Zeynep Kamil Med J 2023;54(3):130–136
DOI: 10.14744/zkmj.2023.55798

ORIGINAL ARTICLE

Duration of abstinence and TMSC are significant predictors for IUI outcome

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ABSTRACT

Objective: The aim of this study is to assess the impact of pre- and intracycle spermiogram parameters on treatment outcome in Intrauterine Insemination (IUI) cycles of couples with optimal female cycle characteristics.

Material and Methods: The study group comprised infertile couples diagnosed as anovulation, grade 1–2 endometriosis, and unexplained or male factor infertility. Women included in this study underwent ovulation induction and women with optimal cycle characteristics were included in the analysis to figure out the individual impact of sperm parameters on cycle outcome in terms of clinical pregnancy.

Results: Precycle sperm concentration, semen volume, duration of abstinence, total progressive sperm counts, and pre- and intracycle progressive motile sperm concentrations were significant predictors for clinical pregnancy. In addition, parameters of abstinence and total progressive motile sperm count evaluated on IUI day were found to significantly predict favorable outcomes. Multivariate regression analysis was conducted to show adjusted associations and analysis revealed that intracycle duration of abstinence and precycle total progressive motile sperm counts were significantly associated with clinical pregnancy.

Conclusion: It is shown that intracycle duration of abstinence and precycle total progressive motile sperm counts were significant predictors for IUI cycles with optimal female partner reproductive properties.

Keywords: Clinical pregnancy, intrauterine insemination, male factor infertility, sperm parameters.

Cite this article as: Yılmaz MB, Devranoğlu B, Kolcu SA, Çelik Z, Özkaya E. Duration of abstinence and TMSC are significant predictors for IUI outcome. Zeynep Kamil Med J 2023;54(3):130–136.

Received: December 21, 2022 **Revised:** January 21, 2023 **Accepted:** January 26, 2023 **Online:** September 29, 2023
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Zeynep Kamil Medical Journal published by Kare Publishing. Zeynep Kamil Tıp Dergisi, Kare Yayıncılık tarafından basılmıştır.

INTRODUCTION

The mechanism of Intrauterine Insemination (IUI) is based on the placement of a high amount of motile sperm in the uterine cavity. The procedure increases the likelihood of conception with the requirements of the presence of patent fallopian tubes, and the absence of active cervical, intrauterine, or pelvic infection with a sufficient number of motile sperm.

In general, the semen sample is collected after 2–5 days of sexual abstinence.[1,2] After incubation for liquefaction, the methods "swim up" or "density gradient centrifugation" are used for sperm processing. In the studies comparing these methods, no statistically significant difference was reported for pregnancy rates.[3] However, the motile sperm rate is generally higher with the density gradient method.[3,4]

In the literature, the minimum total motile sperm count (TMSC) for acceptable conception rates is controversial.[5,6] It has been reported that more than 5–10 m TMSC is associated with higher pregnancy rates.[7,8] If <3–5 m motile sperm counted, pregnancy rates decreased to 1% per cycle. In these oligoastenozoospermia cases, in vitro fertilization (IVF) with controlled ovarian stimulation is recommendable for better outcomes.[9,10]

In many studies prolonged abstinence was found inversely proportional to parameters of sperm motility and morphology,[11] to get the highest chance of fertility it is suggested that semen should be collected between day 2 and day 7 of abstinence from patients who undergo fertility treatment.[12]

In this study, we aimed to evaluate the influence of pre- and intracycle spectrogram parameters on IUI cycle outcome in cycles with optimal female cycle characteristics.

MATERIAL AND METHODS

This retrospective study was conducted in the Department of Reproductive Endocrinology and Infertility of Health Sciences University Zeynep Kamil Women and Children's Health Training and Research Hospital. The study protocol was approved by the Local Ethics Committee of Zeynep Kamil Women and Children's Health Training and Research Hospital (approval reference number:100, June, 03 2020).

The study group comprised of infertile couples diagnosed as anovulation, grade 1–2 endometriosis, and unexplained or male-factor infertility. The patency of fallopian tubes was confirmed by hysterosalpingography (HSG). Semen samples were prepared with the density gradient centrifugation method and evaluated according to World Health Organization (2010) guidelines and <15 m/mL sperm concentration, <40% progressive motility rate (A + B), and <4% normal morphology for Kruger criteria defined as male factor infertility.[13] The term "unexplained infertility" has been used for couples in whom the results of a standard infertility examination are normal (spermiogram, HSG, and ultrasonographic evaluation of reproductive system).[14] Minimal and mild endometriosis was diagnosed as revised AFS criteria.

Those patients with age >35, poor ovarian reserve, bilateral tubal blockage, severe male factor infertility (TMSC <1 million before IUI), and grade 3–4 endometriosis were excluded from the study. However, both patients with lower and higher sperm counts after preparation were included in the study. Cycles with more than two follicular growth >14 mm and <8 mm endometrial thickness were cancelled.

The protocol for ovulation induction was a standard dose of 75IU gonadotrophins. (Gonal-F; Merck Serono, Bari, Italy or Fostimon; IBSA Lamone, Swiss). Administration of gonadotrophin was started on the day of 3–8. Then follicular development was monitorized by transvaginal ultrasound per 2 days. Once a follicle of >17 mm size was developed, recombinant human chorionic gonadotrophin 250 mcg (hCG) (Ovitrelle; Merck Serono, Bari, Italy) was applied and insemination was performed after 34–36 h. If three or more follicles larger than 14 mm were identified, the cycle was cancelled. Patients were informed for the risk of multiple pregnancies and hyperstimulation and advised to avoid intercourse for 2 weeks.

On the day of the procedure, the male partner was suggested to give a semen sample with abstinence of 2–5 days. Before washing, sample parameters were evaluated, and then prepared by the density gradient centrifugation method with gradient-buffered colloid solution SpermGrad-125 (Vitrolife, Sweden) and washing solution SpermRince (Vitrolife, Sweden). With lithotomy position and ultrasound guidance, IUI was performed with a soft insemination catheter (Swemed LAB, International AB, Billdal, Sweden) with a seeding volume of 0.5 mL. After the procedure, the patient was offered 5 min of rest. Luteal phase support was provided with 400 mg daily vaginal micronized progesterone for 14 days (Progestan 200 mg capsule; Koçak Farma, Tekirdağ, Türkiye).

Beta hCG test was carried out on the 2nd week following IUI. When it was >1500–2000 a transvaginal ultrasonography was performed to confirm intrauterine pregnancy. Clinical pregnancy was defined as the presence of fetal cardiac activity.

Archive files of patients who had undergone IUI were scanned and the woman's age, infertility time, and IUI cycle number were recorded. Semen parameters within 6 months before IUI were saved as " pre-cycle" spermiogram and "intracycle" parameters were evaluated on the day of the procedure. After collecting the data the mean age of the woman, number of previous cycles of IUI, and duration of infertility were compared with conceived and un-conceived patients. Pre-cycle and intracycle spermiogram parameters of sperm concentration, total progressive sperm motility, semen volume, abstinence time, and TMSC were compared between cycles with and without successful outcomes.

Statistical Analysis

SPSS version 15 was used for the analysis. To compare groups, the Mann–Whithney U-test or student-t tests were used in binary group comparisons. Paired samples t-test was used to compare the pre and intracycle values of each participant. The Chi-square test was used for analyzing the categorical data. ROC analysis was used to figure out the predictive values of each variable. Logistics regression analysis was carried out to analyze the effects of all factors and factors on delivery type. P<0.05 was used as the statistical significance level.

Table 1: Comparison of mean age, number of previous cycles, and duration of infertility between cycles with and without successful outcome

	n	Mean	SD	p
Age (years)				0.5
Unconceived	236	29.6	4.1	
Conceived	43	29.1	4.6	
# of previous IUI cycle				0.9
Unconceived	224	1.3	0.5	
Conceived	38	1.3	0.4	
Duration of infertility (years)				0.5
Unconceived	232	3.5	2.9	
Conceived	43	3.9	2.8	

#: Number; SD: Standard deviation.

RESULTS

The study included 279 IUI cycles of women with unexplained infertility, anovulation, male factor infertility, and minimum to mild endometriosis. Comparison of mean age, number of previous cycles, and duration of infertility between cycles with and without successful outcomes are shown in Table 1. Spermiogram and sperm morphology parameters before cycle were recorded and reassessed on IUI day before washing, then compared between groups with and without favorable outcomes and presented in Table 2. Both pre-cycle and intracycle sperm concentration, total progressive sperm motility (A + B), and semen volume were higher in the group who conceived. Of these pre-cycle abstinence time and TMSC were significantly higher (3.3 vs. 3.6, p=0.001 and 67.7 vs. 124.3, p=0.001). For intracycle parameters, duration of abstinence was the significative factor of IUI success (3.3 vs. 3.8, p<0.001).

Again groups were compared in terms of ratios of each pre- and intracycle spermiogram parameters (Table 3). TMSC ratio was significantly lower in the group of conceived patients (1.8 vs. 1.1, p<0.001). ROC analysis was conducted to figure out predictive values of each pre- and intracycle spermiogram parameters for clinical pregnancy (Fig. 1a).

Analysis revealed that pre-cycle sperm concentration, semen volume, duration of abstinence total progressive sperm count, and pre- and intracycle progressive motile sperm concentrations were significant predictors for clinical pregnancy. In addition, parameters of abstinence and total progressive motile sperm count evaluated on IUI day were found to significantly predicted favorable outcomes (Table 4 and Fig. 1a). None of the ratios of pre- and intracycle values were found to have significant predictive value for clinical pregnancy (Fig. 1b). Multivariate regression analysis was conducted to show age, IUI cycle number, and infertility time-adjusted associations for clinical pregnancy, and analysis revealed that intracycle duration of abstinence and pre-cycle total progressive motile sperm counts were significantly associated with clinical pregnancy (Table 5). Paired of comparisons of each spermiogram parameters between pre- and intracycle values revealed that there were significant differences in terms of sperm concentrations, motile sperm counts, and ejaculate volume (p<0.05), whereas there were no differences with regard to TPMSC and duration of abstinence (Table 6).

Table 2: Comparison of pre- and intracycle spermiogram and sperm morphology parameters

	n	Mean	SD	p
Pre-cycle sperm concentration (M/mL)				0.03
Unconceived	236	57.3	47.7	
Conceived	43	66.2	39.8	
Pre-cycle total progressive sperm motility (A+B)(%)				0.005
Unconceived	236	41.9	16.7	
Conceived	43	50.5	17.8	
Pre-cycle volume (cc)				0.002
Unconceived	235	3.06	1.4	
Conceived	43	3.7	1.6	
Pre-cycle abstinence (day)				0.001
Unconceived	236	3.3	0.5	
Conceived	43	3.6	0.7	
Pre-cycle normal morphology (%)				0.3
Unconceived	212	19.7	25.09	
Conceived	36	27.1	30.9	
Pre-cycle TMSC				0.001
Unconceived	236	67.7	80.3	
Conceived	43	124.3	85.7	
Intracycle concentration (M/mL)				0.08
Unconceived	232	49.5	34.3	
Conceived	43	59.1	36.2	
Intra cycle total progressive sperm motility (A+B)(%)				0.008
Unconceived	232	52.9	18.1	
Conceived	43	60.5	16.5	
Intracycle volume (cc)				0.07
Unconceived	232	2.7	1.2	
Conceived	43	3.2	1.6	
Intracycle abstinence (day)				<0.001
Unconceived	229	3.3	0.5	
Conceived	42	3.8	0.8	
Intracycle TMSC				0.07
Unconceived	232	73.5	64.7	
Conceived	43	109.1	90.1	

TMSC: Total motile sperm count; SD: Standard deviation.

Table 3: Ratio comparison of each pre- and intra cycle spermiogram parameters

	Group statistics			
	n	Mean	SD	p
TMSC ratio				<0.001
Unconceived	232	1.8	2.2	
Conceived	43	1.1	0.8	
Volume ratio				0.2
Unconceived	231	0.9	0.4	
Conceived	43	0.8	0.4	
Sperm concentrations ratio				0.3
Unconceived	232	1.2	1.3	
Conceived	43	1.05	0.7	
Total progressive sperm motility (A+B) (%) ratio				0.4
Unconceived	232	1.4	0.7	
Conceived	43	1.3	0.6	

TMSC: Total motile sperm count; SD: Standard deviation.

Table 4: Results of ROC analysis which were conducted to figure out predictive values of each pre- and intra cycle spermiogram parameters for clinical pregnancy

	Area under the curve		Asymptotic 95% CI	
	Area	p	Lower bound	Upper bound
Pre-cycle sperm concentration (M/mL)	0.601	0.036	0.521	0.680
Pre-cycle volume (cc)	0.651	0.002	0.564	0.737
Pre-cycle abstinence (day)	0.632	0.006	0.535	0.729
Pre-cycle TMSC	0.732	0.000	0.650	0.814
Intra cycle abstinence (day)	0.672	0.000	0.576	0.767
Intra cycle TMSC	0.630	0.007	0.542	0.717
Pre-cycle total progressive sperm motility (A+B) (%)	0.632	0.006	0.542	0.722
Intracycle total progressive sperm motility (A+B) (%)	0.628	0.008	0.536	0.720

TMSC: Total motile sperm count; CI: Confidence interval.

Table 5: Multivariate regression analysis results of age, IUI cycle number, and infertility time-adjusted associations for clinical pregnancy

	Coefficients[a]		
	SC Beta	t	p
Pre-cycle sperm concentration (M/mL)	-0.191	-1.721	0.086
Pre-cycle volume (cc)	0.039	0.541	0.589
Pre-cycle abstinence (day)	-0.208	-1.750	0.081
Pre-cycle TMSC	0.316	2.490	0.013
Intra cycle abstinence (day)	0.444	3.748	0.000
Intra cycle TMSC	0.052	0.742	0.459
Pre-cycle total progressive sperm motility (A+B) (%)	0.003	0.046	0.963
Intracycle total progressive sperm motility (A+B) (%)	0.052	0.745	0.457

a: Dependent variable: Clinical pregnancy; TMSC: Total motile sperm count; SC: Standardized coefficients.

DISCUSSION

IUI is generally recommended to infertile couples where the woman has at least one patent fallopian tube and the man with the sperm parameters in an acceptable range. The characteristics of couples such as age, duration of infertility, hormonal, and sonographic findings and sperm parameters, other accompanying causes of infertility, ovulation induction processes, and numbers of cycles have been used as predictors of IUI success.[15,16] In this report, we aimed to present the impact of pre- and intracycle spermiogram parameters on IUI cycle outcome in cycles with optimal female cycle characteristics. Our data analysis revealed that pre-cycle sperm concentration, semen volume, duration of abstinence total progressive sperm count, and pre- and intracycle progressive motile sperm concentrations were significant predictors for clinical pregnancy.

In addition, parameters of abstinence and total progressive motile sperm count evaluated on IUI day were found to significantly predicted favorable outcomes. None of the ratios of pre- and intracycle values were found to have significant predictive value for clinical pregnancy. Multivariate regression analysis was conducted to show adjusted associations and analysis revealed that intracycle duration of abstinence and pre-cycle total progressive motile sperm counts were significantly associated with clinical pregnancy.

Sperm parameters, especially sperm count, sperm morphology, total motility rate, and TMSC were emphasized in previous studies, but reference values could not be established to predict IUI success.[17–19] In a study, 10 million TMSC was shown as a good threshold for deciding on IUI or IVF, and it was reported that when TMSC below 10 million, it is more cost-effective to prefer IVF treatment.[7] In another study, it was shown that the pregnancy rate significantly decreased with TMSC below 10 million.[8] In the meta-analysis conducted by Ombelet et al.,[19] the cutoff values of the four most frequently examined sperm parameters were reported to be: TPMSC cutoff value be-

Table 6: Paired of comparisons of each spermiogram parameters between pre- and intra cycle values

	Paired samples statistics		
	Mean	SD	p
Pre-cycle sperm concentration (M/mL)	58.7	46.4	
Intra cycle concentration (M/mL)	51.08	34.7	0.004
Pre-cycle total progressive sperm motility (A+B) (%)	43.3	17.1	
Intracycle total progressive sperm motility (A+B) (%)	54.09	18.1	<0.001
Pre-cycle volume (cc)	3.1	1.4	
Intracycle volume (cc)	2.8	1.3	<0.001
Pre-cycle abstinence (day)	3.3	0.5	
Intracycle abstinence (day)	3.3	0.5	0.9
Pre-cycle TMSC	76.7	83.9	
Intra cycle TMSC	79.1	70.3	0.6

TMSC: Total motile sperm count; SD: Standard deviation.

tween 0.8 and 5 million after washing; sperm morphology cutoff value >4% normal morphology based on using strict criteria; TPMSC cutoff value of 5–10 million for pre-wash samples and the total motility to be at least 30% for pre-washed samples. The authors of this review indicated the necessity of further prospective cohort studies investigating the predictive value of semen parameters on the IUI pregnancy rate.

Contradictory results have been reported in studies investigating the relationship between sperm morphology and IUI results.[20,21] Despite a large population study, which used multivariable model to discriminate accuracy and presented IUI relevant for couples with moderate male factor infertility of sperm morphology below 4%,[22] Sun et al.[23] were reported that IUI did not contribute to pregnancy in patients over 35 years of age with a normal morphology rate of <5%. In our study, we have noticed that the percentage of normal sperm morphology was higher in patients with successful outcomes (19.7% vs. 27.1%), but it was not statistically significant (p=0.3).

Our study data revealed that both pre-cycle and intracycle total progressive motile sperm count were significantly associated with clinical pregnancy. However, following analysis of adjusted associations, pre-cycle total progressive motile sperm count was found to be one of the most important predictors for IUI success as expected (p=0.004).

Analysis of adjusted associations showed us that intracycle duration of abstinence was significantly associated with the favorable outcome (mean 3.37 days [95% CI 3.3–3.44]). In many studies, prolonged abstinence was found inversely proportional to parameters of sperm quality based on the epididymal sojourn of sperm.[11] According to our data, it seems the duration of abstinence was longer in the pregnant group (3,3 days vs. 3,6 days), while total progressive motility (A + B) and sperm volume were increased.

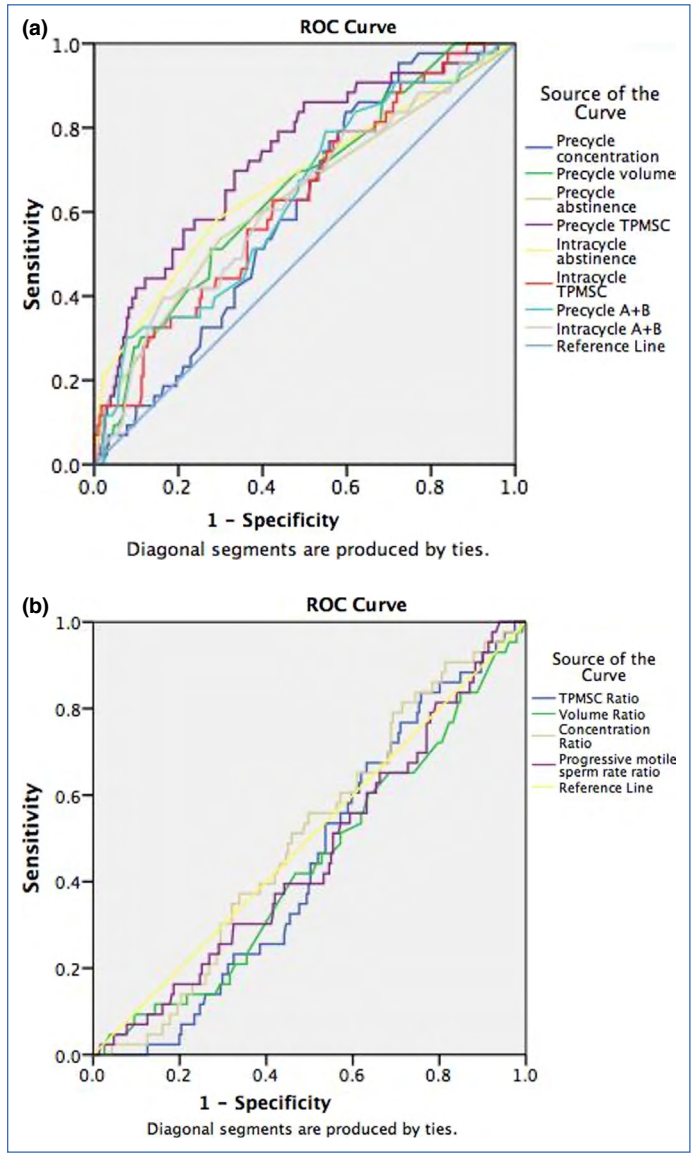

Figure 1: (a) ROC analysis of each pre- and intra cycle spermiogram parameters for clinical pregnancy. **(b)** ROC analysis of ratios of pre- and intra cycle spermiogram parameters to predict clinical pregnancy.

As mentioned above, sperm quality of male IVF patients significantly deteriorated during oocyte retrieval, and an inverse relationship between sperm quality and psychological stress was demonstrated,[24] therefore, we decided to compare semen parameters assessed before and intracycle to investigate the effect of acute stress on IUI success. According to the study on acute immobilization-induced stress on the reproductive endocrine system of rats, it is reported that acute stress activated the hypothalamo-pituitary-adrenal axis with increased adrenocorticoid activity resulted damaged hypothalamo-pituitary-gonadal axis with decreased follicle stimulating hormone, luteinizing hormone, testosterone, and inhibin levels. They demonstrated that the acute stress suppressed sperm motility in 30 min. This mechanism is explained by acute alteration on corticosterone levels, which directly affects epididymis and neuronal system control through the neuronal receptor of the sperm of stressed rats.[25]

Although stimulation potency of the hypothalamo-pituitary-adrenal axis by stress depends on the type, period, or duration of the stressor;[26] on the IUI day psychological stress of patient as anxiety of success and competition over a limited time, there may be a destructive effect on sperm parameters. It was reported that adrenergic activation triggered by psychological stress may result in more vasoconstriction in the testicle with decreased testosterone production and impaired spermatogenesis.[27]

While it is hard to make men to declare their anxiety and stress of sexual performance, studies found self-reported "daily-life-stress" strongly linked with semen parameters.[28] One study reported a negative correlation between stress and semen parameters of volume, concentration, motility, and the rate of normal morphology.[29] The other study did not find any association between stress and decreased fecundability.[30]

In our study, we did not assess the pre- or intracycle anxiety score of a male couple, but analyses of the data showed us that the sperm concentration and sperm volume on IUI day were significantly lower than pre-cycle values. However, although total motility was higher for intracycle assessment, there was no statistically significant difference for total progressive motile sperm count between the two measurements.

This study has strengths of relatively large sample size, pre- and intracycle comparisons, and adjustment for potential confounding. However, our design was retrospective which limited the ability to make causal inferences and assess the anxiety score.

CONCLUSION

In conclusion, intracycle duration of abstinence and pre-cycle total progressive motile sperm counts were significantly associated with IUI cycle success in cycles with optimal female partner reproductive properties.

Statement

Ethics Committee Approval: The Zeynep Kamil Maternity and Children's Training and Research Hospital Clinical Research Ethics Committee granted approval for this study (date: 03.06.2020, number: 100).

Informed Consent: Written informed consent was obtained from patients who participated in this study.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept – MBY, BD; Design – MBY, BD; Supervision – MBY, BD; Resource – MBY, BD; Materials – MBY, BD; Data Collection and/or Processing – SAK, ZÇ; Analysis and/or Interpretation – EÖ; Literature Search – EÖ; Writing – EÖ; Critical Reviews – MBY, BD, SAK, ZÇ, EÖ.

Conflict of Interest: The authors have no conflict of interest to declare.

Financial Disclosure: The authors declared that this study has received no financial support.

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