

ORIGINAL PAPER

doi: 10.5455/medarh.2016.70.39-43

Med Arch. 2016 Feb; 70(1): 39-43

Received: August 25th 2015 | Accepted: October 05th 2015

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The Effects of Total Motile Sperm Count on Spontaneous Pregnancy Rate and Pregnancy After IUI Treatment in Couples with Male Factor and Unexplained Infertility

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ABSTRACT

Introduction: Male infertility factor is defined if the total number of motile spermatozoa (TMSC) < 20 × 10⁶/ejaculated, and unexplained infertility if spermogram is normal with normal female factor. **The aim** of this study was to determine the predictive value of TMSC for spontaneous pregnancy (ST) and pregnancy after treatment with intrauterine insemination (IUI) in couples with male factor and unexplained infertility. **What is known already:** According to the WHO qualification system abnormal spermogram can be diagnosed as oligozoospermia (O), asthenozoospermia (A), teratozoospermia (T) or combination (O+A+T) and azoospermia (A). Although this classification indicates the accuracy of findings its relevance for prognosis in infertile couple and the choice of treatment is questionable. **Materials and Methods:** The study included 98 couples with male infertility factor (bad spermogram) and couples with normospermia and normal female factor (unexplained infertility). Testing group is randomized at: group (A) with TMSC > 3,10⁶ / ejaculate and a spontaneous pregnancy, group (B) with TMSC < 3 × 10⁶ / ejaculate and pregnancy after IUI, plus couples who have not achieved SP with TMSC > 3 × 10⁶ / ejaculate and couples who have not achieved pregnancy. **Main results:** From a total of 98 pairs of men's and unexplained infertility, 42 of them (42.8%) achieved spontaneous pregnancy, while 56 (57.2%) pairs did not achieve spontaneous pregnancy. TMSC was significantly higher (42.4 ± 28.4 vs. 26.2 ± 24, p < 0.05) in the group A compared to group B. Couples with TMSC 1-5 × 10⁶ ejaculate had significantly lower (9.8% vs. 22.2%, p < 0.0001) rate of spontaneous pregnancy in comparison to couples after IUI treatment. Couples with unexplained infertility had significantly higher (56.8% vs. 29.9%, p < 0.01) spontaneous pregnancy rate compared to couples after IUI treatment. Infertile couples had significant pregnancy rate with TMSC 5-10 × 10⁶ / ejaculate (OR = 1.45, 95% CI: 1.26-1.78, < 0.01); with TMSC 10-20 × 10⁶ / ejaculate (OR = 1.36, 95% CI: 1.12 to 1.46, < 0.0001) with TMSC > 20 × 10⁶ / ejaculate (RR = 1.7, 95% CI: 1.56-1.82, < 0.001) after treatment with IUI compared to spontaneous pregnancy. **Conclusion / interpretation:** Based on these results we can conclude that couples with the TMSC > 5 × 10⁶ / ejaculate are indicated for treatment with IUI. TMSC can be used as the method of choice for diagnosis and treatment of male infertility.

Key words: Total motile sperm count. Male infertility. Unexplained infertility. Intrauterine insemination. Spontaneous ongoing pregnancy rate.

1. INTRODUCTION

The World Health Organization (WHO) designated the infertility as

a disease, and its treatment as one of the fundamental human rights. Infertility is defined as the inability

to conceive after 1 year of unprotected relations of couples in the reproductive age (1). Male infertility factor is the most common cause of not having children (2, 3). The diagnosis is based on the results of the analysis of semen. The WHO revised and defined cut-off value for differentiating normal and abnormal semen (4). Van der Steeg and his associates in 2011, tried to check the WHO criteria parameter analysis of seed in 1999 (5) for a spontaneous pregnancy, and it was concluded that the predictive value of the WHO classifications seed analysis was deficient. In other words, although the WHO proposed classification accuracy of seed analysis, relevance for prognosis and treatment choices are bad (6). The parameter for assessing the quality of sperm is the total motile sperm count (TMSC). The total number of mobile sperm has a better predictive value for spontaneous pregnancy (ST) than of the WHO classification system. Sperm morphology parameter is not used in this calculation (7). Van der Weert et al. (2004) showed in a meta-analysis of 16 studies that post wash TMSC between 0.8×10^6 and 5×10^6 has a prognostic value in couples who underwent IUI (8). Badawy et al. (2009) showed that the IUI is less successful when TMSC post wash is low with poor morphology (9). So, there is a need for new parameter values in seed analysis for clinical use in sorting out male infertility factors and selecting the treatment. The majority of the published studies show that IUI is more effective than planned sexual intercourse regardless of whether they are couples with unexplained infertility or male infertility. Intrauterine insemination shows a significant increase in pregnancy rates compared to the planned time sexual intercourse in natural cycles, regardless of the type of infertility (OR = 2.43) (10). The aim of this study was to determine the predictive value TMSC for spontaneous pregnancy (ST) and pregnancy after treatment with IUI in couples with male factor and unexplained infertility.

2. MATERIALS AND METHODS

Study design

Prospective study included examination of 223 infertile couples for marriage infertility with duration longer than 1 year. The study was conducted from January 2013 throughout December 2015 in the Department of Human Reproduction "Dr Hajder" Tuzla. Treatment of infertility was conducted in accordance with the guidelines for infertility (NICE) and consists of the examinations of both partners. All married couples underwent the following examinations: spermogram, analysis of sex hormones in the early follicular phase of the cycle, ultrasonic cycle monitoring (folliculometry), mid-luteinizing progesterone measuring, microbiological and immunological treatment of the infection, hysterosalpingography, diagnostic laparoscopy in indicated cases.

Study population

Total cohort consisted of 223 infertile couples. Of these, 115 pairs were excluded from the study due to female factor infertility, such as disorders of ovulation, tubal disease, endometriosis, cervical factor or sexual dysfunction. The study also excluded couples with a combination of female and male factors, couples in which the

male partner had azoospermia and couples with TMSC $<1 \times 10^6$ / ejaculate. The study included 98 couples with male infertility factor (bad spermogram) and couples with normospermia and normal female factor (unexplained infertility). The study group was randomized to: group (A) with TMSC $> 3 \times 10^6$ / ejaculate and a spontaneous conception; group (B) with TMSC $<3 \times 10^6$ / ejaculate and conception after IUI, plus couples who did not achieve SP with TMSC $> 3 \times 10^6$ / ejaculate and couples who did not achieve pregnancy. Both groups were classified in five subgroups based on TMSC subgroup (1) with TMSC $1-5 \times 10^6$ / ejaculate; subgroup (2) with TMSC $5-10 \times 10^6$ / ejaculate; subgroup (3) with TMSC $10-20 \times 10^6$ / ejaculate; subgroup(4) with TMSC $> 20 \times 10^6$ / ejaculate and subgroup (5) with unexplained infertility

Definition. Infertility is defined as the failure of conception, despite 12 months of unprotected intercourse (Ciglar). Normozoospermia is defined according to the WHO criteria, 2010. TMSC is calculated by multiplying the concentration of sperm / milliliter (SC) x volume (ml) x motility (A + B) divided by 100%. Sperm is normal if TMSC $> 20 \times 10^6$ / ejaculate, lower values are considered abnormal spermogram and define male infertility factor. Infertile couples with normozoospermia and normal female factor were considered unexplained infertility. The normal menstrual cycle is between 25 and 35 days, the level of progesterone in the medium luteal phase is > 27 mmol / l, the duration of the luteal phase > 11 days.

Treatments

Couples with TMSC $> 3 \times 10^6$ / ejaculate, with the chance of conceiving $> 30\%$ (mild cases) were given an advice on planned sexual relations with ultrasound monitoring of follicular growth. Six IUI cycles were offered to couples with TMSC from 1 to 3×10^6 / ejaculate with a forecast of conception $<30\%$ (moderate male infertility), and couples with TMSC $> 3 \times 10^6$ / ejaculate with a conception prognosis $> 30\%$, that did not achieve SP after 6 months of planned sexual intercourse. After six unsuccessful IUI cycles couples were offered in vitro fertilization (IVF) and were excluded from the study. Couples with TMSC $<1 \times 10^6$ / ejaculate were immediately offered in vitro fertilization by intracytoplasmic sperm injection (ICSI) and were excluded from the study.

Outcome measures

The primary objective was to determine the rate of spontaneous pregnancies without treatment and pregnancy rates after IUI treatment. Pregnancy is defined by ultrasound measurement of the fetal heart beat after the 12th week of pregnancy, and increased b-hCG after 1-2 days of the period, the values of which should be doubled when re-measurement after 48-72 hours.

Semen analysis

The men had their spermogram made on arrival at the Institute. In the case of abnormal spermogram, a second sample was analyzed 10 weeks later. Male partners gave a semen sample after 2-3 days of sexual abstinence. The seeds were stored in the Institute in sterile plastic container, or at home and delivered to the laboratory of the Institute within 1 hour. During the analysis volume was measured by a numbered syringes. The sperm was read

in Megler chamber, with an increase of 200 times MAKLER COUNTING CHAMBER for rapid sperm analysis (11). Mobility was expressed as a percentage of: (A) fast forward progressive sperm, (B) slowly forward progressive, (C) non-progressive (D) immovable sperm within the total number of 200 sperm in at least five fields, according to WHO guidelines (WHO, 2010). Sperm morphology was scored according to the criteria of Tygerberg (12), 200 spermatozoas were analyzed. This study used a floating techniques of seed processing that is simple and inexpensive. Treated seed is then inseminated with soft catheter (MedGyn intrauterine insemination catheter, MedGyn Products, Inc., USA).

Statistics

The data were entered into a database. Statistical analysis was performed by application software called SPSS for Windows version 16.0 (Statistical Package for Social Sciences, SPSS, Chicago, IL, USA) that was used for this analysis.

Numerical data were presented as mean + -SD, the absolute number is expressed in%, 95% confidence interval (CI). To test the hypothesis between the groups independent students t-test and Fisher exact test (univariate analysis) were used, with the level of significance of p <0.05. A binary logistic regression was performed for the dependent variable (such as pregnancy), which has two endpoints, i.e. spontaneous pregnancy and pregnancy after treatment IUI. Relative risk ratio (RR) and their 95% confidence interval (CI) was calculated for each subgroup TMSC.

3. RESULTS

The study included a total of 223 couples with marital infertility. After examination of patients, 115 of them (55%) had a female infertility factor, and were excluded from the study. According to the WHO criteria, after seed analysis, 108 pairs (45%) had male infertility factor, of which 10 (11%) had azoospermia and were excluded from the study. The study included 98 (89%) of couples with male and unexplained infertility. After analyzing semen according to the WHO criteria 76 couples (77.5%) had male infertility and unexplained infertility 22 (22.5%) pairs. According to TMSC range, male infertility, i.e.. Abnormal semen was found in 56 (57.1%) pairs, and unexplained infertility and unexplained infertility with normozoospermia 42 (42.9%) pairs. Results of clinical parameters in pairs with male factor and unexplained infertility who achieved the spontaneous pregnancy, pregnancy after treatment with IUI plus pairs which did not achieve any pregnancy are presented in Table 1. Of the total of 98 pairs, 42 (42.8%) achieved spontaneous pregnancy, while 56 (57.2%) pairs did not achieved spontaneous pregnancy. Results of univariate analysis showed significantly lower values of age (p <0.01), duration of infertility (p <0.001), less cigarette smokers (p <0.05), less surgical treated varicocele (p <0.01) in the group A, compared to the group B.

Results of univariate analysis of medium parameter TMSC were significantly higher in the group (A) compared to the group B. In the subgroup A statistically sig-

nificant higher values of the concentration of seeds were identified (SC) (p <0.01), the concentration of the total number of seeds (TSC) (p <0.01), semen motility (A + B)% (p <0.01) and total motile sperm count (TMSC) (p <0.001) in comparison to the group B, while there were no significant differences in the parameters of the volume of the seeds between the groups (Table 2).

After sorting the pairs by categories of the quality of the seeds, the results of univariate analysis showed that couples with TMSC with <5 × 10⁶ / ejaculate had a significantly lower chance of spontaneous pregnancy than couples with TMSC with > 5 × 10⁶ / ejaculate. Couples with TMSC 1-5 × 10⁶ /ejaculate showed significantly lower (p <0.01) chances of the spontaneous pregnan-

Variable	Total cohort N=98 (100%)	Group A N=42 (42.8%)	Group B 56 (57.2%)	P-value
Female age (years)	33±4.1	32.1±4.4	33.8±4.2	p<0.01
Duration of infertility (years)	3.6±1.4	1.3±0.9	5.2±2.6	p<0.001
Smoking (male+female) yes	36%	32%	39.6%	p<0.05
Drinking alcohol yes	16%	15.94%	18.1%	p<0.05
Varicococella yes	2.4%	1.4%	3.2%	p<0.01

Note: Values are the mean ± SD, number %, P<0.05 for A vs. Group B: Group A – spontaneous pregnancy; Group B–achieved pregnancy after treatment with IUI + unachieved pregnancy. Univariate analysis (t-test or x2 test with p<0.05 for Group A vs. Group B).

Table 1. Baseline clinical characteristics of couples with male infertility and unexplained

Variable	Total cohort	Group A	Group B	P-value
Volume (ml)	2.9±1.4	2.8±1.6	2.9±1.4	NS
SC 10 ⁶ /ml	32±1.2	40.6±20.6	29±24.6	p<0.01
TSC 10 ⁶ /ejaculate	92.5±7.4	112.5±15.6	84.1±18.2	p<0.01
Motility (A+B)%	32.8±18.6	35.6±15.2	28±20.6	p< 0.05
Morfology (% normal)	14.7±12.6	15.8±10.2	13.9±12	p<0.8
TMSC 10 ⁶ /ejaculate	34±36.2	42.4±28.4	24±26.2	p<0.001

Note: values are the mean ± SD, Group A- spontaneous pregnancy; Group B–achieved pregnancy after treatment with IUI + unrealized pregnancy; TMSC–Total motile sperm count..Univariate analysis:, comparison between Group A vs. Group B Univariate analysis (t-test with p<0.05 for Group A vs. Group B).

Table 2. The basal characteristics of the parameters of semen analysis in couples with male factor and unexplained infertility

TMSC per category x 10 ⁶ /ejaculate	Total cohort (%)	Group A (%)	Group B (%)	P-value
1 – 5	16.2	9.8	22.2	p<0.01
5 – 10	10.3	11.1	12.1	p<0.9
10 – 20	14.2	13.1	15.7	p<0.9
>20 (normalized)	4.8	4.1	5.4	p<0.08
Unexplained	42.5	56.8	29.9	p<0.01

Note: Values are expressed as a percentage of the number , Group A- spontaneous pregnancy; Group B–pregnancy achieved after treatment with IUI + unrealized pregnancy; TMSC–Total motile sperm count. Univariate analysis:, comparison between Group A vs. Group B Univariate analysis (t-test with p<0.05 for Group A vs. Group B).

Table 3. TMSC groups sorted by the quality seeds categories of couples with male factor and unexplained infertility

cy compared to the pairs after IUI treatment. Couples with unexplained infertility had significantly higher rate of spontaneous pregnancy ($p < 0.01$) compared to pairs after treatment. The difference was not significant (NS) in the categories of TMSC with TMSC 5-10; 10-20: > 20 $\times 10^6$ /ejaculate in a group A compared to the group B (Table 3).

Table 4 shows a comparison between RR spontaneous pregnancies and pregnancies after IUI treatment according to TMSC categories. Infertile couples that are sorted by categories with TMSC 5-10; 10-20; > 20 $\times 10^6$ / ejaculate had a significantly higher pregnancy rates after IUI treatment. Subgroup with TMSC 5-10 $\times 10^6$ / ejaculate had significantly higher (RR = 1.45, 95% CI: 1.26-1.78, <0.01) pregnancy rate in group II than in the group I.

Subgroup with TMSC 10-20 $\times 10^6$ / ejaculate showed higher (RR = 1.36, 95% CI: 1.12 to 1.46, <0.01) pregnancy rate in group (I + II) in comparison with group I. Subgroup with TMSC > 20 $\times 10^6$ / ejaculate showed higher (RR = 1.7, 95% CI: 1.56-1.82, <0.001) pregnancy rate in group (I + II) in comparison to group I. While there were no significant difference t (NS) in TMSC subgroup with 1-5 $\times 10^6$ / ejaculate and subgroup with unexplained infertility after IUI treatment in comparison to the rate of spontaneous pregnancy.

TMSC per category $\times 10^6$ /ejaculate	Group I (%)	Group (I+II) (%)	RR	95% CI	P-value
1 - 5	24	30	1.25	0.95-1.41	NS
5 - 10	40	58	1.45	1.26-1.78	$p < 0.01$
10 - 20	41	56	1.36	1.12-1.46	$p < 0.01$
>20 (normalized)	34	58	1.7	1.56-1.82	$p < 0.001$
Unexplained	62	70	1.13	0.85-1.31	NS

Note: Values are expressed as a percentage of the number with 95% CI. Group I- spontaneous pregnancy; Group II - Group I-spontaneous pregnancy; Group II -Total achieved pregnancy (spontaneous pregnancy + pregnancy after treatment with IUI). TMSC-Total motile sperm count. Binary logistic regression analysis, (RR, 95% CI, $p < 0.05$).

Table 4. Rate comparisons of spontaneous pregnancies (group I) and total pregnancies (I + II) after treatment sorted by TMSC categories in couples with male factor and unexplained infertility.

4. DISCUSSION

According to the new WHO classification system normozoospermia is based on the cut-off values obtained on a sample of 1,953 couples with proven fertility. Although this classification indicates the accuracy, relevance for prognosis in infertile couple and the choice of treatment is questionable. Abnormal spermogram can be diagnosed as oligozoospermia (O), astenozoospermia (A), teratozoospermia (T) or a combination of these, and azoospermia (image x) .. TMSC is obtained by multiplying the volume of semen \times concentration \times the percentage of concentration of A and B sperm divided by 100. TMSC shows a better correlation with spontaneous pregnancy rate (SOPR) than the WHO classification system from 2010. For everyday practice, there are three prognostic groups: couples with TMSC < 5 $\times 10^6$, couples with TMSC 5- 20 $\times 10^6$ and couples with TMSC greater than 20 $\times 10^6$ ejaculate normozoospermia (7). It is significant to point out that there is no difference

in SOPR if TMSC was < 1 $\times 10^6$, 10⁶ \times 1-3 or 3-5 $\times 10^6$. Spontaneous pregnancy are possible in the presence of extremely poor quality of sperm, although most doctors recommend ICSI. However it was noted that a quarter of couples conceived spontaneously. There is still evidence that the semen analysis is weak predictor of pregnancy in the low range (13) 345 consecutive couples presenting for subfertility. It is also illogical that 20% of couples with unexplained infertility and normospermia did not conceive (7). The National Institute for Health and Care (NICE) guidelines in fertility recommended repeated spermogram in the event that the first was abnormal (7).

Results of this study showed that spontaneous pregnancy are present in all subgroups of TMSC. After treatment with IUI there was a statistically significant increase in pregnancy rates in the subgroup of TMSC > 5 $\times 10^6$ ejaculate, while in sub-groups with TMSC < 5 $\times 10^6$ / ejaculate and unexplained infertility after IUI pregnancies were reported, but without statistically significant differences. IUI is less expensive and less invasive than other ART methods, and this therapeutic treatment should be offered to couples with male factor and unexplained infertility as the first choice of treatment (14). Intrauterine insemination shows a significant increase in pregnancy rates compared to the planned time sexual intercourse in natural cycles, regardless of the type of infertility (OR = 2.43). In the analysis of 3,662 cycles showed that intrauterine insemination shows a significant increase in pregnancy rates compared to the planned time sexual intercourse in natural cycles, regardless of the type of infertility (OR = 2.43). It is also proven that the cycles of intrauterine insemination with controlled ovarian hyperstimulation (COH) show higher pregnancy rates (OR = 2.2) compared to the time planned intercourse in a natural cycle (10). Ayala et al. (1996) showed that in the group of 1,055 infertile couples TOPR was significantly higher if the TMSC was greater than 25 $\times 10^6$ ejaculate compared to TMSC under 25 $\times 10^6$ / ejaculate [RR = 6.1, 95% CI: 7.4.-9.7] (15) 055 infertile couples seen at the Texas Institute for Reproductive Medicine and Endocrinology for a total of 9,409 follow-up months. The medians of sperm concentrations (SC. Van der Weert et al. (2004) showed in a meta-analysis of 16 studies that post wash TMSC between 0.8 $\times 10^6$ and 5 $\times 10^6$ ejaculate had prognostic value in couples undergoing IUI (8). Badawy et al. (2009) showed that the IUI was less successful when the total number of motile spermatozoa was low and post wash morphology was bad (9). Hamilton et al. (2015) in their study indicated that TMSC is better diagnostic and prognostic method for male infertility factor (7). Pregnancy after IUI were present in all subgroups TMSC, even in subgroups with TMSC < 1 $\times 10^6$ ejaculate.

5. CONCLUSION

TMSC is a better method for the classification of male infertility factor than the WHO classification system. IUI should be used as first-line therapy method of ART for male infertility factor with TMSC > 5 $\times 10^6$ ejaculate and unexplained infertility.

- **Author's contribution:** All authors contributed in all phases of preparing this article. Final proof reading was made by first author.
- **Conflict of interest: none declared.**

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