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Research Article

SPERM PARAMETERS' PREDICTIVE VALUE IN INTRAUTERINE INSEMINATION SUCCESS: A SINGLE-CENTER EXPERIENCE

Muhamet AFSİN*[1] Ayse Feyda NURSAL[2] Serap Mutlu ÖZCELİK OTCU[3] Dilek YAVUZ[1] Serhat EGE[4]

[1]Dep. of Andrology, Health Sciences University, Gazi Yasargil Edu. and Research Hospital, Diyarbakir, Turkiye
[2]Department of Medical Genetics, Hitit University, Faculty of Medicine, Corum, Turkiye
[3]Dep. of Obstetrics and Gynecology, Health Sci. Uni., Gazi Yasargil Edu. and Res. Hos., Diyarbakir, Turkiye
[4]Department of Obstetrics and Gynecology, Dicle University, Faculty of Medicine, Diyarbakir, Turkiye
* Corresponding author; afsin.muhammet21@hotmail.com

Abstract: *Although intrauterine insemination is a widely used assisted reproductive technique no consensus on sperm parameters affects the probability of pregnancy. Therefore, the purpose of this study was to determine whether semen parameters affect intrauterine insemination (IUI) success. A total of 403 couples (345 negative pregnancies, and 58 positive pregnancies) that underwent 549 intrauterine insemination treatment cycles for heterogenous indications were included in the study. Clomiphene citrate, letrazole, and/or gonadotropins were used for ovarian stimulation in women in this study. The spermiogram tests of the spouse receiving IUI treatment were examined. Clinical pregnancy occurred in 58 of 549 intrauterine insemination (10.56%). Unexplained infertility, polycystic ovary syndrome, and cervical-tubal factors were higher in the positive pregnancy group compared to the negative pregnancy group while the malefactor was more prevalent in the negative pregnancy group compared to the positive pregnancy group (p=0.03). There was no statistical difference between women with positive and negative pregnancies in terms of age (≤ 35 and > 35), body mass index, infertility type, infertility time, endometrial thickness on HCG day, stimulation protocol, cycle numbers, number of insemination, estradiol, prolactin, LH, FSH, and TSH levels (p>0.05). The sperm characteristics in males [ejaculate volume, sperm concentration, total sperm count, motility, immotility, and total progressively motile sperm count] did not significantly differ between positive and negative pregnancy groups (p>0.05). The present study found that the conception probability of intrauterine insemination did not correlate with the spectrogram parameters.*

Keywords*: İntrauterine insemination, infertility, sperm parameters.*

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1. Introduction

Infertility means the inability to conceive after regular intercourse for one year without contraceptives. 10-15 % of the couples are not fertile [1]. Intrauterine insemination (IUI) is the first-line treatment, a cost-effective and noninvasive procedure for treating infertile couples. This treatment is done by inserting a higher concentration of the made sperms into the uterine cavity. Infertile couples have used this technique for many years to treat fertility [2]. They are using the IUI technique combined with controlled ovarian stimulation led to higher hope for infertile couples' pregnancy. For unexplained infertility, it has been reported that the pregnancy rate with IUI has been 9 to 20% [3,4].

Many factors have played a potential role in predicting and optimizing the success of IUI, including duration and type of infertility, endometrial thickness, number of mature follicles, and different seminal parameters.

The first step is a semen analysis is to diagnose male infertility accurately. According to the WHO's guidelines, the volume, concentration, abnormal and normal morphology, viscosity, and motility of spermatozoa are described by standard semen quality assessment [5]. The *sperm morphology using strict criteria, * Total motile sperm count (TMSC), and * Number of motile spermatozoa inseminated (NMSI) are the most frequently examined sperm parameters concerning pregnancy rates in the native sperm sample [6]. Total motility is the most common parameter in the native sperm sample. According to a systematic review, a morphology > 4% and a TMSC > 1 million are of possible prognostic value. In such a case, IUI should be withheld below these cut-off levels [6]. However, the evidence quality was low. The focus of the previous studies has been on the effect of follicle size and hormonal stimulation on the assisted reproduction techniques (ART) outcome and IUI outcome. Bensdorp et al. found a cumulative pregnancy rate of 47% after six IUI cycles with controlled ovarian hyperstimulation in twelve months [7].

Although IUI is a widely used ART, no consensus on sperm parameters affects the probability of pregnancy. Therefore, this study aimed to determine whether semen parameters affect IUI success.

2. Materials and Methods

2.1. Study population

In this study, 549 cycles of 403 infertile patients who underwent IUI after ovulation induction in the Infertility clinic of the Gazi Yasargil Education and Research Hospital, between January and May 2021 were examined. The couples suspected of primary or secondary infertility for 1 year or more and could not achieve spontaneous pregnancy were examined in this study. All participants were aged 40 years and below. Couples with below 1 year of marriage, over 40 years, and men with severe oligozoospermia-azoospermia were excluded from the study. All female patients underwent ovarian stimulation with human gonadotropin (HCG), clomiphene, or letrozole, or a combination of clomiphene or letrozole and HCG (8-10). Body mass index (BMI), estradiol (E2), prolactin, luteinizing hormone (LH), follicle-stimulating hormone (FSH), and thyroid-stimulating hormone (TSH) values were obtained from their files. Hormone analyses of all female patients were performed on the 3rd day of the menstrual period. The spirogram tests of the spouse receiving IUI treatment were examined.

Ethical Statement: The study was approved by Health Sciences University, Ethics Committee. (date and number of approval: 2021/858). This study was carried out according to the current Helsinki Declaration.

2.2. Semen analysis

Sperm samples were collected from the participants who were sexually abstinent for 2-7 days by masturbating into sterile disposable plastic cups without using any lubricant. Semen samples taken from the participants were examined in conformity with World Health Organization (WHO) criteria after liquefaction. The semen samples were first homogenized by pipetting with a Pasteur pipette. Approximately 10 µl of semen was pipetted and placed on the Makler camera (counting chamber) and sealed with a glass lid to determine the count and motility. Spermatozoa in 10 squares were counted through the x20 lens of the light microscope (Olympus CX31), and the result was expressed in millions. It was evaluated that sperm parameters including viscosity, leucocyte count, sperm

concentration, total sperm count, motility, immotilty, and total progressive motile sperm count (TPMSC).

2.3. Semen Preparation with the Density Gradient Separation

The density gradient separation removes seminal fluid and improves sperm quality for IUI. A 2-layer gradient was prepared using approximately 1.0 ml of each 45%-90% gradient in a conical tube. In a conical centrifuge tube, semen was layered onto the gradient and then centrifuged at 1800 rpm for 15 minutes. After the supernatant was removed, an additional 2-3 ml of washing medium was placed on it, and it was centrifuged again at 1800 rpm for 10 minutes. The upper part of the tubes was discarded again, and 0.5 ml of washing medium was added to the lower 0.5-1 ml part. It was withdrawn into the catheter and ready for intrauterine administration.

2.4. Statistical analysis

SPSS 21.0 (IBM SPSS Inc., Armonk, NY, USA) was used for statistical analysis. The normality of data distribution was tested with the Shapiro-Wilk test. When the data did not fit the normal distribution, Mann-Whitney U and Wilcoxon tests were used for pairwise comparison. Data were defined as mean and standard deviation. Student's t-test and Chi-Square test were used for statistical analysis to see whether there was a significant difference between the means of positive and negative pregnancy data. $p \leq 0.05$ was considered significant.

3. Results

A total of 403 couples (345 negative pregnancies, and 58 positive pregnancies) that underwent 549 IUI treatment cycles for heterogenous indications were included in the study. Clinical pregnancy occurred in 58 of 549 IUI (10.56%). There was a significant difference between women with positive and negative pregnancies in the causes of infertility. Unexplained infertility, polycystic ovary syndrome (PCOS), and cervical-tubal factors were higher in the positive pregnancy group than in the negative pregnancy group ($p=0.03$). Also, the malefactor was more prevalent in women with negative pregnancies than in the group with positive pregnancies. There was no statistical difference between women with positive and negative pregnancies in terms of age (≤ 35 and > 35), BMI, infertility type, infertility time, endometrial thickness on HCG day, stimulation protocol, cycle numbers, number of insemination, FSH, LH, E2, prolactin and TSH levels ($p>0.05$). Demographic and clinical findings of women who underwent IUI in the study are given in Table 1.

Table 1. Demographical and clinical characteristics of women who underwent IUI

Characteristics	**Positive pregnancy** (n: 58) (%)	**Negative pregnancy** (n: 345) (%)	**p**
Woman's age (years),			
≤ 35 n %	49 (84.5)	272 (78.8)	
> 35 n %	9 (15.5)	73 (21.2)	0.308
BMI (kg/m²)			
≤ 18.5, n (%)	2 (3.5)	7 (2.1)	
18.5 – 24.9, n (%)	27 (46.5)	167 (48.4)	0.757
25.0 – 29.9, n (%)	18 (31)	123 (35.6)	
≥ 30, n (%)	11 (19)	48 (13.9)	
Infertility type			
Primary infertility, n (%)	38 (65.5)	218 (63.2)	
Secondary infertility, n (%)	20 (34.5)	127 (36.8)	0.811
Cause of infertility			
Male factor, n (%)	5 (8.6)	88 (25.5)	0.03
Unexplained infertility, n (%)	46 (79.3)	234 (67.8)	
PCOS, n (%)	2 (3.5)	6 (1.7)	
Cervical-tubal factors, n (%)	4 (6.9)	10 (2.8)	
Low ovary reserve, n (%)	1 (1.8)	7 (2)	
Endometrial thickness on HCG day			
≤ 8 mm, n (%)	37 (63.8)	230 (66.7)	0.68
> 8 mm, n (%)	21 (36.2)	115 (33.3)	
Number of dominant follicles of day on HCG			
1	40 (68.9)	244 (70.7)	0.5
2	18 (31.1)	101 (29.3)	
Stimulation protocol			
CC (only), n (%)	10 (17.3)	66 (19.1)	0.7
Letrazole (only), n (%)	24 (41.4)	126 (36.5)	
CC + Letrazole, n (%)	8 (13.8)	47 (13.6)	
Gonodotropines (only), n (%)	13 (22.4)	96 (27.9)	
Letrazole + Gonodotropin, n (%)	3 (5.1)	10 (2.9)	
Infertility period, years, mean ± SD	3.13 ± 2.35	3.34 ± 2.62	0. 847
Cycle numbers, mean ± SD	2.94 ± 1.60	3.43 ± 2.07	0.126
Number of insemination, mean ± SD	1.25± 0.36	1.41 ± 0.64	0.534
FSH (IU/L)			
≤ 8, n (%)	53 (91.4)	287 (83.2)	0.119
> 8, n (%)	5 (8.6)	58 (16.7)	
LH (IU/L), mean ± SD	5.84 ± 3.30	7.00 ± 5.48	0,098
E2 (pg/mL), mean ± SD	39.30 ±18.14	37.36 ± 24.13	0.599
Prolactin (µg/L), mean ± SD	16.23 ± 9.06	14.63 ± 9.19	0.395
TSH (mU/L), mean ± SD	1.72 ± 0.86	1.73 ± 1.02	0.69

BMI: Body Mass Index; CC: Clomiphen Citrate; E2: Estradiol; FSH: Folıcule Stimulating Hormone; HCG: Human Corıonic Gonadotropin; LH: Luteinizing Hormone; PCOS: Poly Cystic Ovary Syndrome; SD: Standart Deviation; TSH: Thyroid Stimulating Hormone.

Then, we examined the demographic features and sperm characteristics of the males of positive and negative pregnancy groups. The demographic and sperm characteristics(ejaculate volum, sperm concentration, total sperm count, motility, immotility, and TPMSC) in males did not significantly differ between positive and negative pregnancy groups ($p>0.05$). The results of demographic characteristics and sperm parameters are presented in Table 2.

Table 2. Comparison of demographic characteristics and sperm parameters in the partners with pregnancy (+) and pregnancy (-) women undergoing IUI

Characteristics	**Positive pregnancy** (n: 58) (%)	**Negative pregnancy** (n: 345) (%)	**p**
Age (years),			
≤ 35, n (%)	41 (70.7)	234 (67.8)	0.717
> 35, n (%)	17 (29.3)	111 (32.2)	
Abstinence period (day), mean ± SD	3.07 ± 0.29	3 ± 0.35	0.232
Ejaculate volum (ml), mean ± SD	2.61 ± 1.04	2.95 ± 2.16	0.194
Sperm concentration (million/ml), mean ± SD	56.39 ±39.03	50.65 ± 38.78	0.29
≤ 15, n (%)	6 (10.3)	67 (19.5)	
15-90, n (%)	41 (70.7)	238 (68.9)	0.18
≥ 90, n (%)	11 (19)	40 (11.6)	
Total sperm count (million), mean ± SD	149.69 ± 122.49	140.31 ± 113.65	0.56
≤ 39, n (%)	7 (12,1)	75 (21,7)	0.09
> 39, n (%)	51 (87,9)	270 (78,3)	
Motility (%)			
Progressive (%), mean ± SD	48.05 ± 17.34	52.12 ± 17.73	0.1
≤ 32, n (%)	12 (20.7)	50 (14.5)	0.2
> 32, n (%)	46 (79.3)	295 (85.5)	
Non progressive (%), mean ± SD	8.9 ± 4.6	8.1 ± 5.5	0.31
Immotility (n,%), mean ± SD	42.8 ± 16.56	39.74 ± 16.76	0.19
TPMSC (million), mean ± SD	75.90 ± 79.00	77. 0 ± 70.55	0.99
≤ 30, n (%)	14 (24.2)	121 (35)	0.13
> 30, n (%)	44 (75.8)	224 (65)	

SD: Standart Deviation; TPMSC: Total Progressive Motile Sperm Count

4. Discussion

Although there were revolutionary advances in assisted reproduction, such as intracytoplasmic sperm injection (ICSI), in vitro fertilization (IVF), IUI, and subzonal insemination (SUZI), it remains a noninvasive, inexpensive, and effective first-line therapy for the selected patients with moderate male factor, cervical factor, immunological infertility, unexplained infertility, and infertility because of ejaculatory disorders. It is also suggested as a therapy for ovarian dysfunction, endometriosis, and even tubal factors [11]. Now, male factor infertility is increasing, and the clinical diagnosis is only based on semen parameters. A few studies have only evaluated the predictive value of the WHO criteria for infertile couples' semen [12,13]. Based on the previous studies, a criterion for choosing between IVF and IUI was the total number of motile spermatozoa of semen, suggesting the threshold values of 5-10 million [14]. However, Van Voorhis et al., Akanji et al., and Dorjpurev et al. suggested that IUI is possible when TMSC is more than 10 million. It was also found that motile sperm inseminated ≥ 10 million and normal sperm morphology ≥ 5% are useful prognostic factors of IUI cycles [15-17]. In addition, it was reported that normal sperm morphology ≥ 5% and motile sperm

inseminated ≥ 10 million are useful markers for IUI [18]. However, some studies consider the number of motile sperm counts over one million as an important predictive parameter in IUI [6,19]. According to the publication based on a longitudinal cohort study, the prewash TMSC better correlated with the ongoing pregnancy rate of natural conception than the classification by WHO 2010 [20]. Also, Ates et al. found a correlation between initial progressive motile sperm count and sperm count after washing [21]. According to Yalti's report, the TMSC, percent, and motility of fast motile sperm were the independent predictors of successful IUI [22]. In a study in Turkey, it was found that there was no statistically significant difference between the TPMSC ≤ 10 million and TPMSC >10 million groups and between the morphology ≤ 4% and morphology > 4% groups in terms of clinical pregnancy rates [23]. Irrespective of this, there is still no sufficient evidence concluding that IUI affects the mild male factor infertility [24]. The reports published have been arguable in this field. Several studies found no association between sperm and outcome count after IUI. It was declared that none of the sperm parameters predicted the pregnancy after the first IUI cycle [25]. In an analysis evaluating 20 observational studies, it was reported that there is no clinical difference between those normal and abnormal sperm morphologies in IUI pregnancy success when total motile sperm count and female age are taken into account [26].

Therefore, we aimed to analyze the influence of semen parameters, including sperm concentration, total sperm count, motility, immotility, and TPMSC, on the IUI results. In our study, we evaluated the data of 403 couples as a single center. In the present study, 58 positive pregnancies were achieved from 549 IUI cycles, resulting in a 10.56% rate. Our results are in line with other data. We also examined the features of the women participating in the study. In this evaluation, the cause of infertility in women showed a different distribution in a positive and negative pregnancy. The malefactor was more prevalent in the negative pregnancy group than the positive pregnancy group, while PCOS and cervical-tubal factors were higher in the positive pregnancy group than the negative pregnancy group (Table 1) There was no significant relationship between spectrogram parameters ejaculate volume, sperm concentration, total sperm count, motility, immotility, and TPMSC) and the probability of pregnancy in women who underwent IUI (Table 2).

This study has some limitations. Our study was conducted in a single center and was a heterogeneous group. However, the number of participants was crowded. Another important limitation of our study is that the sperm morphology of these patients could not be examined. It can also be considered an advantage to evaluate many parameters.

5. Conclusion

The present study found that the conception probability of IUI did not correlate with the spectrogram parameters. The current results should be cautiously interpreted, which should be confirmed by further research with advanced study analysis and design.

Ethical Statement:

The study was approved by the Health Sciences University Ethics Committee. (date and number of approval: 2021/858).

Informed Consent:

Written informed consent was obtained from subjects and patients who participated in this study.

Conflict of Interest:

The authors declared no conflicts of interest concerning the authorship and/or publication of this article.

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Author Contributions:

M.A.; A.F.N: Writing - Original draft preparation
M.A, D.Y, S.E., S.M.O: Methodology
M.A.; A.F.N: Formal analysis, Writing
M.A, D.Y, S.E., S.M.O: Conceptualization, Methodology
M.A.: Investigation
A.F.N: Resources
All authors read and approved the final manuscript.

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