Original Article

Short Ejaculatory Abstinence in Normozoospermic Men is Associated with Higher Clinical Pregnancy Rates in Sub-fertile Couples Undergoing Intra-Cytoplasmic Sperm Injection in Assisted Reproductive Technology: A Retrospective Analysis of 1691 Cycles

Sweta Gupta, Vikram J Singh, Ashish Fauzdar, Kamta Prasad, Ajay Srivastava, Kamlesh Sharma

Reproductive Medicine and IVF, Medicover Healthcare Private Limited, New Delhi, India

Abstract

Background: The current WHO abstinence recommendations are ideal only for clinical diagnosis, as in recent years a negative correlation of abstinence duration with good embryo development and clinical pregnancy rate has been seen. **Aim:** The aim of the study was to evaluate the impact of variation in abstinence period on fertilization, embryo development potential, pregnancy, and miscarriage rate in sub-fertile couples undergoing assisted reproductive technology (ART) treatment. **Setting and Design:** A prospective analysis was conducted at a tertiary (level 3) infertility care clinic. **Materials and Methods:** The study included analysis of 1691 cycles for the patient undergoing ART procedures between September 2017 and August 2019. The influence of ejaculatory abstinence (EA) was investigated based on variation in abstinence length with four groups: Group I – 1 day; Group II – 2–5 days; Group III – 6–7 days; and Group IV – EA length of ≥8 days. **Statistical Analysis:** Analysis of variance and Chi-square test were used to calculate *P* value. **Results:** In our primary outcome, we have seen a strong positive correlation of abstinence duration with semen volume, total sperm count, total motile count, and difference between each group was significant. Secondary outcomes showed a significantly higher implantation rate, biochemical pregnancy rate was observed in Group I (1 day) per embryo transfer as compared to longer abstinence groups**.** This resulted in significantly higher clinical pregnancy rates in Group I 30.0% vs. 25.4% in comparison to longer abstinence groups. **Conclusions:** Our study has shown duration of abstinence is negatively correlated with positive β-human chorionic gonadotropin rate, clinical pregnancy rate, and implantation rate. Lower miscarriage rate was also observed with shorter abstinence duration.

Keywords: *Assisted reproductive technology, ejaculatory abstinence, intra-cytoplasmic sperm injection, male infertility, semen parameters*

Introduction

Semen analysis illustrates the evaluation of several characteristics of an ejaculate with an objective of estimating the reproductive probability of an individual.[1-3] Male factor as per the World Health Organization (WHO) standard accounts for 40% of infertility cases. Semen analysis is still considered an important investigation for the evaluation of sub-fertile

Received: 16-12-2020 **Revised:** 18-07-2021
Accepted: 28-07-2021 **Published:** 28-09-2021

Access this article online

Quick Response Code:

Website: www.jhrsonline.org

DOI: 10.4103/jhrs.jhrs_235_20

Address for correspondence: Dr. Sweta Gupta, Medicover Healthcare Private Limited, E-20, Panchsheel Park, New Delhi - 110 017, India.
E-mail: sweta.gupta@medicoverfertility.com

How to cite this article: Gupta S, Singh VJ, Fauzdar A, Prasad K, Srivastava A, Sharma K. Short ejaculatory abstinence in normozoospermic men is associated with higher clinical pregnancy rates in sub-fertile couples undergoing intra-cytoplasmic sperm injection in assisted reproductive technology: A retrospective analysis of 1691 cycles. J Hum Reprod Sci 2021;14:273-80.

couples before undergoing assisted reproductive technologies (ARTs) for infertility treatment. There are many factors that affect semen parameters; one of the factor is abstinence time or the time between ejaculatory events. The standards for testing and analyzing, semen samples should be collected with a minimum of 2 days and a maximum of 7 days of sexual abstinence.[4] Several studies have evaluated the influence of short and long abstinence period on sperm parameters comprising sperm concentration, progressive motility, and morphology.[5-9] Recent studies have occasionally demonstrated improvement of semen parameters, embryo development, and the clinical pregnancy rates with different abstinence intervals.[7,8] While seminal volume may decline with more frequent ejaculatory events, total sperm count, motility, morphology, and vitality may not be significantly affected.[9] There are also contradictory and inconclusive findings of abstinence duration on motility, morphology, and DNA fragmentation rates.[10] Recent studies showed that pregnancy outcomes are clearly impacted by numerous factors including shorter abstinence times and it appeared to be associated with improvements in pregnancy rates following treatment intervention with various ARTs.[11-13]

In current clinical practice as a therapeutic treatment option for infertile couples availing ART procedure including *in vitro* fertilization (IVF) or intra-cytoplasmic sperm injection (ICSI) an abstinence period is advised as per the current recommendations for diagnostic semen analysis only. Although semen analysis remains a gold standard method for evaluating male factor in determining infertile couple, still no semen parameters have been observed to show an ability of predicting fertilization potential of spermatozoa, embryo quality formation, implantation rate ultimately leading to better pregnancy outcomes, and live birth rate**.** Thus, in this study, we wanted to evaluate the impact of various abstinence durations on semen parameters and its role in influencing fertilization potential in ICSI cases, with formation of top-quality embryos, resulting positive pregnancy rate.

Materials and Methods

Methodology of the study

A prospective analysis was conducted at a tertiary (level 3) infertility care clinic undergoing ICSI treatment between, September 2017 and August 2019. The study was approved by the Institutional Ethics Committee (MCRM/01/2017). Written consents were taken from all the couples and ethical standards were adhered as specified by the Helsinki Declaration (2013). During the study period, a total of 2104 ART cycles were analyzed. In the final analysis, a total of 137 cycles were excluded from the analysis-cycles where all oocytes were frozen (n = 18), no eggs/mature oocytes retrieved on day of ovum pick-up (n = 21), frozen donor semen sample (n = 33), cycles where sperms were retrieved surgically (n = 22), and cycles of physiological ICSI technique (n = 5) and 38 cycles with incomplete data for various data variables. The final study included analysis of 1691 cycles for the patient undergoing ICSI. This included 674 donor oocyte cycles. Sample size calculation was not performed; all the eligible patients during the study period were included to maximise number of patients [Flow Chart 1].

Inclusion criteria

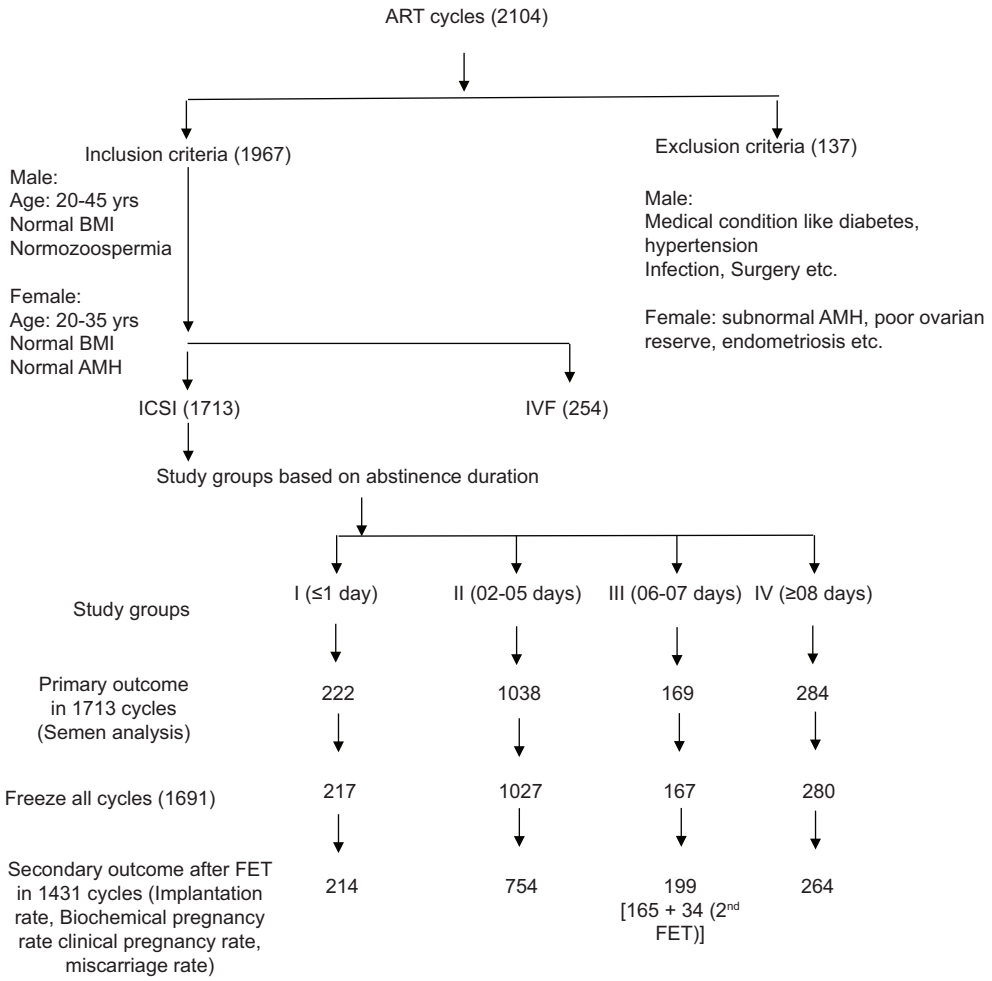
The inclusion criteria followed for this study comprise male partners of the sub-fertile couple with age between 20 and 45 years with normal body mass index (18.5–24.9) with history of either primary or secondary infertility that were able to give semen sample on the day of pick-up through masturbation. The age span included for this study was ranged from 27 to 38 years. The concerned females were tested for a basic fertility work up with normal ovarian reserve (AMH in normal range 2-6.8ng/ml).

Exclusion criteria

As per the exclusion criteria, study excluded male partners with medical disease such as diabetes mellitus, hypertension, mumps, tuberculosis, STD's hydrocele, varicocele, undescended testis, inguinal hernia repair or surgery for hypospadias, congenital absence of vas deferens or genetic and/or chromosomal abnormalities. Females with abnormal characteristics such as poor ovarian reserve, endometriosis, and recurrent implantation failure were excluded from the study.

Ovarian stimulation protocol

All the female partners of the infertile couple planned for ART cycles had undergone controlled ovarian hyperstimulation (COH) through standard gonadotropin-releasing hormone (GnRH) antagonist protocol from their 2nd day of the cycles. For COH was achieved through recombinant follicle stimulating hormone (r-hFSH, Foligraf, 75 IU/150 IU, Bharat Serums and Vaccines Ltd, India) or (r-hFSH, GONAL-$f^{®}$ 150 IU, Merck, India) along with GnRH antagonist, cetrorelix acetate (Cetrotide® 0.25 mg, Merck Serono Ltd, Europe). When at least three or more dominant follicle ≥16–18 mm developed, ovulation was triggered 36 h before oocyte retrieval. Ovulation was triggered with 5000 IU Intramuscular human chorionic gonadotrophin (HUCOG, Bharat Serums Ltd, Ambernath) or Ovitrelle 0.25 microgram (Choriogonadotrophin alfa-r-DNA human chorionic gonadotrophin, Merck



Flow Chart 1: Showing study selection process

Serono Ltd, Europe) or GnRH agonist trigger 0.5 mg buserelin s. c.(Suprefact; Hoechst, Denmark).

Semen collection and processing

The husband semen sample was obtained through masturbation after taking proper history and consent form. The data of abstinence period were collected from filled questionnaire submitted by the male partner at the time of semen sample submission on the day of ovum pickup in the treatment cycle. Advice on duration of abstinence was given but not routinely followed by all patients. Semen analysis and preparation was done within 1 h of collection of semen. Semen sample was washed to free from seminal fluid through density gradient method (Pure Sperm density gradient, SAR Healthline Pvt. Ltd, India) as per the manufactures instructions.[14] In order to study the influence of ejaculatory abstinence (EA) length on semen quality and pregnancy outcomes, four study groups were investigated based on variation in abstinence length (Group I – 1 day; Group II – 2–5 days; Group III – 6–7 days; and Group IV, EA length of ≥8 days). However, aim of forming Group IV was to compare pregnancy rate with Group I and Group IV.

Method for fertilization and embryo development

All oocytes were subjected to intracytoplasmic injection with husband semen sample in this study, as per institutional policy. Fertilization for ICSI cases was assessed after 18 h postinsemination based on the presence of two juxtapose pronucleus (PN) and second polar body appearance.[15] All fertilized oocytes were cultured in individual droplet culture method by using Continuous Single Culture-NX Low-lactate Culture Media for IVF (FUJIFILM Irvine Scientific, USA). All the information from the patient treatment cycle both clinical and embryological parameters including age, follicles obtained, mature oocyte, and grade of embryos were captured from record sheets obtained from medical record department. On day 2 and day 3, embryo assessment and grading were performed based on Istanbul consensus.[15] Grade A embryo is <10% fragmentation, stage specific cell number and no evidence of multinucleation. Grade B is <10%–25%

fragmentation, stage specific cell size for almost all the blastomeres and no evidence for multinucleation. Grade C is >25% fragmentation, cell size is not stage specific and there is evidence for multinucleation.

Embryo cryopreservation and thawing

Day 3 embryos were cryopreserved and only frozen embryo transfer (FET) cycles were included in this study. Vitrification and thawing procedure were performed using the Kitazato Vitrification Kit (Kitazato Co., Fujicity, Shizuoka, Japan). Embryo thawing was done 3–4 h prior to FET.

Endometrial preparation and frozen embryo transfer

For endometrial preparation baseline scan and LH/E2 hormonal assessment was done on Day2/3 of periods, 2 mg oral oestradiol valerate (Progynova, Zydus Cadila Healthcare Ltd, German Remedies); thrice daily was used. After 12 days of endometrial preparation, if endometrial thickness was above 8 mm with triple line, progesterone was supplemented. Luteal phase support was provided by natural micronized progesterone (400 mg) administrated vaginally starting on the night of presumed ovum pickup and continued until the pregnancy test. Vitrified warmed day 3 embryos were transferred into uterine cavity by using a 17.3 cm soft embryo transfer catheter set (COOK K-JETS-7029-SIVF) for transvaginal embryo transfer under ultrasound guidance. If pregnancy occurred, administration continued until the 12th week of gestation. In case of no pregnancy, more attempts were made to achieve pregnancy and success rate was calculated per attempt.

Definitions

1. Primary outcome: In primary outcome, we have compared various semen parameters such as semen volume, sperm concentration, total sperm count, total motile count
2. Secondary outcome: This part of the study mainly includes post FET events such as implantation rate, biochemical pregnancy rate, clinical pregnancy rate, miscarriage rate
3. Fertilization rate: The number of fertilized oocytes (2PN) by the total number of inseminated oocytes (IVF) or mature oocytes (MII) injected
4. Cleavage rate: Total number of embryos cleaved of the total number of fertilized embryos after insemination or injection with sperm
5. Top-quality embryo: Total number of Grade A embryos formed on day 3 of the total cleaved embryos
6. Embryo utilisation rate: The number of embryos utilized (cryopreserved and embryo transfer) per number of 2PN zygotes in the same cycle
7. Positive β-human chorionic gonadotropin (β-hCG) rate: When level of serum β-hCG is ≥50 mIU/ml on day 12 after a day 3 embryo transfer is considered as positive and total number of positive β-hCG divided by number of FET
8. Clinical pregnancy rate: Evidence of a gestational sac as seen on ultrasound divided by the number of FET
9. Implantation rate: The number of sacs seen on ultrasound divided by the number of embryos transferred.

The clinical pregnancy rate and other performance indicators such as fertilization rate, cleavage rate, and embryo development rate were calculated based on Vienna consensus.[16]

Data analysis

In the statistical analysis, categorical variables were presented in number and percentage (%) and continuous variables were presented as mean ± standard deviation quantitative variables were compared using the Analysis of Variance and qualitative variables were compared using the Chi-square test. Univariate and multivariate logistic and linear regression was used to find out the effect of groups on outcome. $P < 0.05$ was considered statistically significant. The data were entered in MS EXCEL spreadsheet and analysis was done using SPSS Statistics (IBM Corp, Released 2012, IBM SPSS Statistics for Windows, Version 21.0, Armonk, NY: IBM Corp).

Results

A total of 1713 couples seeking for fertility treatment were included in this study. The summary of all ART intervention done in the study population is illustrated in Table 1. Total 19,592 COC were harvested from female and 15,864 mature oocytes (MII) were used for the used for the study. Only ICSI cycles were chosen for this study, and total fertilization rate observed across the study was 71.95% with cleavage rate of 99.2%.

The comparison of major semen parameters was done across the study groups of sub-fertile males undergoing ART treatment Table 2. The highest number of males based on the abstinence days was found allocated in Group II 60.5% (1038) followed by 16.5% (284) in Group IV, 12.9% (222) in Group I, and in Group III 9.8% (169) There was significant difference in semen volume (P ‹ 0.005) among all the study groups. The average sperm concentration was 54.5 × 10^6/mL and there was no significant difference between the groups (P = 0.113). The total sperm concentration 111.8 × 10^6 ($P < 0.005$) per ejaculate was

highest in Group IV with maximum abstinence period and significantly different from other groups. Total motile sperm concentration 52.1 ± 59.5 (P = 0.172) was the highest in Group II. It was further noted that in the total motility, progressive motile sperms, and morphology, there was no significant difference among the groups [Table 2].

As a part of treatment intervention through various ART technologies, there was a significant difference in mean number of oocytes harvested (P = 0.024) in each group and oocytes that were injected with husband sperm. The secondary outcome fertilization rate and cleavage rate did not have significant difference among all the groups. The secondary outcome included embryo developmental potential and development of top-quality embryos as a key performance indicator for cleavage stage embryos. The development of good quality embryos (34.2% vs. 25.93%, P < 0.0001) was proportionately higher in Group IV. Grade A embryo formation was the highest in Group IV (26.60%); however, difference was not significant when compared with Group II (19.87% vs 26.60%). The embryo utilisation rate was the highest in Group IV with no significant difference among groups [Table 3].

The primary treatment outcomes of the study were analyzed with total 1431 FET done to avoid effect of stimulation only frozen (FET) cycles were considered for this study. The highest number 52.6% (754) of FET was done in Group II with 53.2% (1761) top quality embryos. On the other hand, a higher implantation rate of 14.6% (75/511) Group I versus 12.8% (226/1761) in Group II (P = 0.008) with positive β-hCG rate of 55.6% (119/214) in Group I versus 39.1% (295/754) IN Group II (P < 0.0001). This resulted into higher clinical pregnancy rates in Group I 30.0% (64/214) vs. 25.4% (192/754) P = 0.009 as compared to other groups. The miscarriage rate of 2.6% (70/264) P = 0.388 and ectopic pregnancy rate of 0.37% (1/264%) P = 0.813 was the highest in Group IV. An additional analysis was done to combine the treatment outcomes of Group II and III with total abstinence period of 2–7days as per the WHO, 2010 recommendations (Group V). It was observed that the primary treatment outcomes including implantation rate, biochemical pregnancy rate, and clinical pregnancy rate were significantly lower in Group V as compared to Group I. The miscarriage and ectopic rate were higher in Group V [Table 4].

The observed unadjusted beta (β) univariate analysis for primary outcomes showed a slight raise in negative correlation for groups with longer abstinence period (>1 day) for implantation rate (β −6.5; 95% confidence interval [CI], −10.2 to − 3.0) P < 0.005, biochemical pregnancy rate (β-1.3; 95% CI, 0.16–0.38) P < 0.005 and for clinical pregnancy rate (β-3.0; 95% CI − 4.9 to − 1.1) p-0.001 in linear correlation with Group I. The adjusted logistic regression analysis also showed a negative beta (β) coefficient correlation in longer abstinence group for biochemical pregnancy (β −6.5 (−9.9 to − 3.0) P = 0.0002), clinical pregnancy rate (β −6.1; 95% CI − 9.9 to − 2.2) p-0.001,

Table 1: Details of treatment intervention of assisted reproductive technologies cycles of the entire study population

Patient characteristics	Mean±SD/*n* (%)
Female age	32.8±5.4
Husband age	35.6±5.5
Methods of fertilization	
Total number of cycles	1691
Self-cycle	1017 (60.1)
Donor oocyte cycle	674 (39.9)
Total number OCC harvested	19,592 (11.6±6.5)
Total MII oocytes injected	15,864 (9.62±5.8)
Overall fertilization (2PN) rate	11,415 (71.95)
Overall cleavage rate	11,319 (99.2)

SD=Standard deviation, OCC=Oocyte-cumulus complexes, MII=Metaphase II, 2PN=2 Pronuclei

Table 2: Comparison of semen parameters (primary outcome) between the different abstinence study groups

Parameters, mean±SD	Group I	Group II	Group III	Group IV	*P**
Abstinence (days)	≤1	2-5	6-7	≥8	
Number (*n*)	222	1038	169	284	
Age	35.5±5.8	35.5±5.4	35.9±5.0	35.8±5.9	
Abstinence days	1±0	3.22±1.05	6.65±0.47	14.98±9.88	<0.05*
Semen volume	1.69±0.75	1.87±0.78	2.10±0.68	2.09±0.87	<0.05*
Sperm concentration $(\times 10)^6$	44.0±26.5	49.92±32.9	48.59±34.3	54.56±32.2	0.113
Total sperm concentration $(\times 10)^6$	74.9±57.3	92.6±70.2	102.3±77.9	111.8±73.3	0.003*
Progressive motility (%)	33.1±15.3	33.1±12.7	32.8±14.6	31.2±11.1	
Total motility (%)	58.9±22.6	58.6±21.7	59.4±22.5	56.0±23.9	
Total motile concentration $(\times 10)^6$	50.4±39.3	52.1±59.5	37.9±46.8	38.9±38.6	0.172
Morphology (%)	3.4±1.7	3.5±1.7	3.3±1.6	3.6±1.6	

**P*-values significant

Table 3: Treatment interventions done through assisted reproductive technologies observed in different abstinence groups[#]

Variables	*n* (%)	Mean oocytes harvested	Total sperm concentration (10^6)	Fertilization rate (2PN)-ICSI	Cleavage rate	Embryo grading (day 3)			Embryo utilisation rate (day 3)
						Grade A	Grade B	Grade A + B	
Group I	217 (12.9)	9.50±5.4	74.9	73.5 (1490/2026)	98.8 (1473/1490)	21.66 (319)	4.28 (63)	25.93 (382)	41.14 (606)
Group II	1027 (61.0)	9.91±6.2	92.6	71.8 (7114/9896)	99.2 (7060/7114)	19.87 (1403)	4.84 (342)	24.72 (1745)	38.20 (2697)
Group III	167 (9.8)	9.09±5.3	102.3	72.8 (1087/1493)	100 (1087/1087)	22.45 (244)	5.24 (57)	27.69 (301)	38.82 (422)
Group IV	280 (16.5)	9.12±4.9	111.8	70.3 (1724/2449)	98.5 (1699/1724)	26.60 (452)	7.59 (129)	34.20 (581)	42.38 (720)
P		0.024*	0.003*	<0.226*	0.640	<0.0001*	<0.0001*	<0.0001*	0.006

[#]Overall, 22 cycles with no embryos for cryopreservation were excluded from all the study groups, **P*-values significant. ICSI=Intra-cytoplasmic sperm injection, 2PN=2 Pronuclei

Table 4: Assisted reproductive technologies treatment outcomes (secondary) observed between the study groups

Variables, *n* (%)	Group I	Group II	Group III	Group IV	Group V (as per WHO)[#]	*P*
Abstinence (days)	≤1	2-5	6-7	≥8	2-7	
FET (1431)	214	754	199	264	953	
Total number of embryos transferred	511	1761	438	599	2199	
Mean number of embryos transferred	2.36±1.1	2.33±1.2	1.82±1.19	2.13±1.20	2.33±1.2	
Embryo sacs	75	226	37	56	263	
β-hCG positive	119	295	48	65	343	
Clinical pregnancies	64	192	30	48	222	
Secondary outcomes, *n* (%)						
Implantation rate	14.6 (75)	12.8 (226)	8.4 (37)	9.3 (56)	11.9 (263)	0.008*
Positive β-hCG rate	55.6 (119)	39.1 (295)	24.1 (48)	24.6 (65)	35.9 (334)	<0.0001*
Clinical pregnancy rate	30.0 (64)	25.4 (192)	15.0 (30)	18.1 (48)	23.2 (222)	0.009*
Miscarriage rate	0.46 (01)	1.7 (13)	1.0 (2)	2.6 (07)	1.57 (15)	0.388
Ectopic	0	0.39 (03)	0	0.37 (01)	0.31 (03)	0.813

[#]An additional fifth column was included by adding treatment outcomes of both group II and group III based on abstinence period of (2-7 days as per WHO Manual, 2010), **P*-values significant. WHO=World Health Organization, FET=Frozen embryo transfer

Table 5: Univariate/multivariate (logistic) regression analysis for calculating odds ratio for outcomes

Primary outcomes, *n* (%)	Group II	Group III	Group IV
Positive β-hCG rate (%)	39.1	24.1	24.6
Unadjusted β (95% CI), *P*[#]	0.66 (0.37-0.69), <0.005	−1.3 (0.16-0.38), <0.005	−1.3 (−0.17-0.38), <0.005
Adjusted OR (95% CI), *P*^	2.3 (0.17-31.9), 0.52	1.2 (0.03-38.9), 0.91	3.0 (0.17-53.4), <0.43
Clinical pregnancy rate (%)	25.4	15.0	18.1
Unadjusted β (95% CI), *P*	−1.8 (−4.9-1.2), 0.23	−3.0 (−4.9-−1.1), 0.001	−1.4 (−2.7-−0.26), 0.017
Unadjusted β (95% CI), *P*	−1.8 (−4.8-−1.1), 0.23	−6.1 (−9.9-−2.2), 0.001	−4.4 (−8.0-−0.87), 0.014
Implantation rate	12.8	8.49	9.3
Unadjusted β (95% CI), *P*	−6.5 (−10.2-−3.0), 0.0003	−6.5 (−8.6-−4.4), <0.0001	−4.1 (−5.5-−2.8), <0.0001
Adjusted β (95% CI), *P*	−6.5 (−9.9-−3.0), 0.0002	−13.2 (−17.5-−8.8), <0.0001	−12.6 (−16.6-−8.5), <0.00001

[#]Unadjusted univariate (linear) regression analysis giving β (95% CI), ^Adjusted multivariate (logistic) regression calculated OR/β analysis with 95% CI, adjusted beta only if univariate comes out to be significant with Group 1 as reference. CI=Confidence interval, OR=Odds ratio, WHO=World Health Organization

and higher odd ratio for implantation rate (adjusted odds ratio [aOR], 3.0; 95% CI 0.17–53.4) $P < 0.43$ with reference to Group I. The ectopic and miscarriages rate were not different in the groups studied [Table 5].

Discussion

In the present study, comparison of EA was done among the study groups of subfertile men and male partners of subfertilie women and pregnancy outcomes were subsequently analyzed in ART cycles across all the study groups. There was increase in semen volume in higher abstinence period compared to other study groups. Thus, there is a strong correlation between longer abstinence time and increased semen volume.[17-19] The total sperm concentration per ejaculate was highest in Group IV with maximum duration of abstinence. Many relevant studies have also demonstrated increase in total sperm count associated with longer abstinence time.[13,17,20-22] A few other studies reported the impact of frequent ejaculation (24 h) and found a fall in total sperm

count, higher level of sperm chromatin immaturity with daily ejaculation, when compared to longer period of abstinence.[9,22-24] Total motile sperm concentration was highest in Group II with the abstinence period of 2–5 days with no significant difference from other groups.

Significant difference was not seen in the total motility, progressive motile sperms, and morphology among all the study groups. Previous studies have demonstrated the association of progressive motility and between abstinence time and motility, with peak progressive motility noted after abstinence of 3 days or less.[25,26] Furthermore, studies have identified an association between abstinence time and morphology with no clear consensus.[27] Few studies also shown an impact of abstinence time on DNA fragmentation rates, which was lower with shorter abstinence period and worsened with longer abstinence.[2,8,28]

The secondary outcome fertilization rate was seen highest in Group I, whereas the embryo development, development of good quality embryos, and embryo utilization were higher in Group IV. This finding is supported with earlier studies.[29] We have cultured embryos till day-3 however sperm genome takes active part in cellular function only after day 3.[30] This might be the reason that we did not find negative correlation between longer abstinence with higher poor quality embryo formation.

Pregnancy outcomes clearly get impacted by numerous other clinical factors, but in recent years, shorter abstinence time also have appeared to be associated with improved pregnancy rates following ARTs. Our study observed significantly higher clinical pregnancy rate after ART with lower abstinence (Group I ≤1 days) as compared to with longer abstinence groups. The pregnancy rate in Group I was higher as compared to a standard abstinence period of 2–7 days. Further, implantation and biochemical pregnancy rate also correlated negatively with longer versus a shorter abstinence group. Positive β-hCG rate, implantation, and clinical pregnancy rates also remained lower in the longer abstinence group even after adjusting potential confounders. Few studies also have shown the similar association of increased pregnancy rate, increased embryo euploidy rate, and lower miscarriage rate with shorter abstinence duration.[8,13,31]

One of the major strengths of this study is that it is one of the largest prospective analysis of ICSI cycles analyzing the influence of abstinence period. One of the important limitations of the current study is that we did not include IVF cycles because of small number of cases, which might have helped us to understand the effect of abstinence on sperm natural selection process during the fertilization and on overall pregnancy rate as sperm also undergo various biochemical and physiological changes with abstinence period. Another limitation is that we have reported the outcome following the first FET cycles in Groups I, II, and IV, and hence, the data on cumulative pregnancy and live birth are not available.

Conclusions

While semen analysis is the cornerstone for testing male fertility, semen parameters may not perfectly prove the fertilizing potential of spermatozoa. Ideal abstinence recommendations to make ART (ICSI) interventions for treatment of infertile couples remain controversial.

The present study shows that short abstinence is unlikely to reduce pregnancy rate in ICSI cycle with normozoospermia. In fact, 1-day abstinence is found to be associated with the highest clinical pregnancy rate. Larger prospective clinical trials should be undertaken including other subgroups like IVF, moderate, and severe oligo-asthenozoospermia to gain further insights on ideal abstinence period for improving the chance of pregnancy in infertile couples undergoing expensive infertility treatment.

Data availability

The supporting data can be made available from Medicover Fertility upon reasonable request.

Acknowledgments

We are grateful for patients' participation.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

References

1. Comar VA, Petersen CG, Mauri AL, Mattila M, Vagnini LD, Renzi A, *et al.* Influence of the abstinence period on human sperm quality: Analysis of 2,458 semen samples. JBRA Assist Reprod 2017;21:306-12.
2. Pons I, Cercas R, Villas C, Braña C, Fernández-Shaw S. One abstinence day decreases sperm DNA fragmentation in 90% of selected patients. J Assist Reprod Genet 2013;30:1211-8.
3. Alvarez C, Castilla JA, Martínez L, Ramírez JP, Vergara F, Gaforio JJ. Biological variation of seminal parameters in healthy subjects. Hum Reprod 2003;18:2082-8.
4. World Health Organization, Department of Reproductive Health and Research. WHO laboratory manual for the examination and processing of human semen. 5th ed. Switzerland: WHO Press; 2010. p. 10-1.
5. De Jonge C, LaFromboise M, Bosmans E, Ombelet W, Cox A, Nijs M. Influence of the abstinence period on human sperm quality. Fertil Steril 2004;82:57-65.

6. Levitas E, Lunenfeld E, Weiss N, Friger M, Har-Vardi I, Koifman A, *et al.* Relationship between the duration of sexual abstinence and semen quality: Analysis of 9,489 semen samples. Fertil Steril 2005;83:1680-6.
7. Agarwal A, Gupta S, Du Plessis S, Sharma R, Esteves SC, Cirenza C, *et al.* Abstinence time and its impact on basic and advanced semen parameters. Urology 2016;94:102-10.
8. Sánchez-Martín P, Sánchez-Martín F, González-Martínez M, Gosálvez J. Increased pregnancy after reduced male abstinence. Syst Biol Reprod Med 2013;59:256-60.
9. Mayorga-Torres BJ, Camargo M, Agarwal A, du Plessis SS, Cadavid ÁP, Cardona Maya WD. Influence of ejaculation frequency on seminal parameters. Reprod Biol Endocrinol 2015;13:47.
10. Hanson BM, Aston KI, Jenkins TG, Carrell DT, Hotaling JM. The impact of ejaculatory abstinence on semen analysis parameters: A systematic review. J Assist Reprod Genet 2018;35:213-20.
11. Colturato S, Abdelmassih S, Carizza C, Nagy P, Abdelmassih V, Abdelmassih R. Influence of sexual abstinence length on sperm parameters and on IVF outcomes in ICSI assisted treatment cycles. Fertil Steril 2007;88:S252.
12. Periyasamy AJ, Mahasampath G, Karthikeyan M, Mangalaraj AM, Kunjummen AT, Kamath MS. Does duration of abstinence affect the live-birth rate after assisted reproductive technology? A retrospective analysis of 1,030 cycles. Fertil Steril 2017;108:988-92.
13. Borges E Jr., Braga DP, Zanetti BF, Iaconelli A Jr., Setti AS. Revisiting the impact of ejaculatory abstinence on semen quality and intracytoplasmic sperm injection outcomes. Andrology 2019;7:213-9.
14. Rhemrev J, Jeyendran RS, Vermeiden JP, Zaneveld LJ. Human sperm selection by glass wool filtration and two-layer, discontinuous Percoll gradient centrifugation. Fertil Steril 1989;51:685-90.
15. Alpha Scientists in Reproductive Medicine and ESHRE Special Interest Group of Embryology. The Istanbul consensus workshop on embryo assessment: Proceedings of an expert meeting. Hum Reprod 2011;26:1270-83.
16. ESHRE Special Interest Group of Embryology and Alpha Scientists in Reproductive Medicine Electronic Address: Coticchiobiogenesi@grupposandonatoit. The Vienna consensus: Report of an expert meeting on the development of ART laboratory performance indicators. Reprod Biomed Online 2017;35:494-510.
17. Welliver C, Benson AD, Frederick L, Leader B, Tirado E, Feustel P, *et al.* Analysis of semen parameters during 2 weeks of daily ejaculation: A first in humans study. Transl Androl Urol 2016;5:749-55.
18. Lehavi O, Botchan A, Paz G, Yogev L, Kleiman SE, Yavetz H, *et al.* Twenty-four hours abstinence and the quality of sperm parameters. Andrologia 2014;46:692-7.
19. Sunanda P. Effect of age and abstinence on semen quality: A retrospective study in a teaching hospital. Asian Pac J Reprod 2014;3:134-41.
20. Mayorga-Torres JM, Agarwal A, Roychoudhury S, Cadavid A, Cardona-Maya WD. Can a short term of repeated ejaculations affect seminal parameters? J Reprod Infertil 2016;17:177-83.
21. Gonzalo A. Influence of ejaculatory abstinence on the characteristics of spermiogram. Systematic review. Rev Chil Obstet Ginecol 2013;78:290-2.
22. Carlsen E, Petersen JH, Andersson AM, Skakkebaek NE. Effects of ejaculatory frequency and season on variations in semen quality. Fertil Steril 2004;82:358-66.
23. Okada FK, Andretta RR, Spaine DM. One day is better than four days of ejaculatory abstinence for sperm function. Reprod Fertil 2020;1:1-0.
24. Uppangala S, Mathai SE, Salian SR, Kumar D, Singh VJ, D'Souza F, *et al.* Sperm chromatin immaturity observed in short abstinence ejaculates affects DNA integrity and longevity *in vitro*. PLoS One 2016;11:e0152942.
25. Wang L. Influence of the abstinence period on human semen parameters. Chin J Androl 2007;21:21-3.
26. AlAwlaqi A, Hammadeh ME. Sexual abstinence and sperm quality. Int J Womens Health Reprod Sci 2017;5:11-7.
27. Bahadur G, Almossawi O, Zeirideen Zaid R, Ilahibuccus A, Al-Habib A, Muneer A, *et al*. Semen characteristics in consecutive ejaculates with short abstinence in sub-fertile males. Reprod BioMed Online 2016;32:323-8.
28. Gosálvez J, González-Martínez M, López-Fernández C, Fernández JL, Sánchez-Martín P. Shorter abstinence decreases sperm deoxyribonucleic acid fragmentation in ejaculate. Fertil Steril 2011;96:1083-6.
29. Colturato S, Abdelmassih S, Carizza C, Nagy P, Abdelmassih V, Abdelmassih R. Influence of sexual abstinence length on sperm parameters and on IVF outcomes in ICSI assisted treatment cycles. Fertil Steril 2007;88:S252.
30. Braude P, Bolton V, Moore S. Human gene expression first occurs between the four- and eight-cell stages of preimplantation development. Nature 1988;332:459-61.
31. Scarselli F, Cursio E, Muzzì S, Casciani V, Ruberti A, Gatti S, *et al.* How 1 h of abstinence improves sperm quality and increases embryo euploidy rate after PGT-A: A study on 106 sibling biopsied blastocysts. J Assist Reprod Genet 2019;36:1591-7.