

Evaluation of Effect of Cervical Mucus Aspiration before Intrauterine Insemination on Pregnancy Rates in Unexplained Infertility

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Abstract: Objective: to evaluate the effect of cervical mucus aspiration before intrauterine insemination on clinical pregnancy rates in cases of unexplained infertility in couples despite having normal standard investigations including semen analysis, tests of ovulation and tubal patency using HSG. Methods: A prospective study of 100 patients diagnosed to have unexplained infertility (1ry or 2ry), the outcome of IUI cycles in 50 patients in whom mucus was aspirated prior to IUI were compared with those of IUI cycles in 50 patients in whom mucus not aspirated (control group). Results: In this study, the pregnancy rate was (18%)(9 pregnancies in 50 patients) in the cervical mucus aspiration group, and (8%)(4 pregnancies in 50 patients) in the control group ($P=0.05$). Mucus aspiration led to significantly increased pregnancy rates for women with unexplained infertility (18% in the aspiration group VS 8% in the control group). Conclusion: Cervical mucus aspiration before intrauterine insemination might improve clinical pregnancy rates by undefined mechanisms till now.

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1. Introduction:

Unexplained infertility refers to a diagnosis made in couples in whom standard investigations including semen analysis, tests of ovulation and tubal patency (hysterosalpingography and laparoscopy) are normal (*Siristatidis and Bhattacharya S , 2007*).

Over the last two decades this approach has no longer been accepted. To diagnose unexplained infertility as acceptance of such an option would, however, mean acceptance of the fact that our most frequently made diagnosis in infertility practice is a non-diagnosis (*Gleicher, 2004*).

Sometimes, nature needs help to start a pregnancy, and the doctor can do this by giving the sperm a piggyback ride through a fine tube into the body; this procedure called intra- uterine insemination (IUI) or artificial insemination with husbands sperm (AIH), and effectively, the doctor is giving nature a helping hand by increasing the chances of the egg and sperm meeting (*Aniruddha and Anjalina 2008*).

Homologous intra-uterine insemination (IUI) is a cost-effective method chiefly used in cases of unexplained or mild male factor infertility. The pregnancy rate per IUI cycle varies between 6% and 22% (*Hughes, 1997*).

Artificial insemination (AI) is another name for intra-uterine insemination (IUI) but can also refer to placing sperm in a woman's vagina or cervix when she is ovulating, the sperm then travels into the fallopian tubes where they can fertilize the woman's egg (*Adam et al., 2006*).

The outcome of IUI may be affected by numerous factors such as timing, type of insemination (single or double), catheter type, modality of ovulation induction, volume of prepared semen, and method of insemination (*Ragni et al., 2004*).

In natural cycles, the quantity and the quality of cervical mucus affect fertilization as the mucus accepts filters, prepares, and releases sperm for successful transport to the ovum and penetration (*Katz 1991*).

Cervical mucus has been reported to have unfavorable effects on pregnancy rates following in-vitro fertilization [IVF] or intra-cytoplasmic sperm injection [ICSI (*Eskandar M.A., A.M. Abou-Setta, M. El-Amin, M.A. (2007)*).

However, and it may also have unfavorable effects in cases of IUI. It has been suggested that mucus aspiration before embryo transfer may lead to higher implantation and pregnancy rates by negating the adverse effects of mucus on the embryo (*Mansour and Aboulghar M.A, 2002*).

On the one hand, pushing possibly hostile cervical mucus into the endometrial cavity with the insemination catheter may harm sperm motility and/or sperm-oocyte interaction, there-by affecting pregnancy rates negatively. In this case, one way to increase pregnancy rates following IVF or ICSI, and perhaps during IUI, may be to bypass the cervical mucus altogether (*Helmerhorst, H.A. van Vliet, T. Gornas, M.J. Finken and D.A. Grimes, (2006)*): On the other hand, cervical mucus may still play a helping role, functioning as a sperm reservoir and a protective

barrier during IUI. This study was conducted to evaluate whether cervical mucus aspiration has any effects on clinical pregnancy rates in IUI cycles.

2. Materials and Methods:

This prospective study was conducted in the Department of Obstetrics and Gynecology, Faculty of Medicine, El-Minia University (Suzan Mubarak University Hospital) during the period from January, 2009 till December, 2009 after being approved by the department ethical committee. 100 patients diagnosed as having unexplained infertility were recruited in the study. The outcomes observed in this study group (50 patients) were compared retrospectively with those of a control group of (50 women) who underwent IUI cycles. All women underwent controlled ovarian hyperstimulation and IUI cycles for primary or secondary infertility. All couples had been trying to conceive for at least 12 months, and all had undergone a fertility workup consisting of a review of their medical history, the confirmation of an ovulatory cycle by assessment of midluteal serum progesterone level, and semen analysis. The patency of at least 1 tube was confirmed by laparoscopy and/or hysterosalpingography. All women underwent an ultrasound examination to rule out pathology of the endometrial cavity before initiation of the IUI treatment, and age of women less than 35 years.

The indications for controlled ovarian hyperstimulation and IUI were unexplained infertility (normal sperm parameters for the man, normal tubal patency and confirmed ovulation for the woman). Clomiphene citrate (CC) or human menopausal gonadotropin (HMG)-were used for ovulation induction.

Treatment with CC was started on 2nd day of the cycle after a baseline vaginal ultrasound examination, but was postponed for 1 cycle for patients with ovarian cysts greater than 15 mm at baseline. The starting CC dose was 100 mg/day for 5 days. It was increased by 25 to 50 mg in the next cycle if the patient only showed monofollicular development, or decreased if she showed 4 or more follicles greater than 14 mm.

Treatment with HMG was started on 4th/ 5th day of the cycle and continued until follicular maturation was achieved. The starting HMG dose was 75 IU/day I.M., combination between HMG and CC used in some women. If no follicle greater than 10 mm was seen on day 9; and if the estradiol levels still corresponded to the follicular phase, the dose was adjusted accordingly. When the dominant follicle reached a mean diameter of at least 18 mm, the patient received 10000 IU of human chorionic gonadotropin (HCG) (Pregnyl; Organon-Turkey) for ovum release.

A single insemination was performed with homologous semen 36 to 48 hours after the administration of HCG. Semen specimens were

produced by masturbation and insemination performed within 1 hour of production. The sperm was washed twice by the gradient method with a sperm wash medium (SupraSperm; MediCult, Copenhagen, Denmark), first for 20 minutes and then for 10 minutes.

Cervical mucus aspiration was performed before sperm insemination in 50 patients, compared with control group in whom no cervical mucus aspiration not done.

Insemination was performed with a sterile Wallace artificial insemination catheter (Smiths Medical International, Hythe, England) and a 1- or 2-mL syringe, and COOK catheter in all patients.

In those who underwent cervical mucus aspiration, the COOK catheter was gently passed through the cervical canal and the sperm suspension slowly pushed into the uterine cavity. Insemination volumes were 1 mL in all cycles. The women remained supine for 10 to 15 minutes after the insemination procedure and were encouraged to have intercourse the following night, progesterone administration as luteal phase support was given to the women.

The technique of cervical mucus aspiration was that used before embryo transfer in IVF and ICSI cycles. In the mucus aspiration group, mucus was removed repeatedly from the internal to the external cervical os by the external soft part of the Wallace catheter until no more was obtained. A Wallace catheter other than the insemination catheter was used for this procedure.

Data are expressed as mean \pm SD. The baseline differences between the study and control groups were analyzed by the independent t test. Homogeneity of variances was calculated by the Levene test and the Lilliefors significance correction test. In contingency tables, the χ^2 test, or the 2-sided Fisher exact test were used. $P < 0.05$ was considered statistically significant. Data were analyzed using SPSS version 9.05 (SPSS, Chicago, Illinois, USA).

3. Results

From table (1) we found that the mean age in both groups 26.2 ± 3.76 and 25.9 ± 2.99 was nearly the same and mean duration of infertility 5.20 ± 3.07 and 4.02 ± 1.44 so there was no significant between the mean ages in both groups.

Number of cases with primary infertility was more than number of cases with secondary infertility in both group.

We found that from table (2) percentage of pregnancy rate in group I (18%) higher than in group II (8%) so cervical mucus aspiration before IUI improve pregnancy rates.

From table (3) we found that pregnancy rate was higher in short term infertility than in intermediate and long term infertility.

From table (4) we found that pregnancy rate was higher in short term infertility than in intermediate and long term infertility.

In group I; From table (5) we found that; pregnancy rate in cases inducted with CC was lower than pregnancy rate in cases inducted with HMG.

In group II; From Table(5) we found that pregnancy rate in cases inducted by CC was nil while in cases inducted by HMG was 2 (4%).

From table (6) we found that pregnancy rate in cases with previous IUI in which cervical mucus aspiration was done was 3 while in cases with previous IUI in which cervical mucus aspiration not done was 0.

From table (7) and figure (5) we found that :Incidence of ectopic pregnancy in successful cases was 11.2%,abortion was 11.4% and only one case of twins 11.2% .

Table (1): Demographic data of studied groups (n = 100).

Distribution of the studied groups according to their characteristics;

Valuables	Group I	Group II	P-value	
Age	(Range)	20-35	19-35	0.66*
	(M±SD)	26.2±3.76	25.9±2.99	
Duration of infertility	(Range)	2-15	2-14	0.01**
	(Mean ± SD)	5.20 ± 3.07	4.02±1.44	
Duration of Marriage (Mean ± SD)		5.72±3.48	4.68±1.99	0.07*
Type of infertility:	Primary	41 (82%)	35 (70%)	-
	Secondary	9 (18%)	15 (30%)	-
Method of induction by:	C.C	10(20%)	12(24%)	-
	C.C+HMG	26(52%)	25(50%)	-
	HMG	14(28%)	13(26%)	-
Previous abdominal surgery	CS	2 (4%)	3 (6%)	-
	Appendectomy	2(4%)	3(6%)	-
Cervical mucus aspiration		Yes	No	-
Pregnancy rate		9 (18%)	4 (8%)	-
HSG Or Laparoscopy		No abnormality		-
Semen analysis		With in normal WHO parameters		-

* Non Significant.

** Significant.

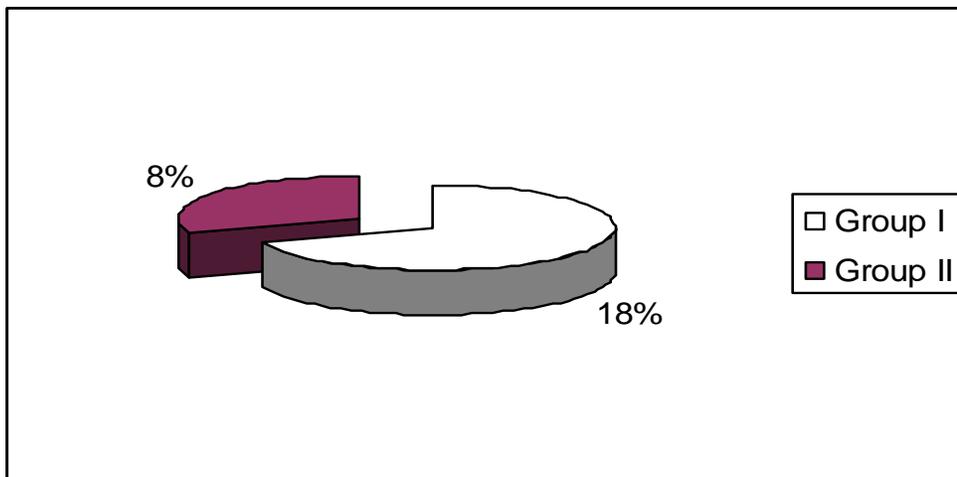


Fig. (1): Pie chart by percentage between pregnancy rate in both groups.

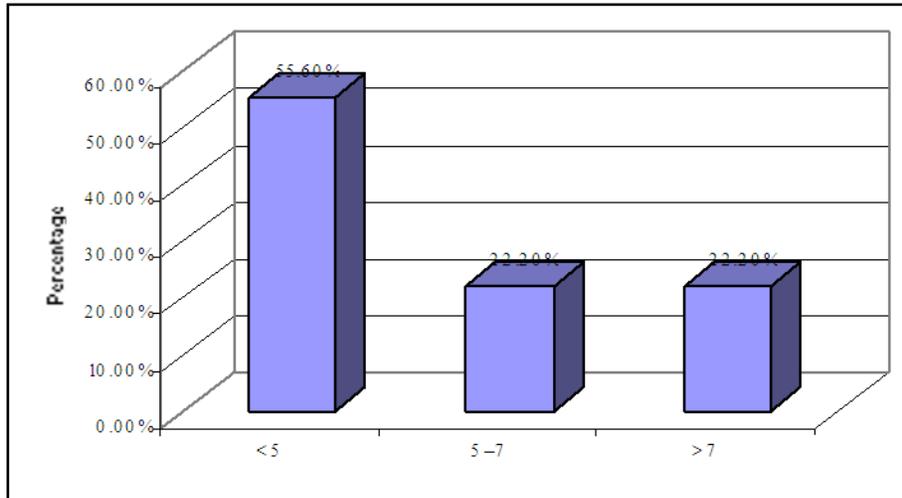


Fig. (2): Bar chart by percentage of duration of infertility on pregnancy rate in group I.

Table (2): Ratio between pregnancy rate in both groups.

Variables	Group I (with Cervical mucus aspiration) (n = 50)	Group II (without Cervical mucus aspiration) (n = 50)
Pregnancy test	9 (18%)	4 (8%)

Table (3): Effect of duration of infertility on pregnancy rate in group I.

Duration of infertility	Number	Percentage
< 5	5	55.6%
5 – 7	2	22.2%
> 7	2	22.2%
Total	9	100%

Table (4): Effect of duration of infertility on pregnancy rate in group II.

Duration of infertility	Number	Percentage
< 5	3	75%
5 – 7	1	25%
> 7	0	0%
Total	4	100%

Table (5): Correlation between method of induction and pregnancy rate in both group.

Groups	C.C	C.C+HMG	HMG
Group I: Number (%) Pregnancy Rate	10 (20%)	26 (52%)	14 (28%)
	2 (4%)	4(8%)	3(6%)
Group II: Number (%) Pregnancy Rate	12 (24%)	25 (50%)	13 (26%)
	0	2(4%)	2(4%)

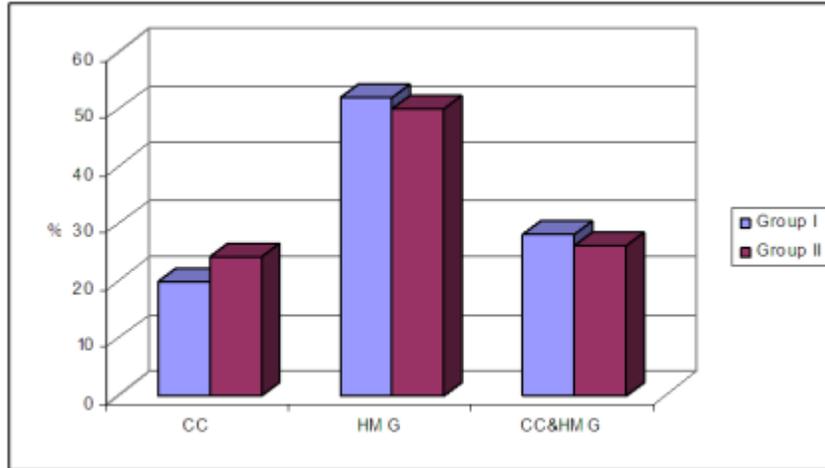


Fig. (3): Comparison between method of induction and pregnancy rate in both group.

Table (6): Comparison between pregnancy rate in cases with previous IUI in both groups.

Group	Serum pregnancy test		Total
	+ve	-ve	
Group I with cervical mucus aspiration	3	8	11
Group II without cervical mucus aspiration	0	11	11

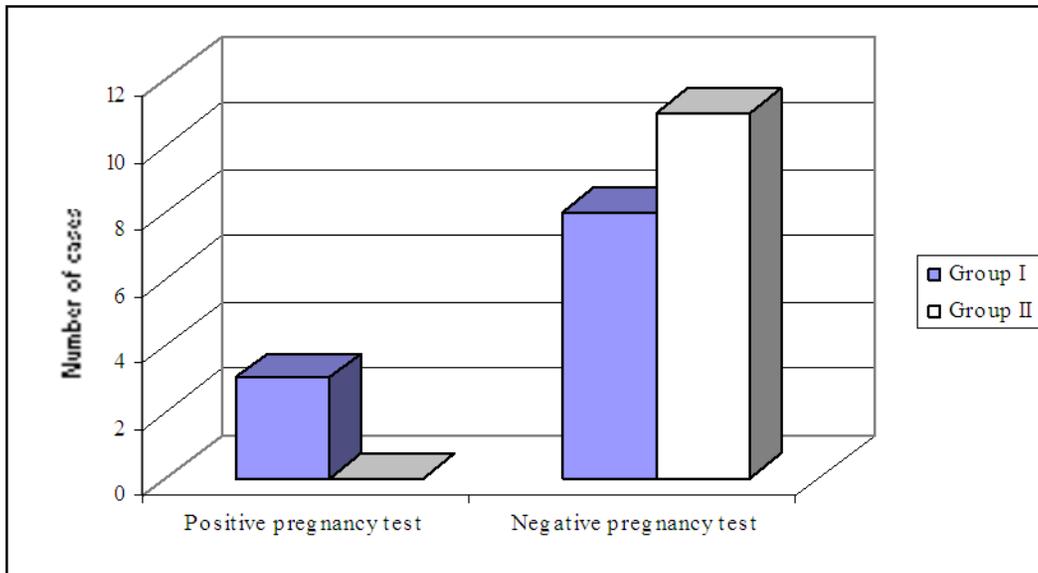


Fig. (4): Bar chart between pregnancy rate in cases with previous IUI in both groups.

Table (7): Outcome of successful cases in group I (n=50).

	Ectopic	Abortion	Multiple pregnancy	Single pregnancy	Total
Positive serum pregnancy test	1 (11.2%)	1 (11.4%)	1 (11.2%)	6 (66.2%)	9 (100%)

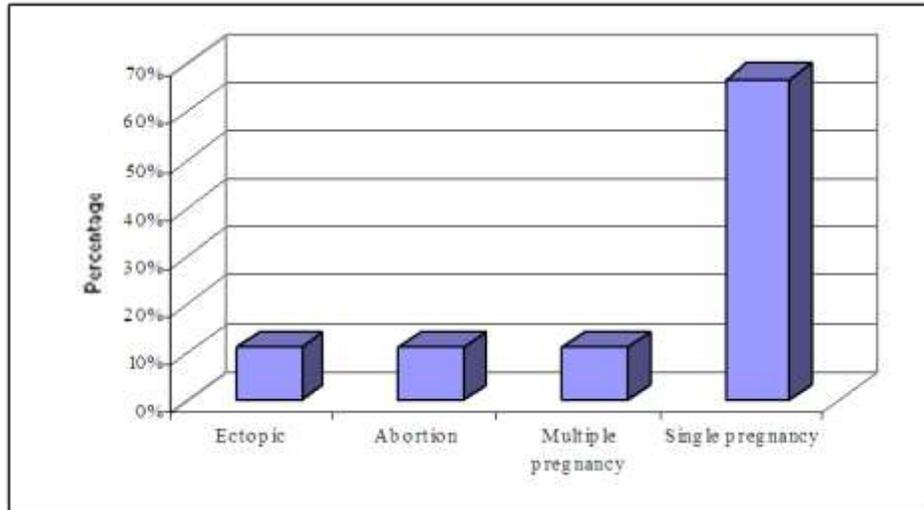


Fig. (5): Bar chart for outcome of successful cases in group I.

4. Discussion:

The aim of the present study was to evaluate the effect of cervical mucus aspiration before intra-uterine insemination (IUI) on the pregnancy rates in couples with unexplained infertility as having normal standard investigations including semen analysis, tests of ovulation and tubal patency using hysterosalpingography, this report demonstrates that cervical mucus aspiration before IUI may improve pregnancy rates (18% in the cervical mucus aspiration group vs 8% in the control group; $P = 0.05$).

Unexplained infertility (UI) may indeed represent the single most frequent female infertility diagnosis, with a reported prevalence of approximately 25-30% of all infertility cases (Evers, 2002; Smith et al., 2003).

Intra-uterine insemination (IUI) with washed sperms has been the most widely used method alternative to non-invasive procedure as IVF and GIFT (Tournaye, 2000).

Intra-uterine insemination (IUI) is the first method of treatment of infertility; mainly unexplained infertility, male infertility and ovulatory dysfunction (Athina Tatsioni et al., 2007).

Escander et al., 2007, found that pregnancy rates increased 2.18-fold with cervical mucus aspiration prior to embryo transfer, the exact mechanisms for this improvement not known; among several suggested mechanisms.

Cervical mucus may prevent embryo from leaving the catheter by acting as a plug at the catheter tip (Katz, 1991).

The mucus may interfere with implantation if pushed or injected into the uterine cavity (Berkanoglu et al., 2006).

Bacteria in the catheter may contaminate the mucus and then the endometrial cavity (Fanchin et al., 1998).

-Induce uterine contraction (Katz, 1991).

In the present study; the pregnancy rate in our group of cervical mucus aspiration was 18% which is better than studies done by Erhan Simsek et al., 2008 which was 15.1%. Karlstrom et al., 1991 which was 15%.

In this study; it was found that duration of infertility affects pregnancy rate in both groups (1 and 2);

In group I; percentage of pregnancy rate in cases with duration of infertility <5 years was 55% while in cases with duration of 5-7 years was 22.2% and duration of >7 years was 22.2%.

In group II; Percentage of pregnancy rate in cases with duration of infertility <5 years was 75% while in cases with duration of 5-7 years was 25% and nil in more than 7 years.

This is in agreement with studies done by Erhan Simsek et al., 2007. Which stated that the cumulative pregnancy rate decreased significantly with increasing duration of infertility.

In this study:

in group I; 11 patients with previous IUI (for 3 cycles) and 3 of them became pregnant.

This is in agreement with study of (Edvinsson et al., 2000) which stated that the rate of conception in IUI increases as the number of cycles increases.

While in group II; No cases became pregnant in comparison with group I and this revealed that cervical mucus aspiration before IUI increased the pregnancy rate.

In this study;

Induction of ovulation by Clomiphene citrate(CC);

"in group I "was 10 cases (20%),"in group 2" ;was 12 cases(24%);NO significant difference in number between both groups.this in agreement with study of (*Erhan Simsek et al., 2008*).

BUT percentage of pregnancy rate was different in group I"was4%(of 18%) while "in group II " was NIL(of 8%).

Induction of ovulation by human menopausal gonadotropin (HMG);

"In group I" was 14 cases (28%),"in group II" was 13 cases (26%) ;NO significant difference in number between both groups.this in agreement with study of (*ErhanSimsek et al., 2008*).

BUT the percentage of pregnancy rate was equal 4% in both groups.

This revealed that ;cervical mucus aspiration increased pregnancy rate in cases inducted by CC despite of it's anti-estrogenic effect on cervical mucus BUT cervical mucus aspiration balanced this effect.

In conclusion, this study suggests that aspiration of cervical mucus before IUI might improve clinical pregnancy rates, and the validity of this hypothesis should be tested by prospective randomized trials specifically targeted toward patients with unexplained infertility in whom cervical hostility is a concern.

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