Original Article

The Effect of the Time Interval From Sperm Processing to Intrauterine Insemination on the Pregnancy Outcomes of Infertile Women

Zahra Rezaei; M.D., Elham Feizabad; M.D., Mehrnaz Valadan; M.D., Saeedeh Ebadizare; M.D.

Department of Obstetrics and Gynecology, Yas Hospital, Tehran University of Medical Sciences, Tehran, Iran

Received February 2022; Revised and accepted July 2022

Abstract

Objective: Intrauterine insemination (IUI) is the first-line treatment in couples suffering from various causes of subfertility and infertility. Considering the relatively low rate of pregnancy achieved with each cycle in this method, optimizing various steps in the process including the time interval from sperm collection to IUI may result in an increased rate of success. The goal of this study was to assess the impact of time intervals from the end of sperm processing to IUI (SP-IUI) on the pregnancy rate in IUI.

Materials and methods: This single-center prospective cohort study evaluated couples with normal male partner sperm analysis and idiopathic female infertility undergoing IUI from 2018 to 2021. Cycles were stimulated using subcutaneous recombinant FSH and oral Letrozole. Ovulation was triggered using GnRH antagonist when the leading follicle's size reached greater than 14mm. The participants were placed in one of the three groups based on SP-IUI: group 1 (0–60 min), group II (60–90 min), and Group III: (>90 min).

Results: 269 couples were included in the study. Sperm processing expectedly resulted in an increased concentration of total sperm count and sperm motility (P<0.001). The rate of chemical or clinical pregnancy, abortion, IUFD, multigestation, pregnancy, term birth, and ectopic pregnancy was not significantly different across study groups (P>0.05).

Conclusion: The results of this study suggest that SP-IUI intervals evaluated in this study do not vary in terms of pregnancy rate or adverse pregnancy outcomes in IUI with normal male partner semen analysis. Hence, infertile couples can be flexible in the collection of semen specimens without time and site (at home or hospital) limitations.

Keywords: Intrauterine Insemination; Sperm Collection; Timing; Idiopathic Infertility; Subfertility

Introduction

Intrauterine insemination (IUI) is the process of transcervical insertion of processed sperm specimens in the uterine cavity at the time of ovulation for the treatment of infertility and subfertility (1, 2). As the least expensive and invasive method of assisted conception, IUI is widely used as the first-line treatment of couples with not only identified causes such as ovulation dysfunction and cervical infertility but also in cases with idiopathic subfertility (3, 4).

Correspondence:
Dr. Saeedeh Ebadizare
Email: ebadisaeedeh5@gmail.com

The data of the European in vitro fertilization (IVF) Monitoring (EIM) Consortium on the use of Assisted reproductive technologies (ART) reports that the use of IUI has led to over 58000 deliveries between 2006-2011, signifying the indisputable role of IUI in the treatment of infertility (5).

Considering the relatively low rate of success compared to alternative methods of ART and the stable rate of IUI success over the years there is a need for optimization of the methods of IUI to achieve higher rates of pregnancy. Several factors influence the outcome of IUI including advancing maternal and paternal age, maternal body mass index (BMI), the total number of motile sperms, endometrial thickness, number of preovulatory follicles, method of ovulation induction, and concomitant use of controlled ovarian hyperstimulation (6-8). Controlled ovarian hyperstimulation in adjunction with IUI has been demonstrated to further improve the marginal but considerable improvement in the achievement of live birth compared to IUI with natural female cycles, with suspected pregnancy rates varying between 8% and 22% (9).

Another factor by which the success of IUI may be affected is the timing intervals from sperm collection to its delivery to the laboratory and from the end of sperm processing to the insemination process. It is recommended that sperm processing and the ensuing insemination process to be performed as soon as possible to reduce the adverse effects of reactive oxygen species produced in the ejaculate on the spermatozoa (10, 11). In this regard, previous studies have demonstrated that delaying semen processing for more than one hour since sperm collection does not allow the achievement of pregnancy (12).

Timing of the insemination process relative to sperm collection and processing is also crucial because the limited life span of spermatozoa (13), time required for sperm chromatin decondensation (14, 15), and sperm motility reduction following sperm washing (16) have been surmised to result in suboptimal cycle fecundity in excessively short or long intervals.

Although the impact of time intervals of the total time of sperm collection to IUI and sperm collection to sperm processing on the pregnancy rate has been examined in previous studies, there have been limited reports on the effect of varying durations of end of sperm processing to IUI interval (SP-IUI) on the pregnancy rate and adverse pregnancy outcomes. As such, the aim of this study is to evaluate the impact of SP-IUI interval on the pregnancy rate and adverse maternal pregnancy outcomes in participants undergoing IUI with controlled ovarian stimulation in the couple in a single-center setting.

Materials and methods

This prospective observational study was conducted on 269 infertile women, who were candidates for IUI in our infertility department, affiliated with Tehran University of Medical Sciences, Yas hospital, from September 2018 to 2021.

Inclusion criteria included women with idiopathic infertility, aged 18 to 42 years, with normal serum hormonal profile, sufficient ovarian reserve, normal hysterosalpingography, and normal male partner sperm analysis (sperm total counts ≥ 5 million/ml and normal morphology sperm count ≥4%). Participants with the polycystic ovarian disease, underlying diseases, severe endometriosis (multifocal, superficial and invasive, firmly and dense, involving the fallopian tubes, ovaries, and cul-de-sac), sperm donor, as well as those withdrawing to participate from the study at any point were excluded from the study.

The study was done in compliance with the Helsinki Declaration and approved by the Tehran University of Medical Sciences Ethics committee (IR.TUMS.MEDICINE.REC.1398.357). The participants signed the informed consent before enrolling.

Male partners were requested to provide a semen sample by masturbation into a sterile container after sexual abstinence for at least 3 days. The semen sample was allowed to liquefy for 20–30 min at room temperature. The semen was subsequently suspended with modified Human Tubal Fluid (mHTF, Irvine Scientific) medium containing 10% serum substitute supplement (Irvine Scientific) centrifuged twice at 1750 rpm for 10 min. The supernatant was discarded and the pellet was suspended in mHTF and incubated at room temperature until insemination. Following sperm washing, a 10 µl semen drop was placed on the Makler Counting Chamber for a secondary analysis beyond the initial pre-washing instance. The number of spermatozoa counted in any strip of 10 squares of the grid was used to obtain sperm concentration (10^6/ml). Sperm analysis and motility grading were performed using WHO 2010 guidelines (17). The time to IUI was recorded until the start of insemination procedure. IUI was performed using Insemi-Cath (Cook Medical). The time interval from

the completion of this stage to the actual intrauterine injection, during which the prepared samples were maintained at room temperature, was recorded.

All women were evaluated on the third day of the menstruation cycle with transvaginal ultrasound (TVS) (4.5-7 MHz vaginal probe, Sono line G-40, Siemens, Germany) for measuring endometrial lining and performing antral follicle count (AFC). On day 3 of the cycle, ovarian stimulation was achieved using recombinant human follicle stimulating hormone (rhFSH) (Gonal-*f*; MerckSerono, Modugno, Italy) 225 IU subcutaneously (SC) and oral Letrozole (Letrofem; Iran hormone, Tehran, Iran) 2.5 mg /day for five consecutive days. Follicular maturation was assessed via serial ultrasound examinations. When the follicle(s) reached ≥14 mm in average diameter, 250mg daily subcutaneous GnRH antagonist, cetrorelix, was administered (CetrotideP P, Serono International, Geneva, Switzerland) until the day of triggering of ovulation. The washed sperm was injected into the woman's uterus 36 hours following ovulation. Women received 200mg progesterone vaginal suppository once a day after the insemination.

The following data were recorded for study patients: age, marriage and infertility duration, subtype of infertility, BMI, and baseline hormone profile (thyroid stimulation hormone (TSH), follicle-stimulating hormone (FSH), luteinizing hormone (LH), prolactin, anti mullerian hormone (AMH)), endometrial thickness (ET), follicle number and size. The participants were placed in one of the three groups based on time interval to insemination: group 1 (0–60 min), group II (60–90 min), and group III: (>90 min).

The study outcomes were chemical pregnancy (assessed in two weeks following fertilization with positive laboratory beta-hCG test), and clinical pregnancy (assessed in six weeks after fertilization with gestational sac visualization via transvaginal ultrasound).

All the statistical analyses were done using Statistical Package for the Social Sciences (SPSS) version 24.0. P-value< 0.05 was considered statistically significant. Differences in baseline characteristics and outcomes between the study groups were analyzed with Analysis of Variance (ANOVA) and Turkey post-hoc multiple comparisons test as appropriate.

Results

Two hundred sixty-nine female patients were included. The mean age of female participants was 30.44±5.13 years (range, 18–42), and the mean age of the male partners was 34.15±5.57 years. The primary infertility duration was reported in 86.2% of participants. The mean BMI was 25.39±3.75 kg/m^2. No significant differences were observed in the demographic information of the three study groups (Table 1).

The total sperm count decreased significantly (P-value<0.001), and the sperm motility increased significantly (P-value<0.001) after sperm washing, while the proportion of sperm with normal morphology did not change significantly (Table 2). The chemical pregnancy rate was 14.13%, and the clinical pregnancy rate was 13.01%. Abortion was found in 11 (4.09%), ectopic pregnancy in 2 (0.75%), and IUFD in 3 (1.12%) of the participants, finally, the live birth rate was 7.1%.

Table 1: The demographic information of the three study groups

Variables	Time intervals from the end of sperm processing to intrauterine insemination			P-value
	less than 60 min	60 to 90 min	more than 90 min	
Age (year±SD)	30.70±5.05	29.78±5.18	30.83±5.13	0.331
BMI (kg/m^2)	24.83±3.4	26.22±4.1	25.12±3.61	0.033
Partner age (year)	33.59±5.28	34.13±5.26	34.70±6.12	0.412
Marriage duration (year)	5.89±3.41	6.1±3.23	5.72±3.39	0.753
Primary infertility duration (year)	2.71±2.50	2.94±2.64	2.78±2.57	0.823
TSH (mIU/ml)	2.40±1.31	2.65±1.29	2.60±1.19	0.386
FSH (IU/ml)	6.16±2.23	6.69±2.49	6.41±2.44	0.332
LH (IU/ml)	6.55±3.43	7.38±4.76	7.21±5.76	0.469
Prolactin (ng/mL)	25.69±13.31	20.38±10.09	23.35±11.24	0.018
AMH (ng/mL)	4.50±3.00	5.01±3.87	4.91±3.83	0.610
Endometrial thickness (mm)	6.73±1.52	6.82±1.96	6.38±1.38	0.163
Follicle number	1.73±0.86	1.77±0.83	1.87±1.00	0.563
Follicle size (mm)	18.12±1.99	17.74±1.90	18.39±2.38	0.116

Table 2: The sperm parameter changing after sperm washing

Variables	Before sperm washing	After sperm washing	P-value
Total sperm count (10^6/ml)	42.11±26.07	29.57±22.56	<0.001
Sperm motility (%)	51.28±21.47	91.61±19.28	<0.001
Sperm with normal morphology	87.04±6.96	87.19±6.93	0.391

Age was not associated with a lower rate of pregnancy in the infertile women (P =0.929) or their partners (P =0.622), whereas marriage duration (P =0.003) and infertility duration more than six years (P =0.040) significantly reduced the pregnancy rate. No significant differences were observed in the study outcomes between the study groups (Table 3).

Discussion

The objective of this study was to investigate the effect of SP-IUI interval length on the pregnancy rate of IUI with controlled ovarian hyperstimulation. Our results demonstrated that the three intervals (<60 min, 60-90 min, >90 min) studied in this investigation did not vary in terms of the study outcomes including chemical and clinical pregnancy as well as live births. Furthermore, pregnancy loss (abortion, IUFD), multigestation, and ectopic pregnancy rate in each interval were not statistically different across the groups. The results of our study may suggest that delaying the insemination process up to at least 90 minutes following sperm processing is not associated with diminished pregnancy rates or adverse pregnancy outcomes with IUI. Clinical pregnancy rates with IUI coupled with ovarian stimulation in the results of each group were similar to the expected rate reported by other studies (9, 16). However, the similar rate of outcomes between the studied groups is a point of interest in this study, considering the controversial reports of the impact of SP-IUI interval duration on IUI outcomes in the literature.

The results of the limited number of previous studies regarding the impact of SP-IUI interval on the success of IUI have been conflicting. In a multi-center study conducted by Fauque et al., the 40-80 minutes SP-IUI interval was associated with higher rates of achieving pregnancy, with values outside this range significantly reducing the probability of clinical pregnancy (15). Furthermore, SP-IUI intervals before 30 minutes and between 31-60 minutes resulted in higher rates of success in IUI primed with human menopausal gonadotropin (12). Pregnancy rates have been also statistically higher with shorter SP-IUI intervals in another study where sperm collection was done close to the laboratory, resulting in a 4% pregnancy rate when SP-IUI was >60 min compared to 16% with SP-IUI interval was ≤60 min (16). On the other hand, a recent study did not detect differences in pregnancy rates per cycle of treatment with varying time intervals of SP-IUI. Pregnancy rate in this study was not diminished with SP-IUI intervals extending up to 3 hours (18). A retrospective study conducted by Song et al. on the impact of total time from sperm collection to insemination process also did not reveal significant differences in ongoing pregnancy rates of IUI using sperm specimens collected at the clinic with short interval time (7.3%) and those collected at home with a relatively long time interval (10.6%) (2). Other retrospective studies with considerable sample sizes have also failed to demonstrate significant negative effects of delayed insemination up to 24 hours after sperm processing (19).

Although the results of this study were compatible with the latter group of reports in which the length of SP-IUI interval was not associated with IUI success, the source of heterogeneity between the studies should be pursued in future studies.

Table 3: The pregnancy outcome comparison in the study groups

Variables	Time intervals from the end of sperm processing to intrauterine insemination			P-value
	less than 60 min	60 to 90 min	more than 90 min	
Chemical pregnancy	16	12	10	0.372
Clinical pregnancy	13	12	10	0.749
Abortion	5	3	3	0.655
IUFD	1	1	1	-
Multigestation pregnancy	2	1	0	0.238
Term birth	6	7	6	0.942
Ectopic pregnancy	1	1	0	0.434

It could be surmised that the variations in results are due to differences in outcome definition (outcome measures of clinical pregnancy; pregnancy confirmation via imaging or laboratory beta-hCG testing), ovarian stimulation protocol, and infertility cause and duration in participants. In line with this, previous studies have demonstrated variable results when comparing the impact of timings with IUI performed in adjunct with Clomiphene citrate and Human menopausal gonadotropin (12).

The single-center nature of this investigation and the restricted number of specialists in this study allowed for consistent procedures in each stage of the process and limited the potential confounding elements such as variations in methods of sperm collection and processing associated with laboratory and the insemination process itself compared to similar procedures in other studies with multi-center data. It is important in this regard to further investigate the impact of sperm collection on IUI timings in respect to the various methods employed for ovarian stimulation as described above. Future studies would benefit from addressing the limitations of this study by adjusting baseline characteristics of the participants and sperm collection to processing intervals in a large-scale randomized study.

Conclusion

The results of this study suggest that SP-IUI intervals evaluated in this study do not vary in terms of pregnancy rate or adverse pregnancy outcomes in IUI with normal male partner semen analysis. Additional randomized studies may be needed to confirm these findings.

Conflict of Interests

Authors have no conflict of interests.

Acknowledgments

This study was extracted from the gynecology resident's thesis of Dr. Saeedeh Ebadizare under the supervision of Dr. Zahra Rezaei.

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Citation: Rezaei Z, Feizabad E, Valadan M, Ebadizare S. **The Effect of the Time Interval From Sperm Processing to Intrauterine Insemination on the Pregnancy Outcomes of Infertile Women.** J Family Reprod Health 2022; 16(3): 199-204.