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# Comparison of semen parameters between pregnant and nonpregnant couples with male factor infertility during intrauterine insemination*

**Aim:** To compare the semen parameters between pregnant and nonpregnant couples with male factor infertility during intrauterine insemination (IUI).

**Materials and Methods:** The study included a total of 156 IUI cycles performed in our center from January 2005 to December 2006 with the indication of male infertility. IUI cycles were divided into 2 groups: group 1 pregnancy (24 cycles) and group 2 (132 cycles) nonpregnancy cycles.

**Results:** In both groups, progressive motility of neither initial nor processed sperm specimens was significantly different ($P > 0.05$). When comparisons of semen parameters in groups were performed in the initial specimen, sperm concentration and total motile sperm count (TMC) were significantly different between the groups ($P = 0.03$, $P = 0.04$, respectively). After processing specimens a definite significant difference was found in sperm concentration and inseminated motile sperm count (IMC) between pregnancy and nonpregnancy cycles ($P = 0.03$, $P = 0.03$, respectively). Although TMC > $10 \times 10^6$ provided a pregnancy rate (PR) of 18% compared with TMC < $10 \times 10^6$ (PR: 10%), no significant differences were detected ($P > 0.05$).

**Conclusions:** In addition to the initial TMC and IMC, sperm concentration in both initial and processed specimens may influence IUI-related pregnancy in male factor infertility.

**Key words:** Male factor, semen parameter, sperm concentration, pregnancy

## Erkek faktör infertilitesine bağlı intrauterin inseminasyon yapılan çiftlerde semen parametrelerinin gebe kalan ve kalmayanlarda karşılaştırılması

**Amaç:** Erkek faktörüne bağlı infertilitesi bulunan, gebeliği olan ve olmayan çiftlerin semen parametrelerinin intrauterin inseminasyon İUİ sırasında karşılaştırmak.

**Materyal ve metodlar:** Çalışma, Ocak 2005 ile Aralık 2006 arasında erkek infertilite tanısıyla kliniğimizde uygulanan 156 İUİ siklusu içermektedir. İUİ siklusları, Grup 1, gebe (24 siklus) ve Grup 2, gebe olmayan (132 siklus) şeklinde iki gruba bölünmüştür.

**Bulgular:** Her iki grupta da, ne başlangıçta ne de işlem görmüş sperm örneklerindeki progresif motilite istatistiksel olarak anlamlı idi ($P > 0,05$). Gruplar arasında başlangıç semen parametreleri karşılaştırıldığında sperm konsantrasyonu ve total sperm sayısı (TSS) belirgin olarak anlamlıydı ($P = 0,03$, $P = 0,04$). Örneklere işlem uygulandıktan sonra gebe olan ve olmayan sikluslarda sperm sayısı ve insemine motil sperm sayılarında belirgin olarak bir farklılık bulundu ($P = 0,03$, $P = 0,03$). $10 \times 10^6$ dan fazla TSS olduğunda % 18' lik bir gebelik oranı sağlanmışken, $10 \times 10^6$ dan daha az TSS'de bu oran % 10 dır, istatistiksel olarak bir fark görülmemiştir ($P > 0,05$).

**Sonuçlar:** Erkek faktörlü infertilitede, TSS ve IMS'ye ek olarak örneklerdeki sperm konsantrasyonu da İUİ' ye bağlı gebelik sonuçlarını etkileyebilir.

**Anahtar sözcükler:** Erkek faktörü, semen parametresi, sperm konsantrasyonu, gebelik

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## Introduction

The most common indications for intrauterine insemination (IUI) are mild to moderate male infertility. Male factor infertility accounts for up to half of all cases of infertility and affects one man in 20 in the general population (1). Many clinicians recommend IUI as the initial assisted-reproductive technology (ART) for couples with male factor infertility when cause-specific therapy has failed. IUI is simpler, less invasive, and less expensive than in vitro fertilization/intra-cytoplasmic sperm injection (IVF/ICSI) (2). Many factors influence its efficacy, and knowledge of these factors is of great importance for IUI success. Male partner assessment begins with the semen analysis. Interpretations of results depend on many variables such as collection technique, method of analysis, and normal values. Traditionally, sperm count, motility, and the percentage of the sperms with normal morphology are the criteria employed to evaluate semen quality. Although the World Health Organization's (WHO) reference values for semen analysis are commonly used to assess sperm quality, predictive sperm parameters and threshold values with respect to semen characteristics for successful IUI are still controversial (3). In studies the predictive value of sperm parameters in IUI shows a wide range of differences among centers.

The aim of this study was to compare the semen parameters in pregnant and nonpregnant couples with isolated male factor infertility during IUI.

## Materials and methods

The retrospective study included a total of 156 IUI cycles performed in 109 couples in our center from January 2005 to December 2006 with the indication of male infertility. A total of 156 IUI cycles were retrospectively evaluated as the pregnancy group (24 cycles) and nonpregnancy group (132 cycles). Male factor was defined by more than one semen analysis that did not meet the WHO criteria for either concentration or motility (i.e. concentration $\leq 20 \times 10^6$/mL and/or progressive motility of ≤50%).

Intrauterine insemination was performed in stimulated cycles. Patients underwent ovarian stimulation using clomiphene citrate or human menopausal gonadotrophin/recombinant FSH. Follicle growth was monitored by transvaginal sonography in all patients during the IUI cycle. When one follicle had at least a diameter of 18 mm, human chorionic gonadotrophin was administered in a single dose of 10,000 IU. After 3 days of abstinence, a semen specimen was collected in a sterile cup and was examined after 30 min liquefaction at 37 °C. Initial volume, sperm concentration, and progressive motility was assessed according to the WHO criteria. The semen samples were processed using density gradient separation in order to remove seminal fluid and enhance sperm quality for IUI. Briefly, a 2-layer gradient was prepared using approximately 1.0 mL each of a 90%-45% gradient (PureCeption, Sage In Vitro Fertilization, Inc., Trumbull, CT, USA) in a sterile conical centrifuge tube. The semen was layered onto the gradient and subsequently centrifuged at 1800 rpm for 15 min. The supernatant was removed, and the pellet was resuspended in approximately 2-3 mL of fresh human tubal fluid (HTF). Following an additional centrifugation at 1800 rpm for 10 min, the pellet was resuspended in 0.5 mL of fresh HTF for the insemination and postwash semen analysis was performed with a Makler Counting Chamber under a phase contrast microscope (Nikon, Japan) for concentration, motility, forward progression, and total motile sperm count. For IUI, 0.3-0.5 mL of sperm suspension was loaded into a Gynetics catheter (Gynetics Medical Products N.V. Hamont-Achel, Belgium) and then it was injected slowly into the uterus. Semen parameters before and after preparation for IUI were evaluated and compared with the presence of pregnancy. Total motile sperm count (TMC) and inseminated motile sperm count (IMC) were calculated as volume × concentration × motility for both initial specimen and processed specimens.

Data were shown as median interquartile range (IQR) for continuous variables and median (min-max) for discreet variables. Categorical variables were presented as percentage. Medians were compared using the Mann-Whitney U test.

## Results

The median age of women was 29 years (21-43) while that of males was 33 years (24-55) at the time of

IUI. The median infertility period was 3 years (IQR: 4.1). The median number of cycles per patients was 1 (IQR: 1). Pregnancy rate per cycle and per patient were 15.4% (24/156) and 22% (24/109), respectively. Baseline characteristics of the groups were similar (Table 1).

The median sperm concentration and total motile sperm count (TMC) were $27 \times 10^6$/mL (IQR: 57.2) and $23 \times 10^6$ (IQR: 49) in the initial specimens, respectively. After processing, the median sperm concentration and IMC were $14.5 \times 10^6$/mL (IQR: 37) and $3 \times 10^6$ (IQR: 10), respectively. When comparison of semen parameters in pregnancy and non-pregnancy cycles were performed in the initial specimens, sperm concentration and TMC were significantly different between the groups ($P < 0.05$). Similar results in sperm concentration and inseminated motile sperm count (IMC) were obtained after processing the specimens ($P < 0.05$). In neither of the groups was initial or post-wash sperm volume or progressive motility different ($P > 0.05$) (Table 2).

The present study also attempted to determine the optimal values of these semen parameters in the pregnancy group. For initial sperm concentration, median sperm concentration ($27 \times 10^6$/mL, IQR: 57.2) was regarded as the reference level and initial sperm count was divided in quartiles. Although a linear correlation was shown with pregnancy rate, no significant differences were detected ($P > 0.05$) (Table 3). As median sperm concentration ($14.5 \times 10^6$/mL) was taken into consideration in processed specimens, sperm concentration > $14.5 \times 10^6$/mL provided a favorable pregnancy rate of 16.9% ($P = 0.02$) (Table 3). The relation between sperm concentration and pregnancy rates is presented in the Figure.

Table 1. Baseline characteristics in pregnancy and non-pregnancy groups.

| | Pregnancy group* | Non-pregnancy group* | P value |
|---|---|---|---|
| Age (years) | | | |
| Women | 30 (23-42) | 29 (21-43) | 0.71 |
| Man | 34 (26-45) | 32 (24-55) | 0.85 |
| Infertility period (year) | 2.7 (1-15) | 3 (1-25) | 0.35 |
| Number of cycle | 1 (1-6) | 1 (1-6) | 0.48 |

* Data are presented as median (min-max)

Table 2. Comparison of sperm parameters between the groups.

| | Pregnancy group* | Non-pregnancy group* | P value |
|---|---|---|---|
| **Initial specimen** | | | |
| Sperm volume (mL) | 3.2 (2.5) | 3 (2) | 0.78 |
| Sperm concentration ($10^6$/mL) | 34 (57) | 25 (34) | 0.03 |
| Progressive motility (%) | 34 (19) | 32 (24) | 0.97 |
| TMC ($10^6$) | 38 (49) | 20 (36) | 0.04 |
| **Processed specimen** | | | |
| Sperm volume (mL) | 0.2 (0.1) | 0.3 (0.3) | 0.18 |
| Sperm concentration ($10^6$/mL) | 27 (37) | 12 (24) | 0.03 |
| Progressive motility (%) | 100 (0) | 100 (22) | 0.06 |
| IMC ($10^6$) | 6 (10) | 2 (6) | 0.03 |

* Data were presented as median (interquartile range = IQR)

Table 3. The association of sperm count and pregnancy rate.

| | Sperm concentration ($10^6$/mL) | Pregnancy rate (%) |
|---|---|---|
| Initial specimen | | |
| I. | <15.7 | 7.7 |
| II. | 15.7-27 | 10 |
| III. | 27-54.2 | 18 |
| IV. | >54.2 | 26 |
| P > 0.05 | | |
| Processed specimen | | |
| | <14.5 | 9.4 |
| | >14.5 | 16.9 |

P: 0.02

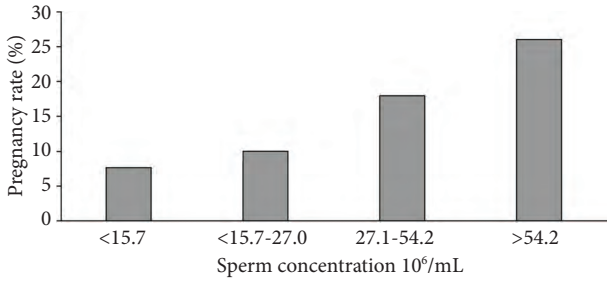


Figure. Relationship between sperm concentration and pregnancy rates.

Because there are several reports describing a threshold phenomenon for the effects of TMC on the success of IUI, we categorized patients depending on the average TMC as ≤10 million and TMC as >10 million. Although TMC > 10 million provided a pregnancy rate (PR) of 18%, the PR was 10% in the TMC ≤10 million group, and no significant differences were detected (P > 0.05) (Table 4). In the present study median IMC was 3 million (IQR: 0.7). The lowest IMC count resulting in pregnancy was 0.3 × $10^6$. According to the literature, IMC was divided into 2 groups (4). Although lacking statistical significance, in the number of IMC > 1 × $10^6$ group, PR was 16.9% but in the IMC of < 1 × $10^6$ group it was 9.4% (Table 4). The insufficient number of patients of the study may have had a negative effect; if more patients were enrolled in the study more meaningful results would be obtained.

Table 4. Association of TMC, IMC, and pregnancy rates.

| TMC ($10^6$) | Pregnancy rate (%) |
|---|---|
| >10 | 18 |
| ≤10 | 10 |
| P > 0.05 | |
| IMC ($10^6$) | Pregnancy rate (%) |
| > 1 × $10^6$ | 16.9 |
| < 1 × $10^6$ | 9.4 |
| P > 0.05 | |

With regard to sperm morphology by Kruger's strict criteria, it was disregarded because of insufficient data from one of the centers and so morphology did not correlate with any of the factors measured in this study.

## Discussion

IUI is generally attempted before resorting to more expensive and invasive assisted-reproductive techniques. In couples with male infertility, IUI more than doubles the pregnancy rate compared with intracervical insemination or timed natural cycles. Although it is well known that different semen parameters may predict success after IUI, there is no consensus about the standardization of semen analysis. Several studies confirmed that sperm morphology using strict criteria and the inseminating motile sperm count (IMC) after sperm preparation is the most important sperm parameter to assess the real impact of semen quality on IUI outcome (5-7). Miller et al. reported significantly lower PR for couples with less than 10 million processed total motile sperm (8). Huang et al. reviewed 939 couples undergoing 1375 cycles of IUI with varying etiologies of infertility. They found that a final post washed total motile sperm count was significantly higher in pregnancy versus non-pregnancy cycles and that this parameter strongly correlated with success after IUI. In this analysis, statistically significant improvements in

pregnancy rates were seen when the post washed total motile sperm count exceeded 5 million (9). Likewise, in our study, IMC level in the pregnancy group exceeded 5 million. However, in other studies including couples with isolated male factor infertility, processed total motile sperm count was not associated with pregnancy (10,11). In our study, we also detected that both initial and processed sperm concentration and TMC were significantly higher in the pregnancy group than in the non-pregnancy group. Similarly, Van Voorhis et al. reported that optimal cycle fecundity rates with IUI were obtained when the average TMC was 10 million and this might be a useful threshold value for decisions about treating a couple with IUI or IVF (12). In another study, Brasch et al. concluded that 3 million TMC was the minimal threshold for conception; however, statistically significant improvements in pregnancy rates were seen when the TMC exceeded 20 million and less than one-third of all pregnancies occurred in cycles with counts less than this value (13). It is clear that controversy still persists regarding the prognostic utility of the TMC and IMC, and the impact on IUI outcome. In our study, sperm motility was not significantly different between groups. In contrast to our study, Zhao et al. reported that sperm motility in the initial specimen is an independent factor influencing IUI-related pregnancy. A forward progression score of 3 to 4 in a processed specimen is necessary for IUI success. They also reported that processed sperm concentration between 51 and 100 million/mL and TMC ranging from 11 to 100 million per insemination offer the best potential for success (14). Likewise, Hendin et al. reported that sperm motility was associated with successful IUI outcome, but that study referred to postwash motility rather than motility in the initial semen specimen (15). In our study we also detected that, in addition to TMC and IMC, sperm concentration both in initial and processed specimens was significantly higher in pregnancy cycles in male factor infertility. Likewise, Dadkhah et al. reported that the higher number of sperms and total motile sperms have a positive relationship with PR (16). In contrast, several studies identified no relationship between sperm concentration and outcome with IUI. For example, Francavilla et al. reported that, in the absence of teratozoospermia, even severe oligospermia did not have a negative impact on outcome after IUI (17). Miller et al. also did not correlate sperm concentration and total motile sperm count, either before or after processing with IUI outcome. The differences may have resulted from the patient population, sperm quality, and sperm preparation techniques.

It is well known that both TMC and IMC in the ejaculate were calculated by multiplying the ejaculate volume by the sperm concentration by the percentage of motile sperm in the sample and therefore when initial and processed sperm concentration increases TMC and IMC are seen to be increased indirectly at the same time; as a result, pregnancy rates are augmented by the effect of these additive factors in male infertility. To date, many studies evaluating the effects of different semen parameters on IUI results have been conducted with varying etiologies of infertility. Isolated male factor infertility was the only reason in our patient population and this was the advantage of our study about the evaluation of the effects of different semen parameters on IUI. On the other hand, we are aware of the disadvantages of retrospective analysis but our results highlight the importance of performing a well organized prospective study on this matter.

In conclusion, if optimal thresholds are determined for isolated male factor, this will help us to encourage patients to proceed with more aggressive ART and avoid the physical, emotional, and financial burdens of IUI. The IUI data presented in this study demonstrate that, in addition to TMC and IMC, sperm concentration in specimens may influence IUI-related pregnancy rates in isolated male factor infertility.

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