position and semi-full bladder, patients instructed to be resting on their back for at least 30 minute following the process of embryo transfer.Patients start progesterone medication after oocyte retrieval. All cases will underwent an initial serum test for B-HCG two weeks after embryo transfer, chemical pregnancy is defined when level of B-HCG is higher than 25 iu, while clinical pregnancy is defined when fetal heart pulsation could be detected from 6 to 7 weeks by TVUS.

As regard baseline characteristics and cycle outcomes of both groups, there were no significant differences in age, BMI, total gonadotropins received, duration of stimulation, the proportion of ICSI, and the diagnosis between groups. The long agonist protocol was used in all but one patient (flushing group) used antagonist protocol. There were no significant differences between the groups regarding the mean number of ovarian follicles ≥16 mm, table(1).

The collected data were coded, tabulated, and statistically analyzed using IBM SPSS statistics (Statistical Package for Social Sciences) (V. 22.0) software version 22.0, IBM Corp., USA, 2013.

Descriptive statistics were done for quantitative data as minimum & maximum of the range as well as mean±SD (standard deviation) for quantitative parametric data, while it was done for qualitative data as number and percentage.

The level of significance was taken at P value < 0.050 is significant, otherwise is non-significant. The p-value is a statistical measure for the probability that the resultsobserved in a study could have occurred by chance.

RESULTS

In our study we found that, Total and differential retrieved oocytes were significantly higher among flushing group than non-flushing group. The study observed 60.5 % oocyte recovery rate with aspiration only (484 oocyte recruited from 800 follicles), compared with 80.9 % with follicular aspiration and flushing (670 oocyte recruited from 828 follicles), It was found that 20.4% more oocytes were obtained with follicular aspiration and flushing than aspiration alone , fig (1).

The average number of oocytes collected per woman randomized in study group (7.3±2.3 versus 5.2±2.6, #P< 0.001) in non flush group, Furthermore, the proportion of mature oocytes recovered from follicles ≥16 mm was significantly more with study group than those with control group (5.8±2.1 versus 4.4±2.4, #P< 0.001).

At the same time immature oocyte retrieved were significantly more in the study group than those in control group (#P< 0.001).

Regarding operative time, operative time (min) was significantly longer among flushing group than non-flushing group. The retrieval time was 1.3 fold higher among those undergoing follicular flushing, with an estimated increase of 5 minutes (P<0.001), fig (2).Regarding total number of produced embryos, total number of produced embryos were significantly higher among flushing group than non-flushing group. In addition, oocytes retrieved by follicular flushing demonstrated a better morphological quality (top quality embryos 296/423 or 70% versus 189/316 or 60%; P<0.001).

The study observed 39.5% fertilization rate with aspiration only (316 oocyte fertilized from 800 follicles), compared with 52.2% with follicular aspiration and flushing (423 oocyte fertilized from 828 follicles), It was found that 12.7% more embryos were obtained with follicular aspiration and flushing than aspiration alone.

The average number of produced embryos per woman randomized in study group (4.7±2.0 versus 3.4±2.2, #P< 0.001) in non flush group, fig (3).

Regarding pregnancy outcome, Pregnancy was non-significantly more frequent among flushing group than non-flushing group. Implantation rates non-significantly more frequent among flushing group than non-flushing group (31.6% versus 26.3% , P=

0.424) and ongoing clinical pregnancy non-significantly more frequent among flushing group(27.4% versus 21.1% , P= 0.31), fig (4).

The study failed to follow women up to obtain data on live births and our pregnancy outcome in general low cause the Assisted Reproductive Technology unit, Ain Shams University maternity Hospital accepting cases with bad prognosis and repeated failed trial of ICSI.

Regarding postoperative adverse events, Postoperative complications were non-significantly more frequent among flushing group than non-flushing group. As 62 cases of 92 required post operativeDiclophin 75 mg Im. in study group versus 49 cases of 93 in control group; P = 0.056, and 9 cases complained of vomiting in the study group versus 4 in control group; P = 0.151, and 15 cases developed post operative hypotension in the study group versus 7 cases in control group P = 0.071, fig (5).

DISCUSSION

The results of this study were in agreement with other studies as regard oocyte yield and produced embryos; the number of embryos obtained is dependent on the number of oocytes retrieved. [16] To maximize the number of oocytes recovered, follicular aspiration followed by 2-ml flush has been suggested.[6] Waterstone and Parson, 1992 reported that the use of double-lumen needles and flushing resulted in 20% more oocytes retrieval.

Bagtharia, found that 40% of the oocytes were retrieved in the primary aspiration without flushing of the follicle, while up to 82% of the oocytes were retrieved with two flushes and 97% were retrieved in up to four flushes. Mendez Lozano, 2008 reported 46.8% oocyte recovery-rate with aspiration only compared with 84.6% with additional follicular flushing in 165 infertile women with low ovarian reserve undergoing 271 consecutive minimal stimulation IVF cycles.[4]

While other studies reported that no difference was observed between the oocyte yield in the flushing and non-flushing groups.[2,8] Also, no difference in the oocyte yield for poor responders (non-flushing group: 83% versus flushing group: 85%, P= 0.70, [2] this study was limited by a small sample size of 30 patients, this disagreements with our results as regard oocyte yield and produced embryos mostly related to operator skills and technical aspects.

Most of the studies reported significantly prolonged operativetime in flushing group, but Bagtharia [4] didn't observe such a significance even when flushing done up to four times per follicle. Procedural times for oocyte retrieval shortened from older to newer studies; however, follicle flushing was associated with a longer procedural time for oocyte retrieval during all time periods in this study. Both of the two most recent trials demonstrated a substantially longer duration of oocyte retrieval in the follicle flushing group of 3 and 4 min, respectively. [2,8]

As regarding implantation rate, clinical pregnancy , and post operative adverse events. Flushing (in our study) non-significantly increased implantation rate and clinical pregnancy. It also non-significantly increased postoperative adverse events.

This results were in agreement with the following studies: Wood, Waterstone&Bagtharia[16,5,4] while Levens *et al.* &Haydardedeoglu *et al.* reported that, no differences in the fertilization of MII oocytes (oocytes that have completed meiosis I) between the flushing and non-flushing groups and reported similar clinical pregnancy rates between the non-flushing and flushing groups.[2,8]

In theory, increasing oocyte yield should lead to an increase in the number of embryos available to select from for embryo transfer, potentially increasing the odds of live birth, **Scott** *et al.*[1] demonstrated that the odds of live birth from ART increased markedly as the total number of oocytes retrieved increased from 1 to 10 oocytes. Similar increases were not observed for higher oocytes retrievals, and with retrieval of between 15 and 30 oocytes the odds of live birth essentially plateaued. Based on this association, little benefit might be expected from follicle flushing in normal-responding patients, where the additional benefit of one to two more oocytes may be negligible.

However, patient groups where a small number of oocytes are available for retrieval may represent patients most likely to benefit from follicle flushing as only one extra oocyte may affect the outcome.

There are a number of theoretical advantages of aspiration only over flushing, such as reduction in the operative time, anesthetic agent and also in the operating cost. There is however, a potential risk of pelvic infection with increased risk of damage to oocytes and patients tissues. These theoretical risks were however not encountered in our study and Wood[16] ,Waterstone and Parson[5] and Bagtharia,[4] except for increase in the operating time, but there was no change in the anesthetic regimen.

CONCLUSIONS

In conclusion, flushing non-significantly increased implantation and clinical pregnancy outcome and was associated with a significant increase in the procedure time for oocyte retrieval, so patient groups where a small number of oocytes are available for retrieval may represent patients most likely to benefit from follicle flushing as only one extra oocyte may affect the outcome. Such patient groups may include poor responders, natural cycle ART and minimal stimulation ART.

A potential weakness of this study, the study failed to follow women up to obtain data on live births.

REFERENCES

1. **Scott RT, Hofmann GE, Muasher SJ, Acosta AA, Kreiner DK, Rosenwaks Z. A (1989):** prospective randomized comparison of single- and double-lumen needles for transvaginal follicular aspiration. J In Vitro Fert Embryo Transfer;6:98 – 100.
2. **Levens ED, Whitcomb BW, Payson MD, Larsen FW(2009):** Ovarian follicular flushing among low-responding patients undergoing assisted reproductive technology. FertilSteril; 91:1381–1384.
3. **Kingsland CR, Taylor CT, Aziz N, Bickerton N (1991):** Is follicular flushing necessary for oocyte retrieval? A randomized trial. Hum Reprod; 6:382 – 383.
4. **Bagtharia S, Haloob AR (2005):**Is there a benefit from routine follicular flushing for oocyte retrieval? J ObstetGynaecol; 25:374 – 376.
5. **Waterstone JJ, Parsons JH (1992):** A prospective study to investigate the value of flushing follicles during transvaginal ultrasound-directed follicle aspiration. FertilSteril; 57:221–223.
6. **El Hussein E, Balen AH, Tan SL (1992):** 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 ObstetGynaecol; 99:841 – 844.
7. **Gembruch U, Diedrich K, Welker B, Wahode J, van der Ven H, Al-Hasani S, Krebs D(1988):**Transvaginalsonographically guided oocyte retrieval for in-vitro fertilization. Hum Reprod;3:59 – 63.
8. **Haydardedeoglu B, Cok T, Kilicdag EB, Parlakgumus AH, Simsek E, Bagis T (2011):.** In vitro fertilization-intracytoplasmic sperm injection outcomes in single versus double-lumen oocyte retrieval needles in normally responding patients: a randomized trial. Fertil Steril;95:812 – 814.
9. **Levy G, Hill MJ, Ramirez CI, Correa L, Ryan ME, DeCherney AH, Levens ED, Whitcomb BW(2012):** The use of follicle flushing during oocyte retrieval in assisted reproductive technologies: a systematic review and meta-analysis. Hum Reprod;27:2373 – 2379.
10. **Lozano DH, Fanchin R, Chevalier N, Feyereisen E, Hesters L, Frydman N, Frydman R (2006):** Optimizing the semi natural cycle IVF: the importance of follicular flushing. J Indian Med Assoc; 104:423 – 427.
11. **Ludwig AK, Glawatz M, Griesinger G, Diedrich K, Ludwig M (2006):** Perioperative and post-operative complications of transvaginal ultrasound-guided oocyte retrieval: prospective study of .1000 oocyte retrievals. Hum Reprod;21:3235 – 3240.
12. **Mendez Lozano DH, BrumScheffer J, Frydman N, Fay S, Fanchin R, Frydman R(2008):** Optimal reproductive competence of oocytes retrieved through follicular flushing in minimal stimulation IVF. Reprod Biomed Online ,16:119– 123.
13. **Veeck LL, ZaninovicN(2003):** An Atlas of Human Blastocysts, 1st edn. FL, USA: CRC Press,.
14. **Wikland M, Enk L, HambergerL(1985):**Transvesical and transvaginal approaches for the aspiration of follicles by use of ultrasound. Ann N Y AcadSci; 442:182– 194.
15. **Wongtra-Ngan S, Vutyavanich T, Brown J(2010):** Follicular flushing during oocyte retrieval in assisted reproductive techniques. Cochrane Database Syst Rev:CD004634.
16. **Wood C, TrounsonAO(2000):** Historical perspectives of IVF. In: Trounson AO, Gardner DK editor(s). Handbook of In Vitro Fertilization. Second Edition. New York: CRC Press,: 4

	Flushing (N=92)	Non-flushing (N=93)
Mean±SD(age)	33.3 ± 4.9 years	33.2 ± 4.8 years
Mean±SD(BMI)	27.2 ± 5	27.8 ± 4.9
Male factor	37 (42.1%)cases	31 (32.6%)cases
Tubal factor	30 (31.6%)cases	24 (25.3%)cases
PCO	5 (5.3%)cases	14 (14.7%)cases
Endometriosis	7 (7.4%)cases	11 (12.7%)cases
Unexplained	13 (13.7%)cases	13 (14.7%)cases
HMG dose Mean±SD	2895.8±817.5 iu	2861.3±867.2 iu
Total no. of follicles	828	800

Table (1): demographic data of the study groups

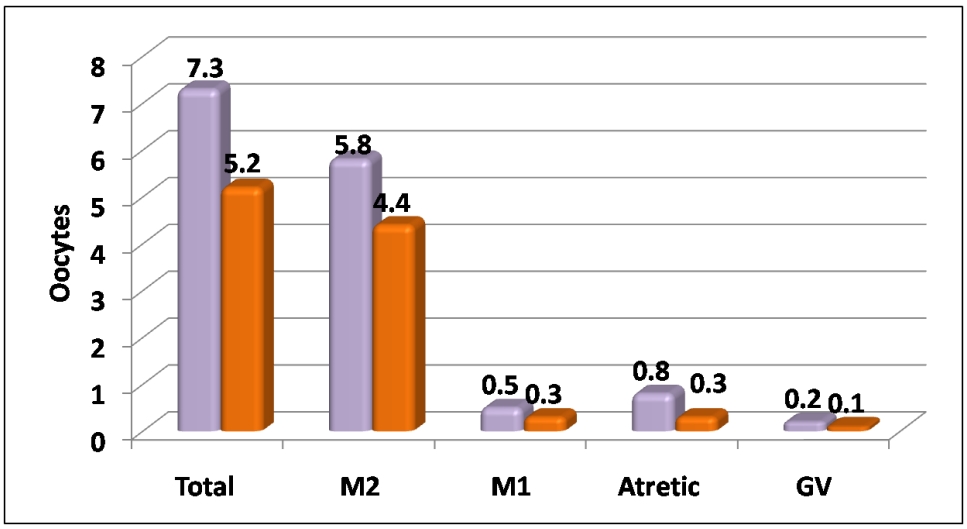

Figure (1): Comparison between both groups regarding retrived oocytes

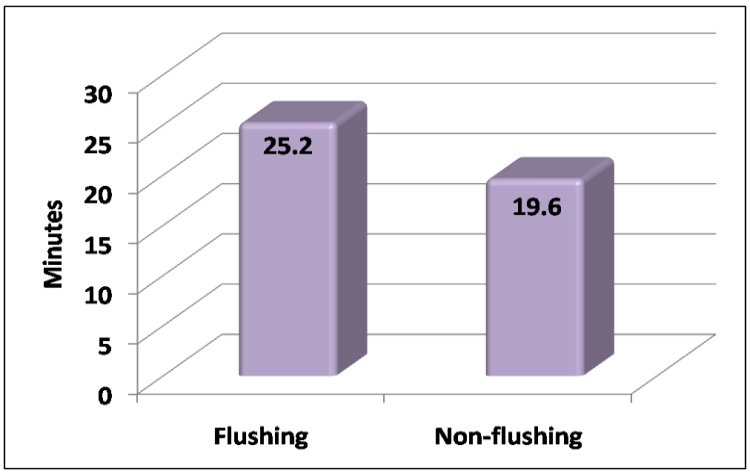

Figure (2): Comparison between study groups regarding operative time (min)

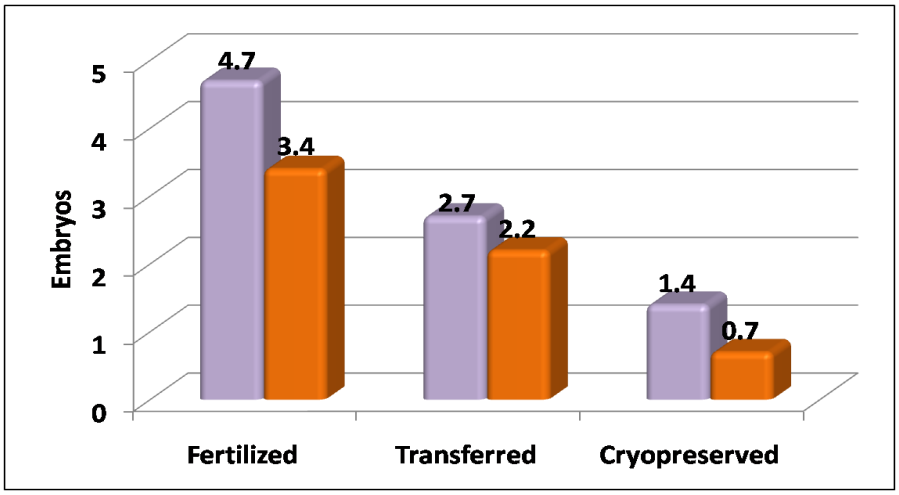

Figure (3): Comparison between both groups regarding total number of produced embryos

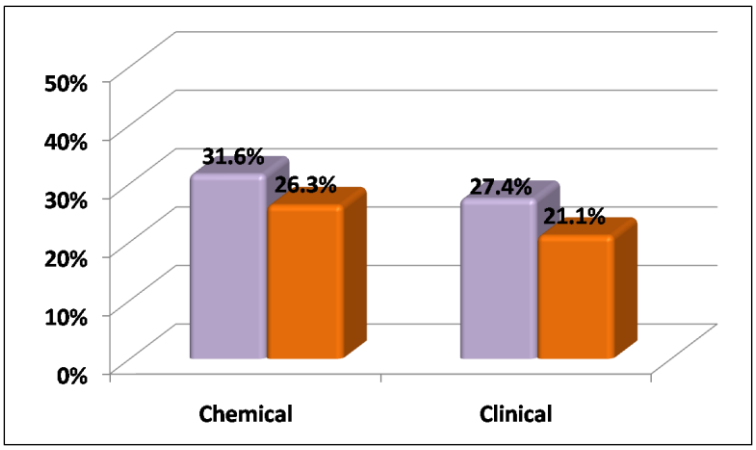

Figure (4): Comparison between both groups regarding pregnancy outcome

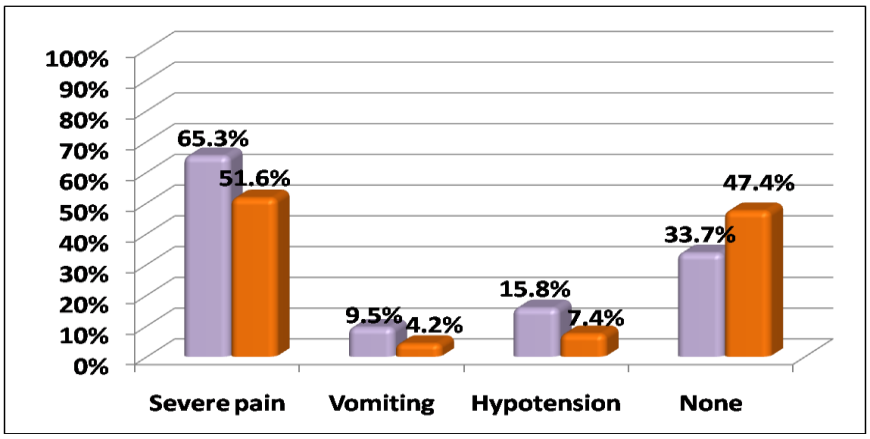

Figure (21): Comparison between both groups regarding postoperative complications