Does duration of abstinence affect the live-birth rate after assisted reproductive technology? A retrospective analysis of 1,030 cycles

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Objective: To study influence of abstinence period on the live-birth rate after assisted reproductive technology (ART).
Design: Retrospective cohort study.
Setting: Reproductive medicine unit, university-level hospital.
Patient(s): A total 1,030 ART cycles evaluated from 2011 to 2015.
Intervention(s): Group I, abstinence period 2–7 days, and group II, abstinence period >7 days, were compared. Two subgroups Ia (2–4 days) and Ib (5–7 days) were also compared with group II.
Main Outcome Measure(s): Primary outcome was live birth per ET. Secondary outcomes included implantation, clinical pregnancy, and miscarriage rates.
Result(s): The live-birth rate (34.1 % vs. 24.1%; odds ratio [OR], 1.6; 95% confidence interval [CI], 1.1–2.4), clinical pregnancy rate (44.4 % vs. 32.7%; OR, 1.6; 95% CI, 1.1–2.3), and implantation rate (26.4% vs. 18.2%) were significantly higher in group I compared with group II. Other secondary outcomes of fertilization rate and miscarriage rate did not differ between groups I and II. The adjusted odds ratio (aOR) for live birth (aOR, 1.6; 95% CI, 1.1–2.5) and clinical pregnancy rates (aOR, 1.7; 95% CI, 1.2–2.5) were significantly higher for group I compared with group II. The live-birth rate was significantly higher in group Ia (36.1% vs. 24.1%) compared with group II.
Conclusion(s): An abstinence period of more than 7 days may impact ART outcomes adversely when compared with an abstinence period of 2–7 days. (Fertil Steril® 2017;108:988–92. ©2017 by American Society for Reproductive Medicine.)
Key Words: Abstinence, IVF, ICSI, live birth

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Semen analysis is an important investigation in the evaluation of the subfertile couple. The abstinence period before semen collection can influence the seminal parameters, with short or long abstinence being linked to abnormal results (1). For standardization, the World Health Organization guidelines recommend a 2–7 days abstinence period before semen analysis during routine infertility workup (2). The European Society of Human Reproduction and Embryology advises 3–4 days of abstinence before semen analysis (3).

The role of abstinence period and its impact on sperm DNA fragmentation has been studied, and conflicting reports have emerged. While one study reported an increase in immature sperm chromatin after 1 day of abstinence, another found reduced sperm DNA fragmentation after a similar duration of abstinence (4, 5). While the effect of abstinence period on seminal parameters and sperm quality has been extensively reported in the literature, its overall impact on clinical outcomes of therapeutic interventions such as in vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI) is not clear (6). One study found higher pregnancy rates in the shorter abstinence group after ICSI and reduction in pregnancy rates after abstinence of ≥5 days (7).

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In clinical practice, advice regarding period of abstinence during assisted reproductive technology (ART) has largely been extrapolated from existing recommendations for diagnostic semen analysis. There is a felt need for greater clarity on the issue of abstinence for couples undergoing ART. The literature investigating the effect of abstinence period on clinical outcomes after ART is sparse. We decided to evaluate the influence of abstinence period on clinical pregnancy and live-birth rates after ART.

MATERIALS AND METHODS

A retrospective study was conducted in the Reproductive Medicine Unit of a university-level teaching hospital. Data from all the ART cycles performed during January 2011 to December 2015 were analyzed. Ethics approval was given by the Institutional Review Board.

We included all ART cycles that resulted in a fresh ET irrespective of indication. Type of ART treatment included ICSI or a combination of IVF and ICSI. We excluded the following: [1] women $\geq$40 years, [2] cycles where surgically retrieved sperms or cryopreserved samples were used for ICSI, [3] IVF only cycles, [4] poor responders ($\leq$3 oocytes retrieved), and [5] cycles where all the embryos were cryopreserved.

The included cycles were divided into two main groups: group I, abstinence between 2 and 7 days (standard abstinence period), and group II, abstinence period of >7 days (long abstinence period). We further divided group I into two subgroups: group Ia, abstinence period of 2–4 days, and group Ib, abstinence period of 5–7 days.

We used standard long GnRH agonist, ultralong, or GnRH antagonist protocols. For controlled ovarian hyperstimulation, 100–300 IU of recombinant FSH (Recagon, Organon) was used, and follicular monitoring was done using serial ultrasounds. When at least three follicles >17 mm developed, 5,000 IU of injected hCG (Pregnyl, Organon) was administered. Oocyte retrieval was planned after 35 hours, after hCG trigger. Between one and three embryos were transferred either at cleavage (day 2 or 3) or blastocyst stage (day 5). For luteal support, micronized P, 400 mg twice a day intravaginally (Naturogest, German Remedies), along with IM P, 100 mg (Gestone, Ferring) twice weekly was given. The serum beta hCG level was checked on day 18 after oocyte retrieval.

Data regarding abstinence detail were collected from questionnaires filled out by the male partner on the day of sample collection during the treatment cycle. These questionnaires were safely kept along with embryological details in the ART laboratory records section. Information regarding other clinical and ART variables such as age, indication, oocyte numbers, embryo quality, and numbers transferred was obtained from the unit ART database. The pregnancy outcomes were collected from the women through e-mails and telephone contacts. Collected data were entered in SPSS, and data were analyzed using STATA, version 13.1 (Statacorp).

Outcomes Measured

The primary outcome was live-birth rate per ET. Live birth is defined as delivery of a live baby after 24 weeks of gestation. Secondary outcomes included fertilization rates after IVF and ICSI, development of top-quality embryos, and implantation, clinical pregnancy, and miscarriage rates.

The fertilization rate is defined as the number of fertilized oocytes by the total number of inseminated oocytes (IVF) or injected oocytes (ICSI). A top-quality cleavage-stage embryo is defined as the total number of grade I embryos on day 2/3 of insemination or injection. Clinical pregnancy is defined as evidence of a gestational sac on ultrasound. Implantation rate is defined as the number of sacs seen on ultrasound divided by the number of embryos transferred. The miscarriage rate is absence of cardiac activity or loss of embryo or fetus before 24 completed weeks of gestation divided by the number of clinical pregnancies.

Statistical Methods

Data were summarized using mean (SD) for continuous variables and frequency (percentage) for categorical variables. Analysis of variance (followed by post hoc test) and χ^2 test were used to check the relation between the duration of abstinence and the outcome variables. A logistic regression was performed for the dichotomous outcomes (live birth, miscarriage, and clinical pregnancy), mutually adjusting the potential confounders such as severe oligozoospermia (<5 million/mL) and asthenozoospermia (progressive motility <1%) and male age. The effect is given as odds ratio (OR) with 95% confidence interval (CI). A multiple linear regression was used to assess the influence of predictors over the continuous outcome, mutually adjusting the confounders and expressed as β (95% CI).

RESULTS

A total of 1,345 ART cycles were performed during the study period. After screening, 315 cycles were excluded for reasons such as [1] female confounders (n = 269), [2] frozen semen sample used (n = 16), [3] IVF only (n = 5), and [4] data unavailable (n = 25). In the final analysis, 1,030 cycles were included, among which group I had 868 and group II had 162 cycles.

There were no significant differences in baseline clinical characteristics between group I and group II (Table 1). Mean duration of abstinence in group I was 4.33 $\pm$ 1.31 days and in group II, 18.4 $\pm$ 29.69 days.

Among ART variables, the dose of gonadotropins, the duration of stimulation, the number of oocytes retrieved, and the mean number of embryos transferred were not significantly different in main group comparisons (group I vs. II). The method of fertilization (ICSI or IVF+ICSI) was significantly different between the two main groups (P=.001). Mean progressive motility was significantly higher in group I (36.71 $\pm$ 20.2 vs. 30.6 $\pm$ 19.1; P<.001) compared with group II (Table 2).

The live-birth rate per ET (34.1 % vs. 24.1%; OR 1.6; 95% CI, 1.1–2.5; P=.01) and clinical pregnancy rate per ET (44.4 % vs. 32.7%; OR, 1.6; 95% CI, 1.1–2.3; P=.008) were significantly higher in group I compared with group II (Tables 3 and 4). The implantation rate (26.4% vs. 18.2%; P<.001) was also significantly higher in group I versus group II. Other secondary outcomes of fertilization rate, development

TABLE 1

Baseline clinical comparisons between main study groups.

Variable	Group I (n = 868)	Group II (n = 162)	*P* value
Female age (y)	31.76 ± 3.83	31.63 ± 4.03	.68
Male age (y)	37.13 ± 4.67	37.95 ± 4.66	.05
Type of infertility			
Primary infertility	619 (71.3)	118 (72.8)	.69
Secondary infertility	249 (28.7)	44 (27.2)	
Indication			
Tubal	142 (16.4)	23 (14.2)	.63
Anovulation	105 (12.1)	17 (10.5)	
Male	204 (23.5)	37 (22.8)	
Unexplained	91 (10.5)	16 (9.8)	
Endometriosis	106 (12.2)	17 (10.5)	
Combined	220 (25.35)	52 (32.1)	
Method of collection			
Masturbation	773 (89.1)	149 (91.9)	.51
Intercourse	94 (10.8)	13 (8.02)	
Vibro (penile vibratory stimulation)	1 (0.1)	0	

Note: Data presented as mean ± standard deviation or n (%), unless stated otherwise.

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of top-quality embryos, and miscarriage rate did not differ significantly between the two main groups (Table 3).

After adjusting for potential confounders, the adjusted OR (aOR) for live birth (aOR, 1.6; 95% CI, 1.1–2.5) and clinical pregnancy rates (aOR, 1.7; 95% CI, 1.2–2.5) were significantly higher in group I compared with group II (Table 4). The fertilization rate and miscarriage rate did not differ significantly among the two groups even after adjusting for potential confounders (Table 4).

In the subgroup analysis, baseline clinical characteristics were comparable between groups Ia (n = 536) and Ib (n = 332) versus group II (Supplemental Table 1). Mean duration of abstinence in group Ia was 3.47 ± 0.63 days and in group Ib, 5.71 ± 0.88 days. Among ART variables, mean progressive motility was significantly higher in both group Ia (37.7 ± 20.2 vs. 30.6 ± 19.1; $P<.001$) and group Ib (35.2 ± 20.3 vs. 30.6 ± 19.1; $P=.05$) when compared with group II. The method of fertilization was significantly different between group Ia and group II ($P=.02$; Supplemental Table 2).

The implantation rates were significantly higher when group Ia (28.01%; $P<.001$) and group Ib (23.7%; $P=.04$) were compared with group II (18.2%). The clinical pregnancy rates per ET were significantly higher in group Ia (45.5%; $P=.004$) and group Ib (42.5%; $P=.048$) compared with group II (32.7%). The live-birth rate per ET was significantly higher when group Ia was compared with group II (36.1% vs. 24.1%; $P=.005$), but there was no significant difference in live-birth rate when group Ib (30.7% vs. 24.1%; $P=.127$) was compared with group II (Supplemental Table 3). The fertilization rates, development of top-quality embryos, and miscarriage rate did not differ significantly between group Ia and Ib versus group II.

TABLE 2

Treatment (assisted reproductive technology) characteristics comparison between main study groups.

Variable	Group I (n = 868)	Group II (n = 162)	*P* value
Total dose, IU	2,304.1 ± 1,099.05	2,161.4 ± 980.7	.15
Duration of stimulation, days	10.11 ± 2.2	9.9 ± 1.9	.30
No. of oocytes obtained	9.61 ± 4.76	9.23 ± 4.07	.34
Progressive motility, %	36.71 ± 20.2	30.6 ± 19.1	<.001
Method of fertilization			
ICSI	679 (78.2)	135 (83.3)	.001
Both IVF and ICSI	189 (21.7)	27 (16.7)	
No. of embryos transferred	2.3 ± 0.63	2.3 ± 0.61	.99

Note: Data presented as mean ± SD or n (%), unless stated otherwise.
*Significant difference.

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TABLE 3

Comparison of outcomes between the main study groups.

Variable	Group I (n = 868)	Group II (n = 162)	*P* value
Live birth per ET,[a] %	34.1 (286/840)	24.1 (38/158)	.014[b]
Fertilization rate (IVF), %	67 ± 28	66 ± 31	.81
Fertilization rate (ICSI), %	76 ± 19	75 ± 19	.58
Cleavage-stage grade I embryo rate, %	47.4 (2,346/4,945)	47.3 (407/861)	.47
Implantation rate, %	26.4 (526/1991)	18.2 (68/373)	<.001[b]
Clinical pregnancy rate per ET, %	44.4 (385/868)	32.7 (53/162)	.008[b]
Miscarriage rate, %	7.3 (63/868)	6.2 (10/162)	.62

Note: Data presented as mean ± standard deviation, unless specified otherwise. ET = embryo transfer; ICSI = intracytoplasmic sperm injection; IVF = in vitro fertilization.
[a] For available data.
[b] $P<.05$.

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DISCUSSION

The current study found a significantly lower live-birth rate after ART in the group with a long abstinence (>7 days) period, compared with the group with a standard abstinence period of 2–7 days. The implantation and clinical pregnancy rates were also significantly lower in the long abstinence group versus the standard abstinence period group. Live-birth, clinical pregnancy, and implantation rates remained significantly lower in the long abstinence group even after adjusting for potential confounders. Within the standard abstinence group, live-birth rate was significantly higher in the subgroup with 2–4 days abstinence period compared with a long abstinence period >7 days, although it was no longer significantly different when the former group was replaced by the 5–7 days abstinence subgroup. The clinical pregnancy and implantation rates were significantly higher for the subgroups with an abstinence period of 2–4 days and 5–7 days when compared with a long abstinence period. The fertilization and miscarriage rates were not significantly different in either the main groups or subgroup comparisons.

Earlier studies have evaluated the influence of abstinence on sperm parameters or IUI outcomes. An abstinence period of

TABLE 4

Linear and logistic regression analysis for outcomes.

Outcome	Group I	Group II	OR (95% CI)/β (95% CI) unadjusted	OR (95% CI)/β (95% CI) adjusted[a]
Live birth per ET (%)	286 (34.1)	38 (24.1)	1.6 (1.1–2.4)[b]	1.6 (1.1–2.5)[b]
Clinical pregnancy per ET (%)	385 (44.4)	53 (32.7)	1.6 (1.1–2.3)[c]	1.7 (1.2–2.5)[c]
Miscarriage (%)	63 (7.3)	10 (6.2)	1.2 (0.6–2.4)	1.3 (0.6–2.7)
Fertilization rate, ICSI (%)	76 ± 19	75 ± 19	0.01 (−0.02 to 0.04)	0.01 (−0.02 to 0.04)

Note: Values presented as n (%) and mean ± SD (logistic regression was performed), unless stated otherwise.
[a] Models were adjusted for progressive motility (<1 %), concentration <5 million/mL, and male age.
[b] $P<.05$.
[c] $P<.01$.

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4–5 days compared with 2–3 or 6–7 days was associated with a higher proportion of progressively motile sperms (8). Another study suggested a 1-day abstinence for male factor infertility and <10 days of abstinence for nonmale factor in order to obtain a higher yield of morphologically normal, motile sperms (1). The results of a retrospective analysis evaluating the influence of abstinence on IUI cycles (n = 417) found the highest pregnancy rate after an abstinence period ≤3 days (14%) and the lowest after an abstinence period of ≥10 days (3%). The investigators found a decrease in pregnancy rates after prolonged abstinence, which was independent of semen parameters. They suggested sperm senescence as the likely reason for the decline that was not readily identified with routine semen analysis (9). A small retrospective study evaluated the influence of the abstinence period (2–4 vs. 5–7 days) on ICSI cycles (n = 131). The investigators in that study did not find any significant difference in fertilization rate (77.5% vs. 72.9%; $P=.1$) and clinical pregnancy rate (44.8% vs. 43.8%; $P=.9$) between the two groups (10). The results of this study are similar to clinical outcomes reported for subgroups Ia and Ib in the current study, even though we compared these subgroups with a long abstinence group of >7 days. Another retrospective study evaluated the influence of 1, 2, 3, 4, 5, 6–10, and >11 days of abstinence on ICSI cycle (n = 445) outcomes. The investigators found the highest and lowest pregnancy rates after 1 and 5 days of abstinence (67.2 vs. 42.1%; $P=.007$), respectively (7). This study suggested lower pregnancy rates after 5 or more days of abstinence and an inverse relationship between abstinence period and ICSI results. Although the abstinence intervals in that study were different from those in the current study, the main conclusion is in agreement with it.

Prolonged exposure to reactive oxygen species from dead spermatozoa and leukocytes is suggested as one of the reasons for the decline in sperm quality and an increase in DNA-damaged sperms with longer abstinence (11–13). It is possible that the use of a potentially higher proportion DNA-damaged sperms for ICSI may reduce implantation and live-birth rates (14). This may be the likely explanation for the association between longer abstinence and reduction in live birth after ICSI.

The current study is one of the first studies evaluating the influence of the abstinence period on clinical outcomes after ART. Among the strengths of the current study are the inclusion of the largest number of ART cycles to date and the reporting of live-birth outcomes. We excluded cycles with female age >40 years or poor responders, which could have influenced the live-birth outcomes. In the current study, ICSI was performed either alone or in combination with IVF for fertilizing the oocytes in the majority of cycles. A subgroup of cycles included male factor infertility with varying levels of semen abnormalities. After adjusting for potential confounders, the live birth and clinical pregnancy rates were still significantly lower in the longer abstinence group compared with in the standard abstinence group, further validating our findings.

Retrospective design remains the one of the important limitations of the current study. The information on abstinence was obtained from self-filled questionnaires and was not verifiable. While the study findings strengthen the common practice of adhering to the standard abstinence period before ART, the exact cutoff day could not be ascertained beyond which a detrimental effect on ART outcomes is significant. Since the majority of cycles used ICSI alone or in combination with IVF, these findings may not be applicable to ART cycles where only IVF was used.

The current study findings suggest an abstinence period of more than 7 days may impact ART outcomes adversely when compared with the standard abstinence period of 2–7 days. Additionally, within the standard abstinence period, an abstinence of 2–4 days is associated with higher live-birth rates compared with longer abstinence >7 days. These findings appear to support the widely practiced advice regarding the standard abstinence period during ART, which is similar to diagnostic semen analysis guidelines (2, 3). The study findings are useful for couples planning for ART treatment. There is a need to conduct larger prospective trials to further validate these findings.

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