Original Article

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The Effects of Ovulation Documentation before Insemination on Intrauterine Insemination Cycle Outcomes: A Retrospective Analysis

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ABSTRACT

Introduction: Ovulation is the key event in Intrauterine Insemination (IUI) cycles. Monitoring ovulation prior to insemination will help to alter insemination time to improve pregnancy rate.

Aim: To compare pregnancy rates and live birth rates in presence and absence of ultrasonographic features of ovulation before insemination in IUI cycles.

Materials and Methods: This was a retrospective cohort study conducted in a Institute of Medical Sciences and SUM Hospital, Bhubaneswar. Three hundred eighty eight IUI cycles performed in the period of January 2017 to December 2018 were analysed. On the day of IUI prior to insemination, transvaginal ultrasonography was done 36-38 hours after ovulation trigger to document ovulation. Presumptive signs of ovulation were documented in 201 cycles (Group A) and there was no feature suggestive of ovulation in 187 cycles (Group B). In all the cycles, single insemination was performed at 38-40 hours after ovulation trigger. Baseline characteristics were compared. Mann-Whitney U test was used to compare continuous variables. Chi-square test and Fisher's-exact test were applied to find out the differences in the categorical variables as well as the pregnancy outcomes among the groups. Pregnancy rate and live birth rate were considered as the primary outcomes.

Results: Pregnancy rate (17.9% vs 18.2%, p-value=0.945) and live birth rate (17.9% vs 16.0%, p-value=0.625) were similar irrespective of ovulation status documented in ultrasonography performed before insemination.

Conclusion: IUI cycle outcomes are independent of the ovulation status documented before insemination.

Keywords: Birth, Pregnancy, Ultrasonography

INTRODUCTION

Intrauterine Insemination (IUI) is one of the commonly practiced procedures in infertility clinics. It is performed with an aim to make the motile fraction of spermatozoa available to the oocyte around the time of ovulation. National Institute for Health and Care Excellence (NICE) guidelines recommend insemination to be performed around the time of ovulation [1]. From biological point of view, insemination in the ovulatory period provides a favourable environment for sperms to receive the cascade of signals to initiate capacitation process and fertilisation [2]. After intercourse, sperms migrate towards the fallopian tubes and bind to the endo-salpingeal epithelium in the isthmus region of oviduct which acts as a reservoir, where spermatozoa remain incapacitated until they get signals of ovulation [3]. Conversely in case of IUI, inseminated spermatozoa do not reside in reproductive tract for a long time as spermatozoa were recovered from peritoneal fluid by laparoscopy 2-4 hour after IUI [4]. Hence, the time difference between ovulation and insemination should be minimum to improve pregnancy rate in IUI cycles [4].

In a prospective cohort study, IUI was performed 36±2 hours after ovulation trigger and pregnancy rate was significantly higher in post-ovulatory patients compared to pre-ovulatory patients [5]. In the above mentioned study, ovulation was documented during insemination. Another prospective study also demonstrated higher pregnancy rate in presence of ovulation documented post-insemination compared to patients without ovulation [6]. These observations are unlikely to benefit the patients without evidence of ovulation as it leaves no option to alter the timing of insemination because the processed semen sample should be inseminated within 90 minutes of semen collection to achieve maximum pregnancy rate [7]. The present study aims to find benefits of documented ovulation prior to insemination in IUI cycles particularly prior to semen collection so that observation of this study will facilitate further research to improve pregnancy rate by delaying semen collection in patients without features of ovulation.

MATERIALS AND METHODS

This was a retrospective study of 388 IUI cycles performed during January 2017 to December 2018 in which evidence of ovulation was monitored prior to semen sample collection in autologous cycles and thawing of donor semen samples in donor sperm cycles.

Since, it is a time bound retrospective study based on hospital records, where a number of infertility patients undergoing IUI with the following inclusion criteria were enrolled over a period of two years.

Inclusion criteria: IUI was advised to couples with unexplained infertility, mild to moderate male factor, minimal and mild endometriosis and ovulatory dysfunction. Artificial Insemination with Donor sperm (AID) was done in cases of males with azoospermia and grossly abnormal semen parameters when the couples were unable to afford In-vitro Fertilisation (IVF). Controlled ovarian stimulation was performed with administration of clomiphene citrate with human Menopausal Gonadotropin (hMG) or letrozole with hMG.

Exclusion criteria: IUI cycles where other ovarian stimulation protocols were followed were excluded from the study due to less sample size and interference in statistical analysis. IUI cycles cancelled for sub-optimal response or hyper-response were also excluded from the study.

In planned IUI cycles, clomiphene citrate 50 mg or 100 mg (Clofert, Svizera Healthcare) or letrozole 2.5 mg or 5 mg (Letroz, Sun Pharma Laboratories) was started from 2nd or 3rd day of cycle for 5 days and hMG injections were used with oral drugs. Injection hMG (75IU) (GMH, Sun Pharma Laboratories) was given intramuscularly on 5th and 7th day of the cycle. Follicular monitoring was done on 9th or 10th day of cycle. Transvaginal ultrasound was done in all cases with Samsung Medison SonoACE R7 with the 4-9 MHz transvaginal probe.

Once the largest follicle reached a size of atleast 17 mm, ovulation trigger was administered and IUI was planned 38-40 hours after ovulation trigger. Urinary human Chorionic Gonadotropin (hCG) 5000IU (Fertigyn, Sun Pharma Laboratories) was used for ovulation trigger in majority of cycles. In presence of more than four follicles measuring more than 13 mm including the dominant follicle(s), 1 mg of injection leuprolide acetate (Lupride, Sun Pharma Laboratories) was administered subcutaneously as ovulation trigger to avoid Ovarian Hyperstimulaton Syndrome (OHSS) risk. If there were more than 3 follicles measuring more than 16 mm, the cycle was cancelled in view of hyper-response. Transvaginal ultrasound was performed on the day of IUI, 36-38 hours after ovulation trigger to document presumptive ovulation. A non-latex probe cover was used during ultrasonography. Ovulation was presumed to occur if there was collapse of dominant follicle(s) with or without free fluid in pouch of Douglas. Male partner was instructed for semen collection after ultrasonography.

Liquefied semen sample was processed by density gradient centrifugation method using commercially available media (Nidacon International, Sweden). An aliquot of 1 mL 40% (v/v) density gradient medium (PureSperm-40) was layered over 1 mL 80% (v/v) density gradient medium (PureSperm-80). A properly mixed liquefied semen sample was transferred to the top of the density gradient media and centrifuged at 300 g for 15 minutes. After centrifugation, the supernatant was discarded. Sperm pellet was suspended in an aliquot of 3 mL of wash medium (PureSperm wash) and the mixture was centrifuged at 200 g for 10 minutes. The supernatant was further discarded, and an additional aliquot of 0.5 mL sperm wash media was added to the sperm pellet. Insemination was completed within two hours of ultrasonography. In case of AID, pre-washed frozen semen sample was thawed and insemination was done. In all cases, insemination was performed at 38-40 hours after ovulation trigger.

Luteal phase support was provided with natural micronized progesterone (200 mg) intravaginally twice daily for 15 days. Urine pregnancy test was done 20 days after IUI to know the outcome. If the pregnancy test was positive, confirmation of pregnancy was done by ultrasonography after two weeks. Clinical pregnancy was defined as the presence of gestational sac with or without a foetal pole in ultrasonography. Ongoing pregnancy was defined as the persistence of foetal cardiac activity beyond 10 completed weeks of gestation. Live birth was defined as delivery of live foetus beyond 20 completed weeks of gestation.

STATISTICAL ANALYSIS

SPSS, Inc version 20.0 (IBM, USA) was used to check the normal distribution of age, endometrial thickness and Total Motile Fraction (TMF) in the groups by Shapiro-Wilk test. Both the groups were compared for major confounding variables like female age, indications for IUI, type of IUI, ovarian stimulation protocol, number of dominant follicles, type of ovulation trigger used and TMF. Since the dataset did not follow the normal distribution, the Mann-Whitney U test was applied to compare age, endometrial thickness and TMF between the groups. Chi-square test and Fisher's-exact test were applied for categorical data to find out the difference in the indication of IUI, protocols of ovarian stimulation, type of IUI, number of dominant follicles, type of ovulation trigger used as well as the pregnancy outcomes among the groups. Pregnancy rate and live birth rate were considered as the primary outcomes. Clinical pregnancy rate, ongoing pregnancy rate, miscarriage rate and multiple pregnancy rates were analysed as secondary outcomes. The p-value ≤0.05 was considered statistically significant.

RESULTS

Three hundred eighty eight IUI cycles were analysed including 102 AID cycles. Ovulation was documented in 201 cycles (51.8%) and represented as group A. Group B included 187 cycles (48.2%) which did not have evidence of ovulation.

Group A and B were comparable for all the parameters such as female age (p-value 0.451), indication (p-value 0.746), ovarian stimulation protocol (p-value 0.115), types of IUI (p-value 0.789) [Table/Fig-1]. IUI cycle characteristics like ovulation trigger (p-value 0.527), number of dominant follicles (p-value 0.300), endometrial thickness (p-value 0.228) and TMF (p-value 0.747) were also similar [Table/Fig-2].

Parameters	Group A (n=201)	Group B (n=187)	p-value
Age in years (Mean±SD)	28.7±4.1	28.9±3.9	0.451*
Indication (n, %)			
Oligo anovulation	17 (8.5%)	10 (5.3%)	
Unexplained	65 (32.3%)	62 (33.2%)	0.746†
Endometriosis	1 (0.5%)	1 (0.5%)	
Male factor	109 (54.2%)	108 (57.8%)	
Combined	9 (4.5%)	6 (3.2%)	
IUI			
AIH	147 (73.1%)	139 (74.3%)	0.789‡
AID	54 (26.9%)	48 (25.7%)	
Ovarian stimulation protocol			
CC+hMG	57 (28.4%)	67 (35.8%)	0.115‡
Letrozole+hMG	144 (71.6%)	120 (64.2%)	

[Table/Fig-1]: Baseline characteristics of the patients.
*Mann-Whitney U test; †Fisher's-exact test; ‡Chi square test; p-value was significant at ≤0.05; IUI: Intrauterine insemination; AIH: Artificial insemination with husband's sperm; AID: Artificial insemination with donor sperm; CC: Clomiphene citrate; hMG: Human menopausal gonadotropin

Parameters	Group A (n=201)	Group B (n=187)	p-value
No. of dominant follicles			
1	130 (64.7%)	133 (71.1%)	0.300*
2	67 (33.3%)	49 (26.2%)	
3	4 (2.0%)	5 (2.7%)	
Trigger			
hCG	199 (99.0%)	186 (99.5%)	0.527*
Leuprolide	2 (1.0%)	1 (0.5%)	
Endometrial thickness (Mean±SD) (mm)	7.9±1.7	7.7±1.8	0.228†
TMF (Mean±SD) (million)	7.07±4.2	6.9±4.3	0.747†

[Table/Fig-2]: IUI cycle characteristics of the patients.
*Fisher's-exact test; †Mann-Whitney U test; hCG: Human chorionic gonadotropin; TMF: Total motile fraction

There was no difference in pregnancy rates (17.9% vs 18.2% p-value=0.945) in presence or absence of ovulation documented before insemination. Clinical pregnancy rates (p-value=0.945), ongoing pregnancy rates (p-value=0.625) and live birth rates (p-value=0.625) were also similar. There were 3 miscarriages and one ectopic pregnancy in group B and none in group A. Group A had one twin pregnancy.

Outcome (n, %)	Group A (n= 201)	Group B (n=187)	p-value
Pregnancy positive	36 (17.9%)	34 (18.2%)	0.945*
Clinical pregnancy	36 (17.9%)	34 (18.2%)	0.945*
Ongoing pregnancy	36 (17.9%)	30 (16.0%)	0.625*
Live births	36 (17.9%)	30 (16.0%)	0.625*
Multiple pregnancies	1 (2.8%)	0	
Miscarriages	0	3 (1.6%)	

[Table/Fig-3]: Comparison of IUI cycle Outcomes between the groups.
*Chi-square test

DISCUSSION

In the present study, insemination was performed at 38-40 hours after the ovulation trigger. Though early literature suggested 32-38 hours post-hCG is the best time for insemination [8], later AboulGheit S, reported similar pregnancy rate following inseminations performed

at 24 hours, 34 hours and 48 hours after hCG trigger in couples with unexplained infertility [9]. Several other studies also reported that insemination timing of short or long intervals after ovulation trigger has no impact on pregnancy rate [10-13]. A meta-analysis of two Randomised Controlled Trials (RCTs) revealed a similar pregnancy rate when IUI is performed at 34-36 hours and 48 hours after ovulation trigger [14].

The study groups were similar for major baseline characteristics which are likely to affect the cycle outcome. There is no difference in pregnancy rate in presence or absence of ovulation documented before insemination. It differs from the prospective study by Kucuk T, with 578 IUI cycles which reported significantly higher pregnancy rate in presence of ovulation documented after insemination (23.5% vs 8.8%, p-value <0.001) [6]. But, in that study, insemination was done at 36-38 hours after the hCG trigger and evidence of ovulation was confirmed after insemination. The current study also contradicts the findings of the prospective cohort study with 1146 first stimulated IUI cycles, where pregnancy rate was significantly higher in presence of ovulation documented during insemination (11.7% vs 6.7%, p-value=0.015) [5]. In the above mentioned study, insemination was scheduled at 34-38 hours after ovulation trigger. In the present study, though ultrasonography to document ovulation was performed at 36-38 hours after ovulation trigger, insemination was performed 2 hours after ultrasonography at 38-40 hours after trigger. Similar pregnancy rate in the present study irrespective of ovulation status may be due to comparatively delayed insemination at 38-40 hours which might have increased the chances of ovulation before IUI. Authors thought to document ovulation before semen collection or thawing donor semen samples in AID cycles so that a difference in pregnancy rate would have prompted the option of delaying semen collection and insemination without affecting the sperm parameters due to long interval waiting after sperm preparation.

The overall pregnancy rate in the present study is 18.0% which is within the reported range of 8-22% in existing literatures [15-17]. The present study claims to be one of the few studies reporting live birth rate (17.0%) after IUI. Erdem A et al., reported a per cycle live birth rate of 11.4% in couples with unexplained infertility and mild male factor [18]. Another prospective study documented a live birth rate of 9.2% in unexplained infertility after IUI [19]. Higher live birth rate in the current study may be due to the fact that it included IUI cycles for all indications and AID cycles as well. The present study reported one twin pregnancy which is 1.4% (1/70) of all clinical pregnancies. The reason for the low multiple pregnancy rates are due to the strict cycle cancellation policy in case of more than 3 dominant follicles.

Limitation(s)

Being a retrospective study, it has its inherent drawbacks. The decision for ultrasonography before IUI was arbitrary. In spite of the above-mentioned limitations, the study may be useful to determine the effectiveness of routine transvaginal ultrasonography to document ovulation in IUI cycles.

CONCLUSION(S)

Intrauterine Insemination (IUI) cycle outcomes remain unaffected by the ovulation status documented before insemination. Hence, ovulation monitoring should not be routinely performed before insemination to improve pregnancy rate in IUI cycles. This approach will save time as well as human resource and avoid inconvenience to the patients due to repeated transvaginal ultrasonography. A well designed Randomised Controlled Trial is suggested in future to verify the results.

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