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Original Research Article

Effect of Vaginal Misoprostol on Success Rate of Intrauterine Insemination

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Abstract

Intrauterine insemination has an important role in the treatment of infertile couples. Usage of vaginal misoprostol therapy at the time of intrauterine insemination was investigated and its tolerability and effects on clinical pregnancy rates was assessed. The objective was to assess the effectiveness of misoprostol after intrauterine insemination on pregnancy success in infertile female, and investigate if misoprostol effect on pregnancy before intrauterine insemination. Eighty-one infertile couples who attended the High Institute of Infertility Diagnosis and Assisted Reproductive Technologies, Al-Nahrain University, and private fertility clinics were enrolled in this study. The period of collection of patients extended from September (2018) until May (2019). Patients were divided into two groups, first group received 100-µg vaginal misoprostol immediately after completion of intrauterine insemination procedure while control group were subjected to ordinary intrauterine insemination procedure without adjunctive therapy. There was no significant effect of active motility, the percentage of pregnancy rate in control group was 5.0% while it was 19.5% in Misoprostol post IUI. Moreover, there is a significant difference in pregnancy rate among all study groups. However, there is no significant result of pregnancy outcome related with sperm parameter before and after activation of semen sample. Misoprostol use after intrauterine insemination has positive impact on pregnancy outcome. Smaller doses of misoprostol can decrease side effects without affecting the outcome. No significant difference in pregnancy rate was noted between pregnancy outcome with mild to moderate oligozoospermia, asthenozoospermia and normozoospermia men which may be attributed to good preparation of semen samples for intrauterine insemination.

Keywords: Intrauterine insemination, pregnancy outcome rate, Misoprostol post intrauterine insemination, sperm parameter.

INTRODUCTION

Infertility is defined according to the World Health Organization (WHO) as a disease of the reproductive system defined by the failure to achieve a clinical pregnancy after 12 months or more of regular unprotected sexual intercourse (WHO, 2010). Infertility is divided into two types viz primary infertility and secondary infertility. Intra-Uterine Insemination is the first-line procedure of assisted reproductive technologies. IUI is simple and low cost (Ganguly et al., 2016). It is a relatively quick procedure and is performed by the doctor without any anesthesia. IUI treat couples with mild male infertility (oligozoospermia, asthenozoospermia) and

cervical factor. Several factors are working to make IUI success rate including age, the duration of infertility, the presence of endometriosis, the endometrial thickness, the sperm preparation time (Jeon et al., 2013; Goldman et al., 2014; Goverde et al., 2000). Sperm washing used with best quality semen samples (good count and motility of spermatozoa) and it is often performed for the intrauterine insemination (IUI) (Natali, 2011). The technique was simply performed by washing the semen with a sterile medium. Thereafter, dilution of the entire mixed semen sample (1:2) with supplemented medium was carried out. Then it was transferred into multiple centrifuge tubes and centrifugation of the sample was done at (300–500g) for (5–10) minutes. Then the supernatants were discarded with re-suspension of the sperm pellets in 1 ml of supplemented medium and centrifuge again at (300–500g) for (3–5) minutes with repetition of the second step. The number of washings can be reduced by using fewer tubes and increasing the volume in each tube, but the centrifugal force and duration of centrifugation should be increased (WHO, 2010). Swim-up method is one of the most commonly used techniques for sperm preparation (Jameel, 2008). It is based on spermatozoa self-propelled active movement from a single centrifuged, pre-washed cell pellet, into an overlaying medium. This procedure should not be considered for intrauterine insemination (IUI) if the initial total motile sperm is less than 30× 106 (Richard, 2010). Misoprostol is a synthetic prostaglandin E1 analog and has been used off-label for cervical ripening and labor induction since the 1980s (Hofmeyr et al., 2010). Misoprostol is a synthetic prostaglandin E1 analogue that causes uterine contractions and softening and dilation of the cervix. It has been used off-label in the management of miscarriage, postpartum hemorrhage, and induction of labor and ripening of the cervix. Misoprostol has advantages being cheap, widely available, and stable at room temperature and having few side effect (Allen et al., 2009).

PATIENTS, MATERIALS AND METHODS

Eighty-one infertile couples who attended the High Institute of Infertility Diagnosis and Assisted Reproductive Technologies, Al-Nahrain University, and private fertility clinics were enrolled in this study. The period of collection of patients extended from September (2018) until May (2019). Couples were randomly selected after explanation of the procedure and taking their verbal consent to participate in this study. Patients were divided into two groups, first group received 100 µg vaginal misoprostol immediately after completion of IUI procedure, while control group were subjected to ordinary IUI procedure without adjunctive therapy. Detailed history taken from all couples involved examination including general, abdominal and genital tract for any abnormality. Hysterosalpingogram for assessment of tubal patency and SFA for male. Male with normozoospermia and mild male factor infertility were included (those with mild oligozoospermia, mild asthenozoospermia, and those with mild teratozoospermia).

In vitro activation technique for intrauterine insemination

A consultant urologist in the institute examined the husbands who were involved in this study. Semen sample was obtained via masturbation after an abstinence period of 3-5 days (De Jonge et al., 2004). It was collected directly into a clean, dry and sterile disposable wide mouth container in a special allocated room for this purpose in the institute or in private laboratories. The sample was transported to the semen examination laboratory immediately to be allowed to liquefy in an incubator at (37 ºC). After complete liquefaction, the semen was analyzed by a macroscopic and microscopic examination using the standardization of WHO 1999 as seen in table 1 and table 2 ((WHO, 1999).

RESULTS

Semen volume of male, liquefaction time, viscosity and appearance of sperm were studied. It was found that the mean semen volume of male partners in the control and Misoprostol post IUI groups were 2.91 ± 1.29 ml and 3.37 ± 1.09 ml, respectively. The difference was statistically not significant (P= 0.087), as shown in Table (3). There was in addition insignificant difference in mean liquefaction time among groups, 37.85 ± 9.69 minutes and 38.29 ± 13.00 minutes, respectively (P= 0.863) according to Table (3). However, there was no significant difference in the distribution of patients according to semen viscosity and appearance among study groups (P = 0.813), these results were shown in Table (3).

Motility, normal morphology and sperm concentration

Motility, normal morphology and sperm concentration was investigated in this study and it was found that the mean of actively motile sperm percentage before activation was 42.75 ± 9.93 and 38.24 ± 17.76 in control and Misoprostol post IUI groups, respectively. The difference was statistically not significant (P = 0.640), as shown in Table (4). In addition, the mean normal morphology sperm percentage before activation was studied, it was found that the mean was 31.00 ± 8.56 and 36.83 ± 11.86 in control and Misoprostol post IUI groups, respectively; the difference was statistically not significant (P = 0.430), according to Table (4). Moreover, the mean

Table 1. Normal values of semen macroscopically examination as (WHO, 1999)

Parameters	Normal values
Appearance	Homogenous opalescent
Volume	2-6 ml
Liquefaction time	Within 60 minutes
Viscosity	Drops ≤ 2cm thread
PH	7.2-8

Table 2. Semen microscopic examination, normal values (WHO, 1999)

Parameters	Normal values
Sperm concentration (m/ml)	(20) million sperm/ml or more
Sperm motility (%)	(50%) or more with forward progression or (25%) or more with rapid progression within (60) min.
Morphologically normal sperm	(30%) or more with normal forms
Round cells	Less than (5) cells/ HPF

Table 3. Semen volume of male, liquefaction time viscosity and appearance of sperm before activation

Seminal fluid characteristic	Control *n* = 40	Misoprostol post IUI *n* = 41	*P*
Volume (ml)	2.91 ±1.29	3.37 ±1.09	0.087 † NS
liquefaction (minute)	37.85 ±9.69	38.29 ±13.00	0.863 † NS
Viscosity and appearance			
Viscous or Milky	13 (32.5 %)	12 (29.3 %)	0.813 ¥ NS
Not	27 (67.5 %)	29 (70.7 %)	

Date were expressed as either mean ± standard deviation or number (%); n: number of cases; IUI: intra uterine insemination; †: Independent samples t-test; ¥: Chi-square test; NS: not significant at P ≤ 0.05

Table 4. Motility, normal morphology and sperm concentrationbefore activation

Seminal fluid characteristic	Control *n* = 40	Misoprostol post IUI *n* = 41	*P*
Pre-preparation active motility	39.75 ± 9.93	38.24 ± 17.76	0.640 † NS
Pre-preparation normal morphology	35.00 ± 8.56	36.83 ± 11.86	0.430 † NS
Pre-preparation concentration (M/ml)	48.70 ± 9.95	50.68 ± 13.52	0.455 † NS

Date were expressed as either mean ± standard deviation or number (%); n: number of cases; IUI: intra uterine insemination; †: Independent samples t-test; ¥: Chi-square test; NS: not significant at P ≤ 0.05

sperm concentration before activation was 43.75 ± 12.69 and 50.68 ± 13.52 in control and Misoprostol post IUI groups, respectively; the difference was statistically insignificant (*P* = 0.455), these results were shown in Table (4).

Agglutination, presence or absence of round cells of sperm

Agglutination, presence or absence of round cells of sperm was investigated in this study. It was found that the agglutination of sperm was significantly more frequent in Misoprostol post IUI group than control group, 65.9 % versus 15.0 %, respectively (*P*< 0.001), as shown in Table (5). However, there was no significant difference in the distribution of patients according to presence or absence of round cells among study groups (*P* = 0.753), according to Table (5).

Seminal fluid characteristics of male partners after activation

In this study, seminal fluid characteristics of male partners after activation were investigated. The seminal fluid characteristics of male partners after activation were shown in Table (6). Sperm concentration following activation showed no significant variation among groups (*P* = 0.938). In addition to that, the mean sperm concentration of control and Misoprostol post IUI groups was 20.45 ± 6.82 million/ml and 26.10 ± 8.27 million/ml

Table 5. Agglutination, presence or absence of round cells of sperm before activation

Seminal fluid characteristic	Control *n* = 40	Misoprostol post IUI *n* = 41	*P*
Agglutination			
Yes	6 (15.0 %)	27 (65.9 %)	<0.001 ¥ NS
No	34 (85.0 %)	14 (34.1 %)	
Round cells			
Yes	36 (90.0 %)	36 (87.8 %)	0.753 ¥ NS
No	4 (10.0 %)	5 (12.2 %)	

Date were expressed as either mean ± standard deviation or number (%); n: number of cases; IUI: intra uterine insemination; †: Independent samples t-test; ¥: Chi-square test; NS: not significant at P ≤ 0.05

Table 6. Seminal fluid characteristics of male partners after activation

Seminal fluid characteristic	Control *n* = 40	Misoprostol post IUI *n* = 41	*P*
Post preparation concentration(M/ml)	25.98 ±5.53	26.10 ±8.27	0.938 † NS
Post preparation active motility %	87.28 ±12.37	88.54 ±11.63	0.637 † NS
Post preparation normal morphology %	48.08 ±10.43	50.02 ±18.23	0.558 † NS

Date were expressed as mean ±standard deviation; IUI: intra uterine insemination; †: independent samples t-test; NS: not significant at *P* ≤ 0.05

Table 7. Method of sperm activation

Seminal fluid Characteristic	Control *n* = 40	Misoprostol post IUI *n* = 41	*P*
Simple	31 (77.5)	24 (58.5)	0.068 ¥ NS
Centrifugation	9 (22.5)	17 (41.5)	

Date were expressed as number (%); *n*: number of cases; IUI: intra uterine insemination; ¥: Chi-square test; NS: not significant at *P* ≤ 0.05

respectively according to Table (6). There was no significant difference in mean actively motile sperm percentage between study groups, 87.28 ± 12.37 and 88.54 ± 11.63, respectively (*P* = 0.637), these results were shown in Table (6). In addition, there was no significant difference in mean normal morphology sperm percentage between study groups, 40.08 ± 10.43 and 50.02 ± 18.23 respectively (*P* = 0.558), as shown in Table (6).

Methods of activation and sperm characteristics

Two methods were conducted in the present study, simple and centrifugation methods. Patients semen samples in all study groups were randomly allocated according to method of activation to insure insignificant difference in the distribution of patients according to method of sperm activation (*P* = 0.068), as shown in Table (7).

In control group, there was significant difference in mean sperm concentration. Simple method resulted in higher concentration than centrifugation method (P = 0.023), but there was insignificant difference in mean actively motile sperm percentage and normal morphology sperm percentage using either activation methods (*P*> 0.05). In addition, in Misoprostol Post IUI group, there was no significant difference in mean sperm concentration, mean actively motile sperm percentage and normal morphology sperm percentage using either activation methods (*P*> 0.05), as shown in Table (8).

Biochemical pregnancy outcome

Biochemical pregnancy outcome according to study groups is shown in Table (9). Highest biochemical pregnancy rate was obtained by Misoprostol post IUI group, followed by control group, 19.5 % versus 5.0 % respectively. The difference in biochemical pregnancy rate between study groups was statistically significant (*P* = 0.047).

Moreover, pregnancy outcome was insignificantly correlated to any of semen characteristics before activation, volume, liquefaction time, sperm concentration, actively motile sperm percentage, normal morphology sperm percentage, presence of

Table 8. Comparison of sperm characteristics according to method of activation

Groups	Characteristic	Simple	Centrifugation	*P*
Control	Number of cases	31	9	---
	post preparation concentration(M/ml)	27.03 ± 5.44	22.33 ± 4.33	0.023 S
	post preparation active motility	86.77 ± 12.49	89.00 ± 12.51	0.641 NS
	post preparation normal morphology	47.52 ± 10.52	50.00 ± 10.48	0.536 NS
Misoprostol Post IUI	Number of cases	24	17	---
	post preparation concentration(M/ml)	26.71 ± 7.89	25.24 ± 8.96	0.581 NS
	post preparation active motility	90.00 ± 9.67	86.47 ± 14.00	0.345 NS
	post preparation normal morphology	50.63 ± 16.87	49.18 ± 20.50	0.806 NS

Date were expressed as mean ±standard deviation; *n*: number of cases; IUI: intra uterine insemination; †: independent samples t-test; NS: not significant at $P \leq 0.05$

Table 9. Biochemical pregnancy outcome according to study groups

Biochemical pregnancy	Control *n* = 40	Misoprostol post IUI *n* = 41	*P*
Positive	2 (5.0 %)	8 (19.5 %)	0.0 47 ¥ S
Negative	38 (95.0 %)	33 (80.5 %)	

Date were expressed as number (%); n: number of cases; IUI: intra uterine insemination; ¥: Chi-square test; S: significant at P ≤ 0.05

Table 10. Correlation between biochemical pregnancy outcome and seminal fluid characteristics before activation

Characteristic	Positive pregnancy *n* = 9	Negative pregnancy *n* = 82	*P*
Volume(ml)	3.14 ±1.39	3.15 ±1.19	0.990 † NS
Liquefaction (minute)	32.00 ±6.32	38.93 ±11.74	0.072 † NS
Post preparation concentration(M/ml)	48.00 ±9.43	49.94 ±12.20	0.630 † NS
Post preparation active motility	35.20 ±12.87	39.52 ±14.57	0.377 NS
Post preparation normal morphology	37.40 ±13.15	35.72 ±9.98	0.633 † NS
Round cells			
Negative	0 (0.0 %)	9 (12.7 %)	0.592 F NS
Positive	10 (100.0 %)	62 (87.3 %)	
Agglutination			
Positive	6 (60.0 %)	27 (38.0 %)	0.327 Y NS
Negative	4 (40.0 %)	44 (62.0 %)	
Viscosity & appearance			
Viscous or Milky	1 (10.0 %)	24 (33.8 %)	0.246 Y NS
No	9 (90.0 %)	47 (66.2 %)	

Date were expressed as either mean ±standard deviation or number (%); *n*: number of cases; †: independent samples t-test; Y: Yates correction; F: Fischer exact test; NS: not significant at $P \leq 0.05$

Table 11. Correlation between biochemical pregnancy outcome and seminal fluid characteristics after activation

Characteristic	Positive pregnancy *n* = 9	Negative pregnancy *n* = 82	*P*
Post preparation concentration(M/ml)	25.40 ± 5.46	26.13 ± 7.23	0.761 † NS
Post preparation active motility	89.50 ± 8.96	87.69 ± 12.34	0.656 † NS
Post preparation normal morphology	57.50 ± 18.17	47.87 ± 14.05	0.054 † NS

Date were expressed as either mean ±standard deviation or number (%); *n*: number of cases; †: independent samples t-test; ¥: Chi-square test; NS: not significant at $P \leq 0.05$

agglutination, presence of round cells and viscosity and appearance ($P > 0.05$), as shown in Table (10).

In Table (11), pregnancy outcome was also not significantly correlated to sperm concentration, actively

motile sperm percentage and normal morphology sperm percentage after activation (P > 0.05).

DISCUSSION

Semen processing techniques have long been an important part of semen preparation in IUI. Semen processing is designed to yield the highest concentration of morphologically and functionally normal sperm (Lee et al., 2018). The swim-up method for recovery of motile sperm is reliable. These and other techniques are designed to recover morphologically better motile sperm. The washing procedure is necessary to remove prostaglandins, infectious agents and leukocytes (Muratori et al., 2019). Two methods were conducted in the present study, simple and centrifugation methods. Patients semen samples in all study groups were randomly allocated according to method of activation to insure insignificant difference in the distribution of patients according to method of sperm activation (*P* = 0.068), as shown in Table (7). In control group, there was significant difference in mean sperm concentration. Simple method resulted in higher concentration than centrifugation method (P = 0.023), but there was insignificant difference in mean actively motile sperm percentage and normal morphology sperm percentage using either activation methods (*P*> 0.05). In addition, in Misoprostol Post IUI group, there was no significant difference in mean sperm concentration, mean actively motile sperm percentage and normal morphology sperm percentage using either activation methods (*P*> 0.05), as shown in Table (8). Biochemical pregnancy outcome according to study groups is shown in Table (9). Highest biochemical pregnancy rate was obtained by Misoprostol post IUI group, followed by control group, 19.5 % versus 5.0 %, respectively. The difference in biochemical pregnancy rate between study groups was statistically significant (*P* = 0.047). The use of misoprostol in women undergoing assisted reproduction has been evaluated by a number of studies. In one study, 253 women were given vaginal misoprostol in a dose of 400 µg during intrauterine insemination (IUI). Those women achieved significantly higher biochemical pregnancy rate than a control group comprising 241 subfertile women, 17% versus 9% (Brown et al., 2001). These results are in accordance with the findings of the present study. However, the authors of the current study used a dose of 100 µg rather than 400 µg to overcome pain associating sever uterine contraction with higher doses of misoprostol. In another study evaluating the use of 200 µg vaginal misoprostol, the pregnancy rate was significantly higher than that of the control group, 31 % versus 20 % (Barroso *et al*., 2001). Again, these results are consistent with the findings of the current study. On the contrary, to the finding of the present study and the later mentioned studies, some authors found no significant rise in biochemical pregnancy rate following vaginal administration of 200-µg misoprostol to subfertile women post IUI (Sorouri et al., 2015). In addition, the results of the present study are inconsistent with that of (Moslemizadeh et al., 2009), who found no significant difference in biochemical pregnancy rate following post IUI 200 µg misoprostol. Other authors have noted the significant effect of vaginal misoprostol on infertile women undergoing IUI on the results of biochemical load (Chikkagowdra et al., 2013). The available data from this study and other previous studies that support the significant effect of using Misoprostol in subfertile women undergoing IUI, this observation must have some scientific explanation. Indeed, almost all body fluids contain some amount of prostaglandins and seminal fluid is one of the richest body fluids in its prostaglandin content (Moslemizadeh et al., 2009). Entry of seminal fluid into vagina causes a lot of effects that may aid fertilization such as increasing myometrial contractility, potential relaxation of tubal isthmus, improved spermatozoon–oocyte binding penetration and attenuation of the female immune response to spermatozoa. This may all facilitate fertilization potential (Niringiyumukiza et al., 2018). Misoprostol was used in women undergoing IUI because of statistically significant effect on improving biochemical pregnancy rate, and the negligible or minimal side effects associated to the use of misoprostol. The need for more research work is suggested with the inclusion of larger sample size and performing a multicenter study. Moreover, pregnancy outcome was insignificantly correlated to any of semen characteristics before activation, volume, liquefaction time, sperm concentration, actively motile sperm percentage, normal morphology sperm percentage, presence of agglutination, presence of round cells and viscosity and appearance (*P*> 0.05), as shown in Table (10). Moreover, pregnancy outcome was insignificantly correlated to any of semen characteristics before activation, volume, liquefaction time, sperm concentration, actively motile sperm percentage, normal morphology sperm percentage, and presence of agglutination, presence of round cells and viscosity and appearance. Pregnancy outcome was also not significantly correlated to sperm concentration, actively motile sperm percentage and normal morphology sperm percentage after activation. In Table (11), pregnancy outcome was also not significantly correlated to sperm concentration, actively motile sperm percentage and normal morphology sperm percentage after activation (P > 0.05). Significant statistical association between pregnancy outcome and misoprostol use reflects an independent effect subjected by the drug that was not modified by other variables such as demographic, hormonal, endometrial and semen quality. The majority of couples in the present study were doing the procedure of IUI for the first time. Couples with previous IUI are less frequent because they may have sought other

techniques such as IVF or ICS.

CONCLUSIONS

Misoprostol use after IUI has positive impact on pregnancy outcome. Smaller doses of misoprostol can decrease side effects without affecting the outcome. No significant difference in pregnancy rate was noted between pregnancy outcome with mild to moderate oligozoospermia, asthenozoospermia and normozoo-spermia men which may be attributed to good preparation of semen samples for intrauterine insemination.

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