

Prospective comparative trial to define the optimum time for insemination during stimulated intrauterine insemination cycles

Original
Article

Hany A Elkallaf

Departments of Obstetrics and Gynecology, Faculty of Medicine, Benha University

ABSTRACT

Objectives: To determine the clinical pregnancy rate (CPR) of women who underwent clomiphene citrate (CC) and human menopausal gonadotropin ovarian stimulation (OS) followed by intrauterine insemination (IUI) at varied times after hCG ovulation triggering and to determine the appropriate IUI timing.

Patients and Methods: 152 women with primary infertility secondary to male subfertility were randomly allocated into groups (I-IV) according to timing of IUI at 34-35, 35-36, 36-37, and 37-38hr after ovulation triggering. OS consisted of 5-days oral CC 100 mg once daily since day-3 of the menstrual cycle followed by daily injection of human menopausal gonadotropin 150 IU since day-8 until hCG injection. Ovulation was monitored using TVU for evident ovulation with a dominant follicle size >18 mm. IUI was repeated for three cycles and women were evaluated for clinical pregnancy rate (CPR) and abortion and multiple pregnancy rates were recorded.

Results: 44 women had clinical pregnancy for a total CPR of 28.9%. CPR% /patient was 15.8%, 34.2%, 42.1% and 23.7% and CPR%/cycle was 6, 14, 20 and 11 in the four groups, respectively with significant differences in favor of group III. Three women had ectopic and six women had multiple pregnancy and five women had abortion. Statistical analyses defined high number of performed IUI cycles as significantly negative, while longer time till IUI after hCG injection as significantly positive predictor for trial success. Kaplan-Meier regression analysis defined 37 hr (95% CI: 36.5-37.5) after hCG injection as the appropriate time to get the best chance for IUI success.

Conclusion: IUI after CC + GN ovarian stimulation is an appropriate policy for infertile couples secondary to male subfertility. Total motile sperm count threshold at ≥ 5 million gives acceptable outcome. Semen injection at 37 hr after hCG ovulation triggering gives the highest CPR.

Key Words: Clinical pregnancy rate , Combined ovarian stimulation , Intrauterine insemination, Timing of semen injection, .

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Corresponding Author: Hany A Elkallaf, *Departments of Obstetrics and Gynecology, Faculty of Medicine, Benha University*, E-mail: kaainih@yahoo.com

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INTRODUCTION

The clinical pregnancy rate (CPR) of the frequently used fertility treatments for couples with male subfertility is variable.^[1] In-vitro fertilization (IVF) may be associated with higher livebirth rates than expectant management or unstimulated intrauterine insemination (IUI) cycles^[2]. Letrozole is more effective than clomiphene citrate (CC) for the induction of ovulation in IUI cycles^[3]. However, there is no conclusive evidence of a difference in livebirth rates between IVF and IUI + gonadotropins (GN) or between IVF and IUI + CC^[2]. Moreover, IUI clinical pregnancy rates were significantly higher by antagonist protocol than by CC^[4].

Intrauterine insemination involves the direct intrauterine placement of sperm at time of ovulation in natural menstrual cycle or following ovarian stimulation (OS)^[5]. IUI is especially relevant for

couples with moderate male factor infertility^[6] as it increases the chance for maximum number of healthy sperm to reach the site of fertilization and efficiently performed IUI gives pregnancy rates equally good to IVF^[2, 7]. Moreover, IUI yields a higher cumulative pregnancy rate than intra-cervical insemination in both natural and stimulated cycles^[8].

Sperm parameters were intensively studied as predictors for success of IUI and the best chance of clinical pregnancy was found to occur when both motility and morphology values are above normal thresholds^[9]. The effect of number of inseminations is another point of discussion as no difference in CPR was previously reported with two inseminations compared to one insemination^[10] and the use of double IUI was found to be disappointing as it requires a second appointment and insemination, thus making the treatment more complex and expensive, without a

clear evidence of a benefit^[11]. Time lag between semen processing and insemination is an additional factor for success of IUI trial. The time interval from the end of sperm processing to IUI was found to be a significant factor influencing the CPR^[12].

Furthermore, time of semen injection in relation to time of ovulation triggered by human chorionic gonadotrophin (hCG) administration is still a matter of debate as it was documented to be the most important subject which can determine the success of IUI^[13]. Wang et al.^[14] found that pregnancy rates were similar when IUI was performed at either 24 or 36 hr after hCG injection in ovarian stimulated cycles and Guzik^[15] compared IUI at 33 vs. 39 hours after hCG administration and concluded that one well-timed insemination within several hours of ovulation represents a reasonable balance between efficacy and cost.

Objectives

The current study aimed to determine CPR of women underwent CC ovarian stimulation followed by IUI at varied times ranging between 34 and 38 hr after hCG injection so as to determine the appropriate timing for IUI after ovulation being triggered.

Design

Prospective comparative trial

Setting

Tertiary referral hospital, KSA

PATIENTS AND METHODS

Inclusion criteria in the current study included primary infertility for more than two years, secondary to male factor and women who failed to conceive unaided or with repeated IUI cycles. The study protocol

was approved by the Local Ethical Committee. All couples were eligible for evaluation for inclusion and exclusion criteria. Exclusion criteria for male partner included presence of varicocele of grade more than 2 as judged by scrotal ultrasound, obstructive lesions hampering giving seminal sample for examination and processing, hormonal disorders, and males have processed total motile sperm count (PTMS) of <5 million^[16], or presence of infection. Women with endocrinal disorders, infertility for causes other than male factor and uterine abnormalities were excluded. Couples had infertility for less than two years or secondary to immunological causes or infrequent intercourse were also excluded.

Preliminary evaluation included clinical examination and transvaginal ultrasonography and estimation of baseline serum levels of FSH, LH, E2 and prolactin. Males were referred to Andrology Clinic for evaluation of exclusion criteria. Enrolled couples were randomly, using sealed envelopes labeled by the group title, prepared by a blinded assistant and chosen by the couples into four groups according to timing of insemination. Group I, II, III and IV included women who received insemination at 34-38, 37-36, 36-35, 35-, respectively.

All women received ovarian stimulation in the form of oral CC 100 mg once daily since day-3 of the menstrual cycle for 5 days followed by daily injection of human menopausal gonadotropin (hMG, Pergonal, Serono) 150 IU starting on day-8 until hCG administration. Ovulation was monitored and assured using TVU for evident ovulation with a dominant follicle size >18 mm. For males, semen was obtained 3-days abstinence and sperms were prepared according to Density Gradient Sperm Wash Method and processed after tracking of the dominant follicles^[17] for the duration assigned for each group. IUI was repeated for three cycles and women were evaluated for clinical pregnancy rate (CPR) and abortion and multiple pregnancy rates were recorded.

Table 1: Patients' data recorded at time of study enrollment

Data	Group I	Group II	Group III	Group IV	P=	
Age (years)	28±3.2	28.3±2.6	28.5±3.3	28.2±3.5	0.917	
Duration of infertility (years)	4±1.1	3.8±1.2	4.1±1.3	4.1±1.7	0.672	
BMI parameters	Weight (kg)	79.6±9	79.2±9.2	78.2±10.3	80.2±9.9	0.838
	Height (cm)	168.3±1.8	168.2±1.7	169.4±2.7	168.8±2.5	0.103
	BMI (kg/m ²)	28.1±3.1	27.9±3.2	27.3±3.6	28.1±3.3	0.656
Number of previous cycles		2±0.8	2.2±0.85	2.2±0.8	2.3±0.8	0.673
	FSH (mIU/ml)	5.88±2.3	6.07±3	6.24±2.7	6.12±4.55	0.867
Baseline serum hormonal levels	LH (mIU/ml)	8.3±2.1	7.9±1.5	8.5±2.3	8.9±3.5	0.451
	Prolactin (ng/ml)	27.3±22.3	27.1±18.5	25.1±15	24.8±14.3	0.328
	E2 (IU/ml)	53.4±16.5	58.7±18	54.1±16.3	60.9±16	0.155

Data are presented as mean±SD; BMI: body mass index; p value indicates inter-group difference; p<0.05 indicates significant difference

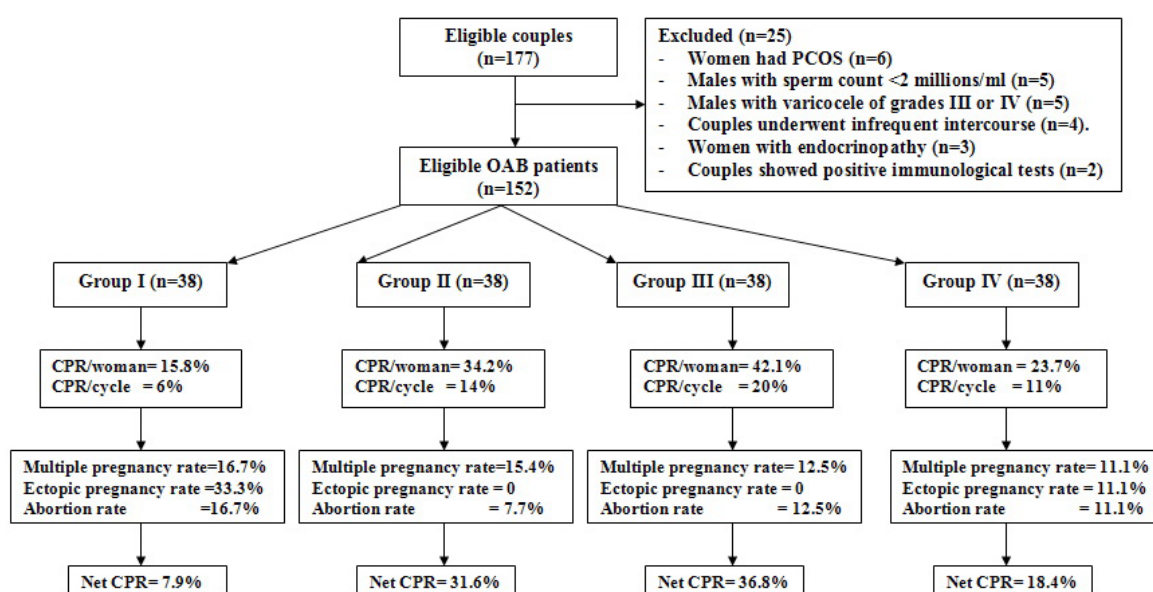


Fig. 1: Flow chart of the study

Statistical analysis

Obtained data were presented as mean±SD, numbers and percentages. The results were analyzed using paired t-test and Chi-square test (X2 test). Possible relationships were investigated using Spearman's linear regression. Sensitivity and specificity of estimated

parameters as predictors were evaluated using the receiver operating characteristic (ROC) curve analysis judged by the area under the curve (AUC) compared with the null hypothesis that AUC=0.05. Statistical analysis was conducted using the IBM SPSS (Version 23, 2015) for Windows statistical package. P value <0.05 was considered statistically significant.

Table 2: Outcomes of ovarian stimulation of enrolled patients throughout the duration of the study

Item	Group	Group I	Group II	Group III	Group IV	P=
Endometrial thickness (mm)		9.1±1.4	8.3±1.6	8.9±1.5	9.1±1.2	0.688
E2 (IU/ml)		235±63	240.7±62.1	229.3±69.2	227.3±69.7	0.815
Cycles	Number	99	90	77	81	
	Frequency (/patient)	2.6±0.5	2.4±0.7	2±0.7	2.1±0.6	0.006
	P1		0.021	0.0008	0.001	
	P2			0.0009	0.029	
Follicles	Number					
	Frequency / Value	129	118	123	132	
	P1	1.32±0.3	1.46±0.7	1.74±0.7	1.76±0.7	0.005
	P2			0.009	0.008	
	P3				0.586	
Frequency/Patients	3.4±0.9	3.1±0.8	3.2±1.1	3.5±1	0.688	

Data are presented as mean±SD and numbers; p value indicates inter-group difference; p<0.05 indicates significant difference; P1 indicates significance versus group I, P2 indicates significance versus group II, P3 indicates significance versus group III

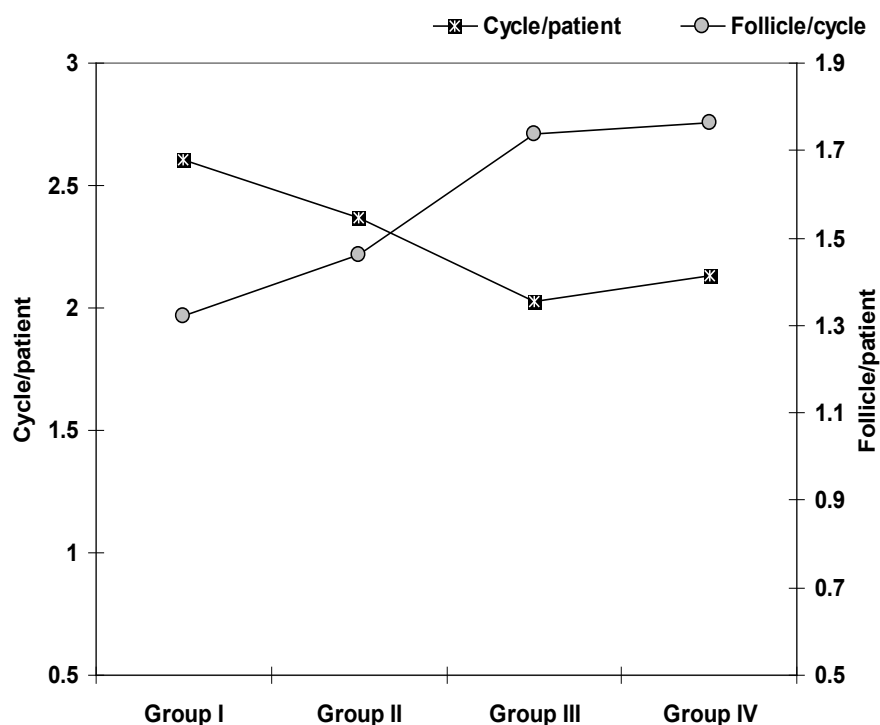


Fig. 2: The distribution of IUI cycles in relation to number of patients and mature follicles

RESULTS

The study included 177 women who were eligible for evaluation ; 25 women were excluded for not fulfilling the inclusion criteria and 152 women were randomly allocated into four studied groups (Fig. 1).

Women included in the study showed non-significant differences as regards inclusion data Table (1).

Mean endometrial thickness and estimated E2 serum levels showed non-significant difference between patients of studied groups throughout duration of the

Table 3: IUI cycle outcome data of studied patients

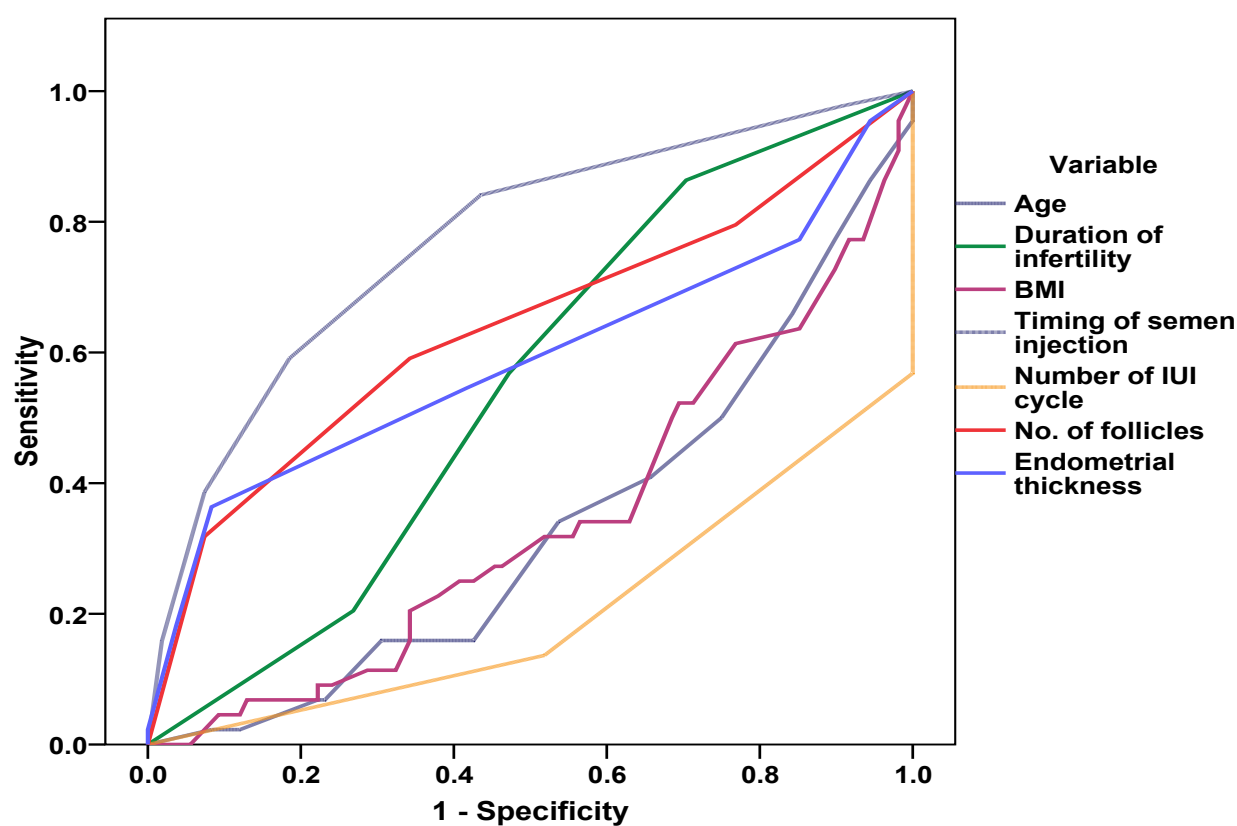
Item	Group	Group I	Group II	Group III	Group IV	P=
Total clinical pregnancy						
Number of cycles		99	90	77	81	
CPR	CPR (%)/cycle	6%	14%	20%	11%	0.026
	CPR (%)/patient	15.8%	34.2%	42.1%	23.7%	0.0003
Ectopic pregnancy rate		2 (33.3%)	0	0	1 (11.1%)	0.211
Multiple pregnancy rate		1 (16.7%)	2 (15.4%)	2 (12.5%)	1 (11.1%)	0.315
Abortion rate		1 (16.7%)	1 (7.7%)	2 (12.5%)	1 (11.1%)	0.723
Net CPR		3 (7.9%)	12 (31.6%)	14 (36.8%)	7 (18.4%)	0.009

study. Through 347 stimulation cycles, the studied women gave 502 follicles >18 mm for a frequency of 2.28 cycle/ woman and 1.45 follicle / cycle and 3.3 follicle/ woman with non-significant difference between studied women as regards the number of mature follicles/patient. The number of cycle/patient and follicle/cycle showed significant variance between

the four groups. The number of cycle/patient was significantly higher in group I compared to other groups and in group II compared to groups III and IV with non-significantly higher frequency in group IV compared to group III. The number of follicle/cycle of OS was significantly higher in III and IV compared to groups I and II with non-significantly higher frequency

Table 4: Spearman's correlation and ROC curve analysis of studied parameters for prediction of IUI trial success

Parameter	Spearman's correlation		ROC curve analysis		
	Rho	p	AUC (\pm SE)	p	95% CI
Age	-0.266	0.001	0.331 (\pm 0.048)	0.001	0.238-0.425
Duration of infertility	0.078	0.340	0.548 (\pm 0.048)	0.354	0.453-0.643
BMI	-0.247	0.002	0.343 (\pm 0.049)	0.002	0.247-0.439
Number of IUI cycles	-0.509	0.0008	0.205 (\pm 0.043)	0.0006	0.121-0.289
Number of follicles	0.224	0.005	0.637 (\pm 0.054)	0.008	0.531-0.743
Endometrial thickness	0.146	0.073	0.589 (\pm 0.058)	0.086	0.476-0.702
Timing of semen injection	0.442	0.0009	0.771 (\pm 0.403)	0.0004	0.686-0.855

**Fig. 3:** ROC curve analysis for variables as predictors for IUI trial success

in groups II and IV in comparison to groups I and III, respectively, between studied groups in favor of group III Table (2), (Fig. 2).

Throughout the duration of the study, 44 women had clinical pregnancy for a total CPR of 28.9%. CPR (%) per patient was 15.8%, 34.2%, 42.1% and 23.7% in groups I-IV, respectively, with significant ($p=0.0003$)

difference between groups in favor group III (Fig. 1). Moreover, CPR (%)/cycle was 6, 14, 20 and 11 in the four groups, respectively, with significant ($p=0.026$) difference in favor of group III. Three women had ectopic pregnancy; two in group I and one in group IV, while no woman in groups II and III had ectopic pregnancy with non-significant difference ($p=0.211$) between groups I and IV. Six women had

Table 5: Regression analysis of studied parameters for prediction of IUI trial success

	Parameter	β	p
Model 1	Number of cycles	-0.468	0.0007
	Timing of semen injection	0.340	0.0009
	Number of follicles	0.204	0.001
	Age	-0.134	0.027
Model 2	Number of cycles	-0.487	0.0007
	Timing of semen injection	0.359	0.0008
Model 3	Number of follicles	0.203	0.001
	Number of cycles	-0.487	0.0007
Model 4	Timing of semen injection	0.377	0.0005
	Number of cycles	-0.549	0.0006

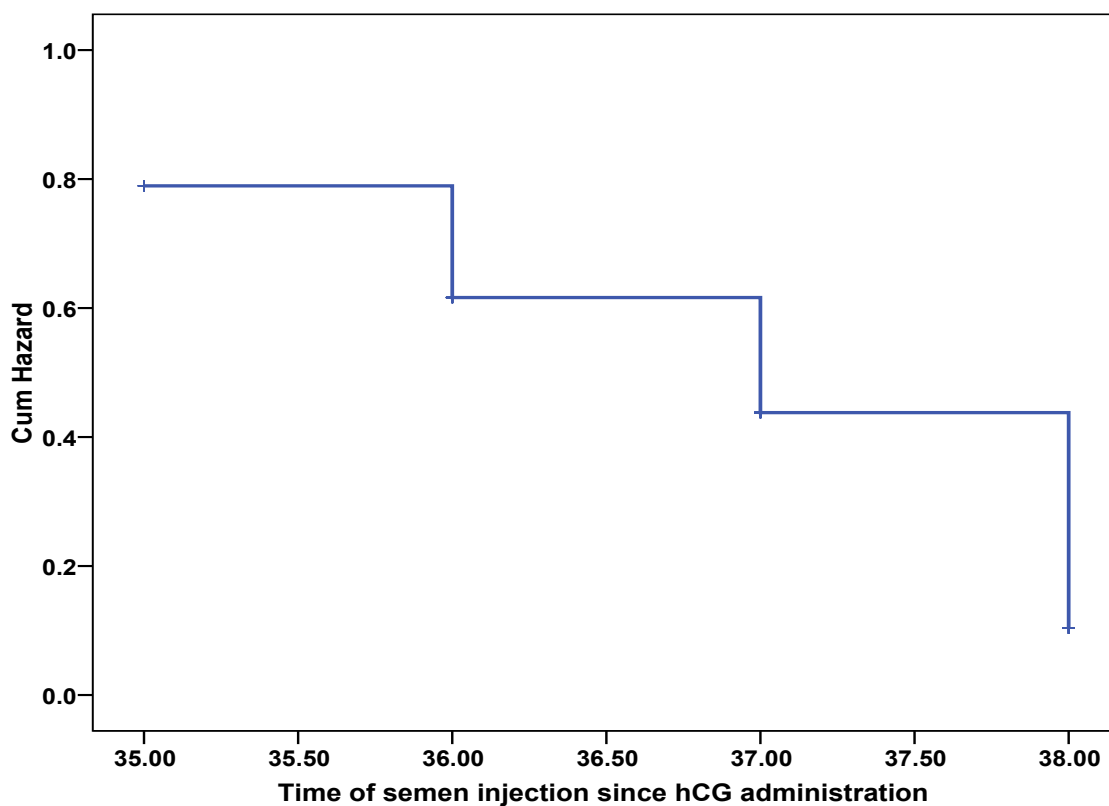


Fig. 4: Kaplan-Meier regression analysis for appropriate time of semen injection to get the best chance for IUI trial success

multiple pregnancy; one case in groups I and IV and two in each of groups II and III. Five women had abortion, two in group III and one in each of the other groups. Net pregnancy rate varied significantly among the studied groups and being the highest in groups III and II Table (3), (Fig. 1).

Spearman's correlation analysis for clinical pregnancy showed a positive significant correlation with outcome of OS protocol as evidenced by the detected positive correlation with number of follicles and endometrial thickness and with timing of semen injection, while there was negative significant correlation between age

of female partner and her BMI and with number of performed IUI cycles. ROC curve analysis confirmed that timing of IUI is the best parameter for its success (Fig. 3).

Regression analysis using stepwise method defined the high number of performed IUI cycles as the persistently significant negative predictor for trial success, while the more longer time till semen injection after hCG administration as the persistently significant positive predictor for trial success.

Kaplan-Meier regression analysis for timing of semen injection defined 37 hr (95% CI: 36.5-37.5) after hCG as

the appropriate time to get the best chance for IUI trial success (Fig. 4).

DISCUSSION

The current study included 152 women with primary infertility secondary to male factor and all women were gynecologically within the acceptable range for getting pregnancy. The rationale of the study was to induce ovarian stimulation (OS) of a nearly normal woman for compensation of deficient male factor to augment the chance for being pregnant. Such rationale coincided with that recently reported by Irani et al.^[18] who documented that couples whose total number of motile spermatozoa in the ejaculate before semen preparation is below the threshold may benefit from a superovulation.

The applied OS protocol consisted of 5-days course of daily oral CC (100 mg) since day-3 of the menstrual cycle, then daily injection of human menopausal gonadotropin (GN; 150 IU) starting on day-8 until hCG administration. Review of literature showed contradictory outcomes concerning CC + GN or GN alone where Sinha and Agrawal^[19] reported that GN alone appears to give better results than CC + GN, but combination is cost-effective. Moreover, Kamath et al.^[20] after a meta-analysis of published studies reported no conclusive evidence indicating that CC with or without GN differed from GN in GnRH agonist or antagonist protocols, but the use of CC led to a reduction in the amount of GN required and the incidence of OHSS.

On contrary to these reports and in line with the effectiveness of CC + GN combination, Hembram et al.^[21] found that CC + hMG is cost effective OS protocol and could be the preferred in couples with unexplained infertility undergoing IUI with no significant complications. Moreover, Banker et al.^[22] documented that GN, either alone or the combination with CC, gives a higher CPR and a lower abortion rate following IUI. Furthermore, Irani et al.^[18] concluded that the response to superovulation with CC determines each patient's total motile sperm count (TMSC) threshold required for satisfactory outcomes and couples whose TMSC is below the threshold may benefit from a superovulation with GN.

The current study included only males with ≥ 5 million sperms with good forward motility; a limit which is coincident with the previously documented as the threshold value^[16]. In line with the applied TMSC threshold value, multiple recent studies assured the possibility of getting acceptable clinical pregnancy rate (CPR) with such limit where Madbouly et al.^[23] documented that a post-wash TMSC of ≥ 5 million sperm is significantly associated with a high pregnancy rate. Also, Ruitter-Ligeti et al.^[24] and Hassan et al.^[25] documented that the decrease in TMSC did not negatively affect pregnancy rates. Moreover, Punjabi et al.^[26] reported that CPR of 13.8% versus 4.4% with > 2 versus < 2 million of rapid progressive spermatozoa.

The reported total CPR was 28.9% and ectopic and multiple pregnancy rates were 2% and 3.9%, respectively, with an abortion rate of 3.3%; so net CPR was 24.3%. In line with these figures, Irani et al.^[18], Sinha and Agrawal^[19], Hembram et al.^[21] and Banker et al.^[22] reported pregnancy rates of 17.8%, 18.9%, 23.3% and 14.55%, respectively, with a miscarriage rate 5.43%^[22].

The group-related CPR was 15.8%, 34.2%, 42.1% and 23.7%/patient and was 6, 14, 20 and 11/cycle in groups I-IV, respectively, with significant inter-group difference in favor of group III with significant difference between groups as regards net CPR, being the highest in groups III and II. Statistical analyses defined timing of semen injection in relation to ovulation triggering as the significant specific and the higher number of conducted IUI cycles as the most significant sensitive parameter for success of IUI trial. Kaplan-Meier regression analysis defined 37 hr as timing for semen injection after hCG triggering as the appropriate time to get the best chance for IUI trial success.

These results are in accordance with Jansen et al.^[27] who documented that there is no negative effect on pregnancy rate when IUI of processed sperm is delayed until the next day. Moreover, Lee et al.^[28] reported that insemination at least 36 h after ovulation is associated with increased pregnancy rate compared to IUI performed ≤ 36 h following ovulation.

Interestingly, correlation analyses for CPR showed a negative significant correlation with age of female partner and her BMI and with number of performed IUI cycles. Similarly, Liang et al.^[12] using Logistic multivariate analysis showed that CPR was decreased with the increased age of the women and Liu et al.^[29] found that cycle protocol and number, female age, percentage of progressively motile sperm and sperm morphology are the main factors affecting the CPR following IUI. Zhang et al.^[30] detected lower pregnancy rates with increased female age and duration of infertility. Also, Huyghe et al.^[31] documented that statistically detected significant influence of female BMI on CPR with the highest CPR was reported in women with average BMI and the lowest in obese women. Recently, Na et al.^[32] found the cumulative pregnancy and live birth rates tended to decrease, while abortion and ectopic pregnancy rates and cesarean delivery rate gradually increased with increased BMI and suggested that it is important to achieve a normal BMI prior to IUI.

CONCLUSION

IUI after CC + GN ovarian stimulation is an appropriate policy for infertile couples secondary to male subfertility. Total motile sperm count threshold at ≥ 5 million as a threshold gives acceptable outcome. Semen injection at 37 hr after hCG ovulation triggering gives the highest CPR than other evaluated times. Wider scale studies are mandatory to establish such time cutoff point and its applicability for men with TMSC < 5 millions.

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