**Human Reproduction, Vol.30, No.5 pp. 1110–1121, 2015**
Advanced Access publication on March 18, 2015 doi:10.1093/humrep/dev058

human reproduction

ORIGINAL ARTICLE *Infertility*

# Total motile sperm count: a better indicator for the severity of male factor infertility than the WHO sperm classification system

**J.A.M. Hamilton[1,*], M. Cissen[1], M. Brandes[3], J.M.J. Smeenk[2], J.P. de Bruin[1], J.A.M. Kremer[3], W.L.D.M. Nelen[3], and C.J.C.M. Hamilton[1]**

[1]Jeroen Bosch Hospital, 's-Hertogenbosch, The Netherlands [2]St. Elisabeth Hospital, Tilburg, The Netherlands
[3]Radboud University Medical Center, Nijmegen, The Netherlands

*Correspondence address. Tel: +31-642358109; E-mail: jozefienhamilton@gmail.com

*Submitted on November 16, 2014; resubmitted on February 9, 2015; accepted on February 20, 2015*

**STUDY QUESTION:** Does the prewash total motile sperm count (TMSC) have a better predictive value for spontaneous ongoing pregnancy (SOP) than the World Health Organization (WHO) classification system?

**SUMMARY ANSWER:** The prewash TMSC shows a better correlation with the spontaneous ongoing pregnancy rate (SOPR) than the WHO 2010 classification system.

**WHAT IS KNOWN ALREADY:** According to the WHO classification system, an abnormal semen analysis can be diagnosed as oligozoospermia, astenozoospermia, teratozoospermia or combinations of these and azoospermia. This classification is based on the fifth percentile cut-off values of a cohort of 1953 men with proven fertility. Although this classification suggests accuracy, the relevance for the prognosis of an infertile couple and the choice of treatment is questionable. The TMSC is obtained by multiplying the sample volume by the density and the percentage of A and B motility spermatozoa.

**STUDY DESIGN, SIZE, DURATION:** We analyzed data from a longitudinal cohort study among unselected infertile couples who were referred to three Dutch hospitals between January 2002 and December 2006. Of the total cohort of 2476 infertile couples, only the couples with either male infertility as a single diagnosis or unexplained infertility were included ($n = 1177$) with a follow-up period of 3 years.

**PARTICIPANTS/MATERIALS, SETTING, METHODS:** In all couples a semen analysis was performed. Based on the best semen analysis if more tests were performed, couples were grouped according to the WHO classification system and the TMSC range, as described in the Dutch national guidelines for male infertility. The primary outcome measure was the SOPR, which occurred before, during or after treatments, including expectant management, intrauterine insemination, in vitro fertilization or intracytoplasmic sperm injection. After adjustment for the confounding factors (female and male age, duration and type of infertility and result of the postcoital test) the odd ratios (ORs) for risk of SOP for each WHO and TMSC group were calculated. The couples with unexplained infertility were used as reference.

**MAIN RESULTS AND THE ROLE OF CHANCE:** A total of 514 couples did and 663 couples did not achieve a SOP. All WHO groups have a lower SOPR compared with the unexplained group (ORs varying from 0.136 to 0.397). Comparing the couples within the abnormal WHO groups, there are no significant differences in SOPR, except when oligoasthenoteratozoospermia is compared with asthenozoospermia [OR 0.501 (95% CI 0.311–0.809)] and teratozoospermia [OR 0.499 (95% CI: 0.252–0.988)], and oligoasthenozoospermia is compared with asthenozoospermia [OR 0.572 (95% CI: 0.373–0.877)]. All TMSC groups have a significantly lower SOPR compared with the unexplained group (ORs varying from 0.171 to 0.461). Couples with a TMSC of $< 1 \times 10^6$ and $1–5 \times 10^6$ have significantly lower SOPR compared with couples with a TMSC of $5–10 \times 10^6$ [respectively, OR 0.371 (95% CI: 0.215–0.64) and OR 0.505 (95% CI: 0.307–0.832)].

**LIMITATIONS, REASON FOR CAUTION:** To include all SOPs during the follow-up period of 3 years, couples were not censured at the start of treatment.

**WIDER IMPLICATIONS OF THE FINDINGS:** Roughly, three prognostic groups can be discerned: couples with a TMSC <5, couples with a TMSC between 5 and 20 and couples with a TMSC of more than 20 $\times$ $10^6$ spermatozoa. We suggest using TMSC as the method of choice to express severity of male infertility.

**STUDY FUNDING/COMPETING INTEREST(S):** None.

**Key words:** semen analysis / total motile sperm count / World health organization criteria / spontaneous ongoing pregnancy rate / male subfertility

# Introduction

Male factor infertility is the most common cause of involuntary childlessness (Hull *et al*, 1985; Brandes *et al.* 2011a). The diagnosis is based on the results of a semen analysis. The World Health Organization (WHO) has defined cut-off values to differentiate between normal and abnormal semen. Forced by the increasing doubt about the composition of the reference populations used in earlier WHO manuals, the WHO modified its criteria in 2010 (Cooper *et al.*, 2010). The new cut-off values were assessed in a population of 1953 men who had recently fathered a child or whose wives conceived. The study population was retrieved from different countries worldwide and with the condition that the time-to-pregnancy was <1 year (WHO, 2010). Values below the fifth percentile of this group of fertile man are considered as abnormal (Table I).

Based on these cut-off values the WHO uses a descriptive nomenclature, including oligozoospermia (O), asthenozoospermia (A) and teratozoospermia (T) and combinations of these factors to classify the different forms of male factor infertility.

Ideally, a medical classification system correlates with the clinical outcome. In case of male infertility this can be the spontaneous pregnancy rate or the pregnancy rate after treatment. Thus far, only a few studies evaluated the predictive value of the WHO criteria in an infertile population. Polansky and Lamb (1988) did not find any significant correlation between semen parameters and probability of conception. Van der Steeg *et al.* (2011) tried to validate the 1999-WHO criteria (WHO, 1999) for spontaneous pregnancy chance in a large longitudinal cohort of infertile couples and concluded that the predictive value of the WHO classification of semen analysis was poor. In other words, although the WHO classification suggests accuracy, the relevance for the prognosis of the couple and the choice of treatment is poor (Esteves *et al.*, 2012).

A different way to express sperm quality is the calculation of the total motile sperm count (TMSC), which is obtained by multiplying the volume of the ejaculate in milliliters by the sperm concentration and the proportion of A (fast forward progressive) and B (slow progressive) motile sperms divided by 100% (Smith *et al.*, 1977; Ayala *et al.*, 1996). The sperm parameter morphology is not taken into account in this calculation. In a recent paper of Deveneau *et al.* (2014), the TMSC was studied in an intrauterine insemination (IUI) program and morphology did not have a predictive value for the pregnancy rate.

The TMSC can be measured before (prewash) and after (postwash) sperm preparation. Van der Weert *et al.* (2004) showed in a meta-analysis of 16 studies that a postwash TMSC of between 0.8 $\times$ $10^6$ and 5 $\times$ $10^6$ has a prognostic value in couples who underwent IUI. Badawy *et al.* (2009) showed that IUI is less successful when both total number of inseminated motile spermatozoa is low and postwash morphology is poor. Although decision-making in daily practice is usually based on the TMSC, the correlation between the TMSC classification and spontaneous pregnancy rate is hardly investigated (Ayala *et al.*, 1996).

Therefore, there is a need to validate the different criteria used to classify male factor infertility for predicting spontaneous pregnancy. In this study we aim to compare the predictive value of the WHO 2010 criteria (WHO, 2010) and the predictive value of the prewash TMSC for the spontaneous ongoing pregnancy rate (SOPR) and for the ongoing pregnancy rate after treatment, in a cohort of infertile couples with male and unexplained infertility. Relevant confounding variables will be taken into account.

# Materials and Methods

## Study design

For this study the dataset of the Brandes-cohort study was used (Brandes *et al.*, 2011a,b). In short, the cohort consists of a longitudinally collected, unselected group of infertile couples referred by their general practitioner for the first time for a fertility work-up to a gynecologist in one of three

**Table I Cut-off values of sperm parameters according to the WHO 1999 and 2010 criteria and nomenclature.**

| | WHO 1999 | WHO 2010 | Nomenclature if below cut-off value |
|---|---|---|---|
| Volume | 2 ml | 1.5 ml | Hypospermia* |
| Sperm concentration | 20 $\times$ $10^6$ spermatozoa/ml | 15 $\times$ $10^6$ spermatozoa/ml | Oligozoospermia** |
| Motility (A + B)*** | 50% | 32% | Asthenozoospermia |
| Morphology | 30% normally formed | 4% normally formed**** | Teratozoospermia |

*No ejaculate is aspermia.
**If there are no spermatozoa in the ejaculate it is called azoospermia.
***A-motility is fast forward progressive, B-motility is slow progressive.
****According to the Tygerberg criteria (Kurger *et al.*, 1988).

hospitals in the Netherlands (two large regional training hospitals: Jeroen Bosch Hospital, 's-Hertogenbosch and St Elisabeth Hospital, Tilburg and the Radboud University Medical Center, Nijmegen) between January 2002 and December 2006. Infertility was defined as failure to conceive despite 12 months of unprotected intercourse. The follow-up for all couples was 3 years. Couples for whom no ongoing pregnancy was documented and who were no longer followed in our clinics were contacted by telephone to find out whether they had started treatment elsewhere and had achieved a pregnancy in the meantime, either spontaneously or after therapy. All information, including the patient characteristics were longitudinally registered in an electronic patient record (Fertibase®, STB, Houten, The Netherlands). To guarantee a standardized and a complete dataset, this information was collected at the first visit using predefined questionnaires. All couples were informed about this study, but written consent was not necessary according to the hospitals' Medical Ethical Committee (approval obtained August 2007).

## Fertility work-up

The fertility work-up was performed according to the standing Dutch national guidelines at that time (NVOG, 2004) and consisted of a history of both partners, a semen analysis, ultrasonographic cycle monitoring, a measurement of midluteal progesterone, a chlamydia antibody test (CAT), a hysterosalpingography and, if indicated, a diagnostic laparoscopy. Also a timed postcoital test (PCT) was part of this work-up, but in case of known poor semen quality, the test was often omitted.

In the Netherlands, the TMSC is used to assess the semen quality. At that time a TMSC of more than $20 \times 10^6$ spermatozoa was considered as normospermia, while a TMSC below this level was defined as male infertility. A normal menstrual cycle is between 25 and 35 days, has a midluteal progesterone level, timed by ultrasound 1 week after the ovulation, of at least 27 nmol/l and a luteal phase of at least 11 days duration. Repeated abnormal findings are diagnosed as ovulation disorder.

Cervical infertility is diagnosed if the PCT is negative as a result of poor cervical mucus or if the test is negative despite the presence of normal mucus and normospermia.

Tubal infertility is defined as uni- or bilateral tubal occlusion or obstructed ovum pickup mechanism due to pelvic adhesions diagnosed by hysterosalphingography. Endometriosis includes revised American Fertility Society (AFS) stage III and IV endometriosis (AFS, 1985).

Uterine infertility is diagnosed when uterine defects, such as fibroids or intrauterine adhesions, are present, as diagnosed by ultrasound, laparoscopy or hysteroscopy. Sexual problems include both male and female sexual disorders, causing inability to have normal intercourse. Unexplained infertility is diagnosed if all other female and male causes are excluded.

## Study population

The total cohort consisted of 2476 infertile couples. Of them, 1239 couples were excluded because of female infertility factors such as ovulation disorders, tubal disease, endometriosis, cervical factor or sexual dysfunction (see Fig. 1). Also couples with a combination of female and male infertility factors, and couples in whom the male partner was diagnosed with azoospermia were excluded. This left a study population of 1177 couples with either a male factor infertility as the only diagnosis (except couples with azoospermia) or normospermia (unexplained infertility) for analysis.

The data of the remaining couples were processed into two different ways: first the study population was classified according to WHO criteria. Subsequently, the same study population was classified according to the TMSC reference. To evaluate both classification systems independently, it was decided not to include a control group consisting of couples with 'real unexplained' infertility in which the semen analysis was normal according to both classification systems. Therefore, both classification systems created their own reference group of normospermia, i.e. unexplained infertility.

## Semen analysis

The semen analysis was ordered at the first visit to the clinic. In case of abnormal semen analysis, a second sample was ordered 6 weeks later. Male partners were advised to collect a sperm sample after 2–3 days of sexual abstinence. The semen was stored at home or in the hospital in a sterile plastic container, and delivered to the laboratory within 1 h. During the analysis the volume was measured using a graded tube. The concentration was measured in an improved Neubauer chamber, with a magnification of 200×. Motility was scored manually, as percentages of (A) fast forward progressive, (B) slow forward progressive, (C) non-progressive and (D) immotile spermatozoa in 200 spermatozoa in at least five power fields per replicate, according to the WHO manual (WHO, 2010). The sperm morphology was scored according to the Tygerberg criteria (Kurger *et al.*, 1988), after analyzing 200 spermatozoa. The hospitals in this study collaborate in

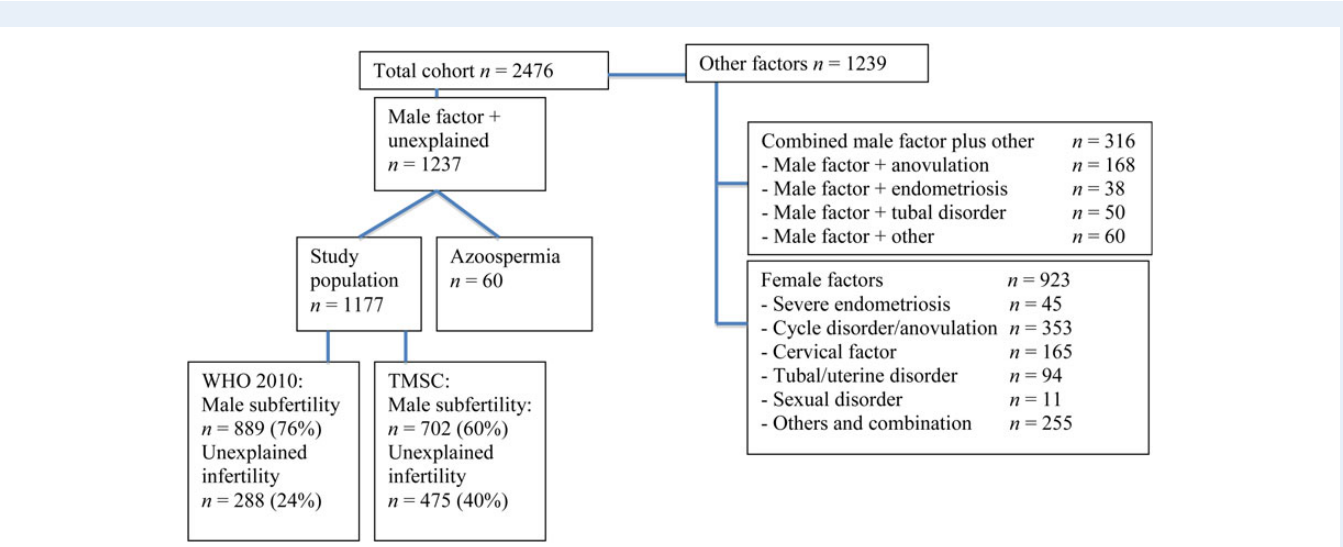



**Figure 1** Flow chart of the study population. TMSC, Total motile sperm count; WHO, World Health Organization.

a nationwide quality control system organized by the 'Stichting Kwaliteitsbewaking Medische Laboratoriumdiagnostiek' (SKML), a Dutch foundation for quality assessment in clinical laboratories (www.skml.nl), to standardize and reduce practice variation as much as possible for the semen analysis. In this SKML collaboration semen samples are sent four times a year to different laboratories for cross-referencing. As recommended by the Dutch guidelines (Netwerkrichtlijn, Nederlandse Huisartsen Genootschap, NVOG, 2011), the best semen analysis was used to classify the couples. Only prewashed semen results were used for classification described here.

## WHO groups

Couples were grouped according to the different WHO diagnostic categories as defined in the latest (WHO, 2010) manual including azoospermia, oligozoospermia, asthenozoospermia, teratozoospermia and the combinations of diagnoses such as oligoasthenozoospermia (OA), oligoteratozoospermia (OT), astenoteratozoospermia (AT), oligoasthenozoospermia (OAT) and normospermia. Table I shows the cut-off values for volume, concentration, motility and morphology according to the WHO 1999 and 2010 criteria. The semen analysis will be classified according to the abnormal semen parameter. If all parameters are above the cut-off value, the semen is defined as normospermia and the couples are diagnosed with unexplained infertility. The couples for which an abnormal semen analysis improved to normospermia in a subsequent semen analysis are referred to as the 'normalized' group. This group will be evaluated separately from the control group, i.e. couples with unexplained infertility.

## TMSC groups

The same couples were also grouped according to the TMSC, calculated by multiplying the sample volume by the density and the percentage of A and B motility divided by 100% (Netwerkrichtlijn Nederlandse Huisartsen Genootschap, NVOG, 2011). Because a validated classification is missing, couples were divided into the following groups according to the degree of male infertility: group 1 TMSC $<1 \times 10^6$ spermatozoa, group 2 TMSC $1-5 \times 10^6$, group 3 TMSC $5-10 \times 10^6$ and group 4 TMSC $10-20 \times 10^6$. A TMSC of $>20 \times 10^6$ is considered normal. Group 5 consisted of couples for whom the first semen analysis was abnormal, but for whom the best test was normalized to a TMSC of $>20 \times 10^6$.

## Treatments

Couples were intentionally treated according to the Dutch national clinical guidelines on male infertility and unexplained infertility of the Dutch Society of Obstetrics and Gynaecology (Nederlandse Huisartsen Genootschap *et al.*, 2011). In these guidelines the choice of treatment for couples with a TMSC $>3$ million is based on the prognosis which was calculated according to the prognostic model of Eimers *et al.* (1994) and from 2004 the model of Hunault *et al.* (2004), which includes the same parameters. Six months of expectant management was advised for couples with a pregnancy chance assessed to be $>30\%$ (mild cases). Six IUI cycles were offered to couples with a prognosis of $<30\%$ (moderate male infertility) or couples with a pregnancy chance $>30\%$ and after 6 months of expectant therapy. After six unsuccessful IUI cycles in vitro fertilization (IVF) was offered. Couples with a TMSC $1-3 \times 10^6$ were offered the same treatment as couples with a prognosis of $<30\%$, as the prediction models were only validated for TMSC $>3 \times 10^6$. Severe cases (TMSC $<1 \times 10^6$) were directly offered intracytoplasmic sperm injection (ICSI). ICSI was also performed in cases where previous IVF resulted in a fertilization rate of $<10\%$. In view of the lack of evidence of success, medical treatment to improve sperm quality was not given. If a second semen analysis 6 weeks after the initial abnormal sample showed normal parameters, the 'normalization' had occurred spontaneously.

Fertility investigations and IUI are fully reimbursed by the insurance companies. IVF and ICSI are only reimbursed for the first three cycles.

## Outcome measures

The primary outcome measure was the SOPR, defined as natural conception resulting in a fetal heartbeat at 12 weeks of gestation by an ultrasonographic evaluation. Non-spontaneous pregnancy is considered as a pregnancy after treatment or no pregnancy at all. Spontaneous ongoing pregnancies (SOPs) could occur before, during or after treatment. The secondary outcome measure was the total ongoing pregnancy rate (TOPR) including the mode of conception for treatment-dependent pregnancies, i.e. IUI, IVF and ICSI.

## Statistics

Patient characteristics, both demographic and infertility-related, were given for the total cohort and for couples who did and did not conceive a SOP. According to the available literature, only variables which are commonly related to spontaneous pregnancy chance were included, i.e. male and female age, duration and type of infertility, female BMI, smoking habits, CAT and PCT results and semen parameters (Hunault *et al.*, 2004; Van der Steeg *et al.*, 2007; Brandes *et al.*, 2011c). For comparison between the groups the independent *t* test or $\chi^2$-test were used. A *P*-value of $<0.05$ was used to indicate a statistical significance. Specific variables from the male history, i.e. drugs use, a history of orchidopexy, large varicocele or varicocelectomy, chemotherapy, orchitis, torsio testis or refertilization, were also analyzed (data not shown). As the numbers of these variables were too small, no statistical significance was found and they were not included.

First, a univariate analysis was performed to calculate the relation between the chosen variables and the SOP. Variables with a significance-value of $P < 0.15$ were selected for the multivariate analysis.

Subsequently, a binary logistic regression was performed, as the dependent variable (pregnancy type), has two outcome measures, i.e. a SOP versus a non-SOP. This analysis with backward selection was used to identify variables that influence the SOP. For the results of this multivariate analysis a *P*-value of $<0.05$ was considered as significant. To calculate a correlation between two confounding variables the Pearson and Spearman, Mann–Whitney *U*-test and $\chi^2$-test were used. Using the Pearson and Spearman test, a cut-off value of 0.6 was used. Using the Mann–Whitney *U*-test and the $\chi^2$-test a *P*-value of 0.05 was considered as significant. If a correlation was shown, only one variable was selected for further analysis.

Crude odds ratios (OR) and their 95% confidence interval (CI) were calculated as the risk of SOP for each TMSC and WHO group. The group with unexplained infertility was used as reference for both types of semen classification. Eventually, the ORs of SOP, adjusted for the possible confounding factors proven with the multivariate analysis, were calculated for all individual TMSC and WHO groups. In view of the correlation between the WHO and TMSC classification, the ORs of the TMSC groups were calculated without selecting the WHO groups.

Again the couples with unexplained infertility were used as reference. The significance was calculated by comparing the individual subgroups. The SPSS 16.0 program (Statistical Package for the Social Sciences; SPSS, Chicago, IL, USA) was used for this analysis.

Additionally, both the TOPR and the mode of conception were calculated per WHO group and TSMC group. These results were visualized using Excel 2000 (Microsoft Office).

# Results

When the WHO criteria were used, 889 couples (76%) were diagnosed with male factor infertility as single diagnosis at the time of first semen analysis and 288 couples (24%) had a normospermia. When on the other hand, TMSC was used to diagnose male infertility 702 men (60%) had abnormal sperm at the time of first analysis, and 475 (40%) had a normospermia.

The descriptive statistics for the total cohort and for the couples who did and did not achieve a spontaneous pregnancy are shown in Table II. Of the study population of 1177 couples, 514 achieved SOP (43.7%). Of the 663 couples who did not achieve a spontaneous pregnancy, 335 couples became pregnant after treatment and 328 couples did not become pregnant at all.

Several variables show a significant relationship to spontaneous pregnancies in the univariate analysis. Both mean female and male ages are lower in the SOP group (both $P < 0.0001$). Compared with the group with noSOP, the median duration of infertility is significantly shorter in the group with a SOP ($P < 0.0001$). In the group with a SOP, also a higher percentage of secondary infertility and non-smoking men is seen.

The mean TMSC is significantly higher in the group with SOP. Also, type of infertility of the couple, smoking habits of the male, the PCT, sperm concentration, motility and morphology of the semen and the TMSC showed a significant relationship to SOP in the univariate model. Looking at the WHO results, only the OA, OAT and unexplained groups correlate with SOP (Table II).

Subsequently, a multivariate analysis was performed. For the parameters which remained significant after the multivariate analysis, *P*-values are shown in Table II. Confounding factors were female and male age, duration and type of infertility, the results of the PCT and WHO classification. No correlation was found between those confounding factors. The adjusted ORs for each type of the WHO and TMSC group are shown in Tables III and IV. The tables also show the differences between the ORs of the subgroups. If couples are diagnosed with abnormal or normalized semen according to the WHO classification, their chance to achieve a SOP is significantly lower than in couples with unexplained infertility (Table III). Compared with each other, the ORs of the abnormal WHO subgroups are not significantly different, except that couples with OAT compared with couples with A or T and couples with OA compared with A have a lower OR of SOPR ($P < 0.05$).

Couples with a TMSC of $<5 \times 10^6$ have a significantly lower chance of SOP than couples with a TMSC of $>5 \times 10^6$ (Table IV). Couples with a TMSC $<1 \times 10^6$ had the same SOPR as couples with a TMSC of $1-5 \times 10^6$ (0.735 with 95% CI of 0.437–1.234). Couples with normalized sperm, after an initial abnormal semen analysis, tend to have a similar outcome as couples with sperm of lower quality. Couples with unexplained infertility have a significantly higher chance of SOP compared with couples with a TMSC $<20 \times 10^6$ or normalized sperm.

Figures 2 and 3 show the TOPR including mode of conception (IUI, IVF and ICSI) and the SOPR and the mode of conception per WHO group and TMSC group, respectively. Both the WHO and TMSC groups are ordered according to an increasing SOPR. Again we have shown that couples in any of the subgroups have a lower SOPR than couples with unexplained infertility. Looking at the abnormal TMSC groups, the SOPR varies between 23 and 42% compared with a SOPR of 60% for the couples with unexplained infertility. The TOPR of couples in these groups of male infertility varies between 61 and 74%. The SOPR of couples with male infertility according to the WHO classification varies between 18 and 44%, while the TOPR of couples in these groups varies between 63 and 82% and are not significantly different. There is a reversed correlation between the proportion of SOP and pregnancies after ICSI. In other words, the lower the SOPR, the higher the contribution of ICSI to the TOPR. Also the contribution of IUI and IVF increases with higher SOPR. The TOPR of the couples with normalized semen analysis is lower (68% and 74%, respectively, using the WHO and TMSC classification) than the TOPR of the couples with unexplained infertility (72 and 79%, respectively, using the WHO and TMSC classification), but this is not significant.

In Fig. 4 the level of agreement and discrepancy of the two classification systems are shown. The bars on the right and left show the outcome if the two systems are in agreement. The middle bars show the outcome if both systems give contradictory results. The SOPR and TOPR are higher when the TMSC is normal compared with abnormal, regardless of the result of the WHO classification. This is not the case in the opposite direction. If the semen is normal according to the WHO criteria, but abnormal according to the TMSC classification, then both the SOP and TOP are worse (respectively, 37.5% and 62.5%), compared with results that are classified as abnormal by WHO criteria in combination with a normal TMSC (respectively, 46.3% and 75.0%).

# Discussion

In this study, couples with unexplained infertility have, after correction for confounding factors, a higher SOPR than couples in any of the WHO classes of male infertility or any of the TMSC groups below $20 \times 10^6$ spermatozoa. In between the various WHO groups with male infertility the SOPRs were not significantly different. The TMSC classification, on the other hand, shows a significant correlation with the SOPR. In addition, the TMSC is easy to calculate. Therefore, we conclude that the TMSC is more useful than the WHO classification system for expressing the severity of male factor infertility.

For daily practice, three prognostic groups can be discerned: couples with a TMSC $<5 \times 10^6$, couples with a TMSC between 5 and $20 \times 10^6$ and couples with a TMSC of more than $20 \times 10^6$ spermatozoa (normospermia). It is striking to see that there were no differences in SOPR whether the TMSC was $<1 \times 10^6$, $1-3 \times 10^6$ or $3-5 \times 10^6$ (data not shown). Spontaneous pregnancies occur even in the presence of extremely poor sperm quality. Most physicians will recommend those couples to start with ICSI straight away, as we did. Yet, it is remarkable that about a quarter of those couples conceived spontaneously. Once again it is shown that the semen analysis is a poor predictor of pregnancy in this low range (Van der Steeg *et al.*, 2011).

If the best semen analysis is in the normal TMSC range, after the first semen analysis was abnormal (normalized group), the pregnancy chances of these couples seem to be at the level of the couples with consistently poorer semen quality. This shows that multiple semen analyses seem to have no additional prognostic value. One abnormal semen analysis already determines the prognosis. However this observation should be evaluated further in a larger group. This is in contrast to the Dutch national network guideline and National Institute for Health and Care Excellence (NICE) fertility guidelines (National Institute for Health and Care Excellence, 2013) that advise repeating the test if the first one is abnormal. These guidelines refer to the study of Opsahl *et al.* (1996), who found that about 10% of men with an abnormal semen at first analysis eventually show a normal sperm count when more samples are examined. The NICE fertility guidelines recommended performing more tests, but did not validate their statement with pregnancy rates.

The WHO reference limits are determined in a large group of fertile men, who recently fathered a child. Men with semen results below these limits, however, are not necessary infertile. On the other hand, sperm results above the reference limits do not guarantee the occurrence of a pregnancy, as other factors might influence the outcome. We think that criteria to assess semen quality should be based on

**Table II Characteristics of couples in the study with male infertility and unexplained infertility.**

| | Total cohort, $N = 1177^{\S}$ | Spontaneous ongoing pregnancy, $N = 514$ | No spontaneous ongoing pregnancy (=no pregnancy or pregnancy after treatment $N = 663$ | *P*-value* | *P*-value** |
|---|---|---|---|---|---|
| Female age (years) | | | | | |
| Mean ± SD | 31.9 ± 4.5 | 31.2 ± 4.4 | 32.4 ± 4.5 | 0.000 | 0.017 |
| Male age (years) | | | | | |
| Mean ± SD | 34.7 ± 5.7 ($n = 1175$) | 33.7 ± 5.2 | 35.4 ± 5.9 | 0.000 | 0.009 |
| Duration of infertility (years) | | | | | |
| Mean ± SD | 1.7 ± 1.5 | 1.4 ± 0.8 | 1.9 ± 1.8 | 0.000 | 0.000 |
| Female BMI ($kg/m^2$) | | | | | |
| Mean ± SD | 24.1 ± 4.8 ($n = 1077$) | 24.1 ± 4.7 | 24.2 ± 4.9 | 0.603 | |
| Type of infertility couple | | | | | |
| Primary | 865 (73.6%) | 342 (66.7%) | 523 (79.0%) | 0.000 | 0.017 |
| Secondary | 310 (26.4%) ($n = 1175$) | 171 (33.3%) | 138 (21.0%) | | |
| Smoking (male) | | | | | |
| Yes | 380 (34.7%) | 149 (31.4%) | 231 (37.3%) | 0.045 | |
| No | 714 (65.3%) ($n = 1094$) | 325 (68.6%) | 389 (62.7%) | | |
| Smoking (female) | | | | | |
| Yes | 530 (47.9%) | 220 (45.6%) | 310 (49.7%) | 0.183 | |
| No | 576 (52.1%) ($n = 1106$) | 262 (54.4%) | 314 (50.3%) | | |
| CAT | | | | | |
| Positive | 105 (8.9%) | 39 (7.6%) | 66 (10.0%) | 0.223 | |
| Negative | 904 (76.8%) | 392 (76.3%) | 512 (77.2%) | | |
| Not executed | 169 (14.3%) | 83 (16.1%) | 85 (12.8%) | | |
| PCT | | | | | |
| Positive | 547 (46.5%) | 291 (56.6%) | 256 (38.6%) | 0.000 | 0.011 |
| Negative | 228 (19.4%) | 65 (12.6%) | 163 (24.6%) | | |
| Not executed | 402 (34.2%) | 158 (30.8%) | 244 (36.8%) | | |
| Parameters best semen | | | | | |
| Volume (ml) | | | | | |
| Mean ± SD | 3.0 ± 1.6 ($n = 1068$) | 3.0 ± 1.5 | 3.0 ± 1.7 | 0.977 | |
| Sperm concentration ($10^6$/ml) | | | | | |
| Mean ± SD | 39.7 ± 47.0 ($n = 1068$) | 46.5 ± 47.6 | 34.8 ± 46.0 | 0.000 | |
| Motility, progressive | | | | | |
| Mean ± SD | 32.1 ± 19.0 ($n = 1067$) | 35.3 ± 18.9 | 29.8 ± 18.7 | 0.000 | |
| A+B (%) | | | | | |
| Morphology (% normal) | | | | | |
| Mean ± SD | 15.8 ± 17.6 ($n = 829$) | 17.0 ± 17.5 | 14.8 ± 17.7 | 0.078 | |
| TMSC, $N = 1070$ | | | | | |
| Mean ± SD | 43.5 ± 72.4 ($n = 1070$) | 55.6 ± 77.1 | 34.8 ± 67.6 | 0.000 | |

*Continued*

**Table II** *Continued*

| | Total cohort, N = 1177§ | Spontaneous ongoing pregnancy, N = 514 | No spontaneous ongoing pregnancy (=no pregnancy or pregnancy after treatment N = 663 | P-value* | P-value** |
|---|---|---|---|---|---|
| TMSC per category ($10^6$) | | | | | |
| 0–1 | 160 (13.6%) | 37 (7.2%) | 123 (18.6%) | 0.000 | |
| 1–5 | 182 (15.5%) | 48 (9.3%) | 134 (20.2%) | 0.000 | |
| 5–10 | 134 (11.4%) | 56 (10.9%) | 78 (11.8%) | 0.641 | |
| 10–20 | 164 (14.0%) | 66 (12.8%) | 99 (14.9%) | 0.305 | |
| Normalized | 61 (5.2%) | 20 (3.9%) | 41 (6.2%) | 0.078 | |
| Unexplained | 475 (40.4%) | 287 (55.8%) | 188 (28.3%) | 0.000 | |
| WHO per category | | | | | 0.012 |
| O | 90 (7.6%) | 34 (6.6%) | 56 (8.4%) | 0.241 | |
| A | 294 (25.0%) | 128 (24.9%) | 166 (25.0%) | 0.958 | |
| T | 60 (5.1%) | 26 (5.1%) | 34 (5.1%) | 0.957 | |
| O-A | 181 (15.4%) | 52 (10.1%) | 129 (19.5%) | 0.000 | |
| O-T | 17 (1.4%) | 3 (0.6%) | 14 (2.1%) | 0.029 | |
| A-T | 67 (5.7%) | 26 (5.1%) | 41 (6.2%) | 0.408 | |
| O-A-T | 143 (12.1%) | 37 (7.2%) | 106 (16.0%) | 0.000 | |
| Normalized | 37 (3.1%) | 15 (2.9%) | 22 (3.3%) | 0.697 | |
| Unexplained | 288 (24.5%) | 193 (37.5%) | 95 (14.3%) | 0.000 | |

Female and male age, duration of infertility were calculated at the day of first presentation.
CAT, chlamydia antibody test; PCT, postcoital test; TMSC, total motile sperm count ($\times 10^6$); O, oligozoospermia; A, asthenozoospermia; T, teratoszoospermia; normalized, normospermia at the best semen analysis, after a previous abnormal test; unexplained, normospermia at the first semen analysis.
§Unless shown otherwise, owing to missing data. Percentages are given for the available data.
*Univariate analysis, comparison between 'spontaneous ongoing pregnancy' and 'non-spontaneous ongoing pregnancy'.
**Multivariate analysis with $P < 0.10$.

pregnancy chances in infertile couples after exclusion of other causes of infertility and should be corrected for confounding factors such as female age and duration of infertility.

Ombelet *et al.* (1997) compared different semen parameters in fertile and infertile men and based on receiver operating characteristic curves, they concluded that sperm morphology was best able to predict which group (fertile or infertile) a person belongs to. However, no attempt was made to correlate the findings with pregnancy chance. It is interesting to see that their suggestions for reference limits come very close to the current WHO criteria. In our study morphology was not seen as discriminative, despite the fact that our hospitals participate in a national program to standardize the laboratory interpretation. This is in agreement with the recent study of Deveneau *et al.* (2014) in an IUI program.

The semen analysis is a good predictor if it correlates with pregnancy chance. There is hardly any study that tried to validate the semen analysis with SOPR. Polansky and Lamb (1988) did not find any significant influence of any semen characteristic on the probability of a spontaneous conception in a cohort of 1089 infertile couples. Ayala *et al.* (1996) showed in a cohort of 1055 infertile couples that the TOPR was significantly higher if the TMSC was more than $25 \times 10^6$ compared with a TMSC below $25 \times 10^6$ [relative risk 6.1 (95% CI: 4.7–7.9)]. In both studies total pregnancy rates were calculated regardless of female infertility factors and treatment given.

Van der Steeg *et al.* (2011) carried out a large multicenter cohort study and measured the pregnancy rate within 12 months. They censored couples at the start of treatment or on the last date of contact in case of expectant management. In 41% of the couples the man had a normospermia, in the remaining 59% the man was diagnosed with abnormal sperm yet, the spontaneous pregnancy rate was 24% in the first group and 23% in the second group. In their study the different WHO categories did not show a clear pattern of success rate. OAT had the lowest pregnancy rate with 12% in 12 months.

The reason for the lack of discriminating potential might be that the WHO criteria make use of cut-off points, while the pregnancy rate increases continuously with increasing values for individual parameters. By dichotomizing the sperm results the effect of the slope is ignored. For example, when a couple is diagnosed with OAT but the semen parameters are just below the cut-off values, the WHO classification will categorize this couple in the same group as a couple with extremely poor parameters. No discrimination will be made, while the TMSC takes the absolute value of three semen parameters into consideration simultaneously. This was nicely shown by van der Steeg *et al.* (2011). They concluded that the cut-off values as defined by the WHO are not good predictors, as the spline curves of the different sperm parameters they produced clearly show that the actual values are more informative than purely dichotomizing the parameters, as the WHO does. Van der Steeg *et al.* (2011) made a prediction model which incorporates several sperm parameters and the TSMC, in fact, comes close to this model. In the prognostic model for spontaneous pregnancy of Hunault *et al.* (2004) motility was the only sperm parameter included into the model.

**Table III Chance of spontaneous pregnancy in relation to the WHO groups.**

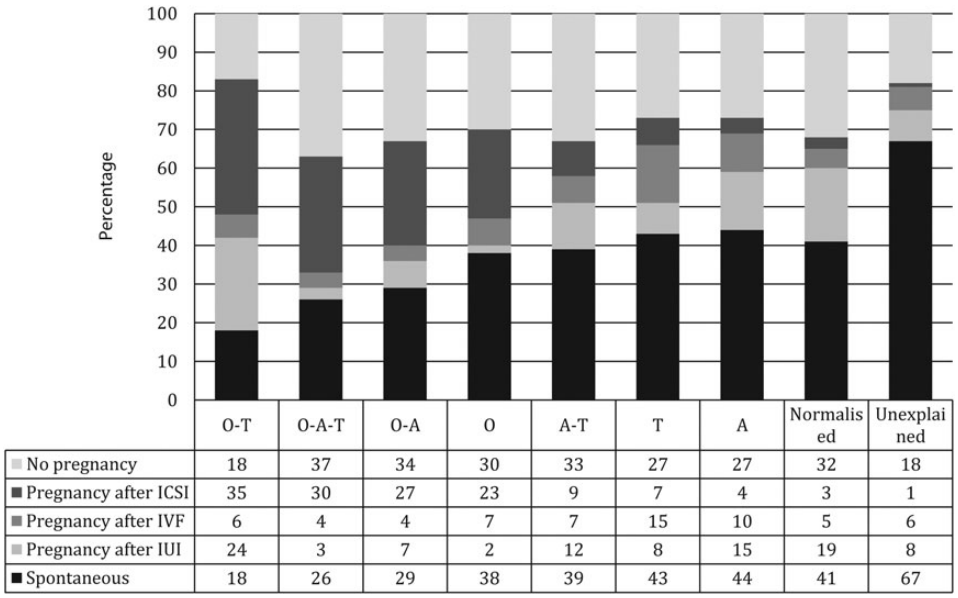
| WHO group | O-T | O-A-T | O-A | O | A-T | A | T | Normalized | Unexplained |
|---|---|---|---|---|---|---|---|---|---|
| O-T | 1 | 0.686 (0.181–2.604) | 0.601 (0.161–2.247) | 0.397 (0.103–1.531) | 0.409 (0.104–1.617) | 0.344 (0.94–1.251) | 0.342 (0.086–1.356) | 0.395 (0.092–1.690) | **0.136 (0.037–0.497)** |
| O-A-T | | 1 | 0.877 (0.525–1.465) | 0.580 (0.319–1.052) | 0.597 (0.310–1.149) | **0.501 (0.311–0.809** | **0.499 (0.252–0.988)** | 0.576 (0.254–1.304) | **0.198 (0.120–0.328)** |
| O-A | | | 1 | 0.661 (0.379–1.155) | 0.681 (0.366–1.267) | **0.572 (0.373–0.877)** | 0.569 (0.298–1.089) | 0.657 (0.300–1.438) | **0.226 (0.143–0.357)** |
| O | | | | 1 | 1.030 (0.519–2.043) | 0.865 (0.519–1.443) | 0.861 (0.426–1.741) | 0.993 (0.432–2.284) | **0.324 (0.200–0.584)** |
| A-T | | | | | 1 | 0.840 (0.471–1.497) | 0.836 (0.394–1.775) | 0.965 (0.402–2.316) | **0.332 (0.183–0.603)** |
| A | | | | | | 1 | 0.995 (0.554–1.789) | 1.148 (0.546–2.414) | **0.395 (0.275–0.568)** |
| T | | | | | | | 1 | 1.154 (0.477–2.789 | **0.397 (0.220–0.716)** |
| Normalized | | | | | | | | 1 | **0.344 (0.162–0.733)** |
| Unexplained | | | | | | | | | 1 |

Odds ratio (95% CI) after adjustment for female and male age, duration and type of infertility and the results of the PCT, using binary logistic regression analysis.
Data are bold if significant.

**Table IV Chance of spontaneous pregnancy in relation to the TMSC groups.**

| TMSC group | 0–1 | 1–5 | 5–10 | 10–20 | Normalized | Unexplained |
|---|---|---|---|---|---|---|
| 0–1 | 1 | 0.734 (0.437–1.234) | **0.371 (0.215–0.640)** | **0.383 (0.226–0.651)** | 0.521 (0.259–1.049) | **0.171 (0.105–0.280)** |
| 1–5 | | 1 | **0.505 (0.307–0.832)** | **0.522 (0.321–0.847)** | 0.709 (0.365–1.377) | **0.233 (0.149–0.365)** |
| 5–10 | | | 1 | 1.032 (0.634–1.681) | 1.403 (0.723–2.722) | **0.461 (0.296–0.719)** |
| 10–20 | | | | 1 | 1.359 (0.712–2.594) | **0.447 (0.298–0.671)** |
| Normalized | | | | | 1 | **0.329 (0.180–0.600)** |
| Unexplained | | | | | | 1 |

Odds ratios (95% CI), after adjustment for female and male age, duration and type of infertility and the results of the PCT, using binary logistic regression analysis.
Data are bold if significant.




| | O-T | O-A-T | O-A | O | A-T | T | A | Normalised | Unexplained |
|---|---|---|---|---|---|---|---|---|---|
| No pregnancy | 18 | 37 | 34 | 30 | 33 | 27 | 27 | 32 | 18 |
| Pregnancy after ICSI | 35 | 30 | 27 | 23 | 9 | 7 | 4 | 3 | 1 |
| Pregnancy after IVF | 6 | 4 | 4 | 7 | 7 | 15 | 10 | 5 | 6 |
| Pregnancy after IUI | 24 | 3 | 7 | 2 | 12 | 8 | 15 | 19 | 8 |
| Spontaneous | 18 | 26 | 29 | 38 | 39 | 43 | 44 | 41 | 67 |

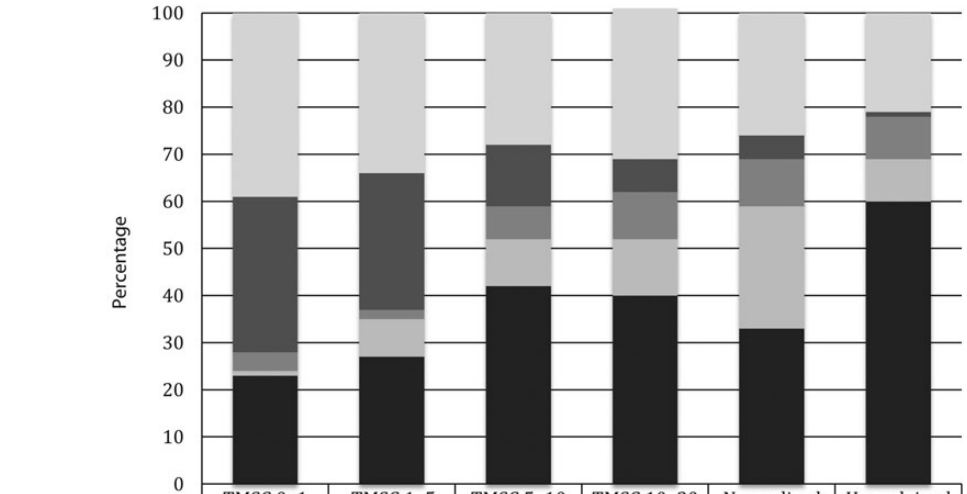
**Figure 2** TOPR and mode of conception, as percentage of total per WHO category. WHO classes sorted according to the increasing spontaneous ongoing pregnancy rate. O, oligozoospermia; A, asthenozoospermia; T, teratozoospermia; Normalized, normospermia at the best semen analysis, after a previous abnormal test; unexplained, normospermia at the first semen analysis; ICSI, intracytoplasmic sperm injection; IVF, in vitro fertilization; IUI, Intrauterine insemination.

A strength of our study is that the spontaneous pregnancy rate was measured and that this rate was corrected for confounding factors. This was done in an unselected longitudinal cohort of infertile couples. The study is therefore representative for couples referred with an infertility problem for the first time. We did exclude other female infertility diagnoses. The follow-up time was 3 years. Couples who had stopped treatment in our hospitals were contacted to ask whether any pregnancy had occurred after they had discontinued treatment.

A weak point of our study is that it is an observational study in which besides expectative management various treatments were given. To assess the spontaneous pregnancy chance in the different groups of male factor infertility, ideally, no treatment is given for a longer period of time. In general practice this is hardly possible. It is hard to believe that enough patients would be willing to participate in such a study, particularly when potent treatments such as IUI, IVF and ICSI are available.

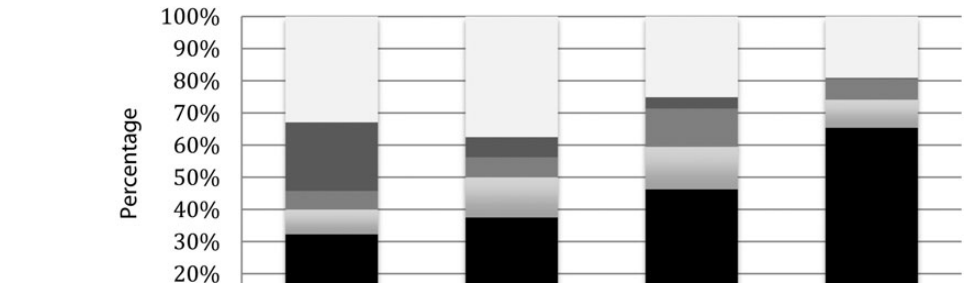
In our study couples were not censored at the moment that treatment was started. Censoring couples at the time the treatment was started, as van der Steeg *et al.* (2011) did, might seem more appropriate at first glance. However, this gives an underestimation of the SOPR in the long run, as spontaneous pregnancies which occur during and after treatments are not included. The follow-up period in our study was 3 years. Most spontaneous pregnancies occur in the first year (Brandes *et al.*, 2011a) yet the cumulative spontaneous pregnancy curve continues to rise, even after IUI, IVF and ICSI are discontinued.

It is difficult to say how many couples would have become pregnant if treatment was not started. We have shown that quite a number of spontaneous pregnancies occur during and after treatment, even in couples that according to the national guidelines must be offered ICSI directly. Despite the fact that we started with 90% of couples with a TMSC of $<1$ with ICSI, 25% conceived spontaneously over a period of 3 years.




| | TMSC 0–1 | TMSC 1–5 | TMSC 5–10 | TMSC 10–20 | Normalized | Unexplained |
|---|---|---|---|---|---|---|
| No pregnancy | 39 | 34 | 28 | 32 | 26 | 21 |
| Pregnancy after ICSI | 33 | 29 | 13 | 7 | 5 | 1 |
| Pregnancy after IVF | 4 | 2 | 7 | 10 | 10 | 9 |
| Pregnancy after IUI | 1 | 8 | 10 | 12 | 26 | 9 |
| Spontaneous | 23 | 27 | 42 | 40 | 33 | 60 |

**Figure 3** TOPR and mode of conception as percentage of total per TMSC group.




| | TMSC abnormal - WHO abnormal | TMSC abnormal - WHO normal | TMSC normal - WHO abnormal | TMSC normal - WHO normal |
|---|---|---|---|---|
| No pregnancy | 205 | 6 | 57 | 59 |
| Pregnancy after ICSI | 133 | 1 | 8 | 1 |
| Pregnancy after IVF | 36 | 1 | 27 | 20 |
| Pregnancy after IUI | 48 | 2 | 30 | 27 |
| Spontaneous | 201 | 6 | 105 | 202 |

**Figure 4** Results showing where the TMSC and WHO classification systems overlap or disagree. The bars on the right and left show the outcome if the two systems are in agreement. The middle bars show the outcome if both systems give contradictory results. TMSC normal – WHO normal = 'real unexplained' infertility.

The results of the semen analysis are not only used to determine the prognosis, but also to choose the appropriate mode of treatment. Because there is no specific WHO class with the poorest outcome, as the SOPRs among various WHO male infertility classes are comparable, it is questionable whether the WHO criteria can be used to determine the proper treatment strategy. We recommend using the TMSC

classification, as is common practice in the Netherlands. Yet, we lack proper randomized controlled trials (RCTs) to choose the right therapy for each couple. In the INeS-trial (IUI, Natural cycle and Single embryo transfer) which compared (i) IUI with controlled ovarian hyperstimulation, (ii) modified natural cycle IVF with single embryo transfer (SET) and (iii) IVF with SET in couples with unexplained infertility and mild male infertility, there were no significant differences in pregnancy rate between the three groups (Bensdorp *et al.* 2013). As IUI is much cheaper and less invasive, this therapy should be offered as the first choice treatment (van Rumste *et al.*, 2014). For moderate and severe male infertility, in the Netherlands the MASTER-trial (Male Subfertility Therapy Effectiveness RCT's) is currently ongoing, in which two RCTs for different TMSC classes of male infertility will be performed (Cissen and de Bruin, 2014). In the moderate range of male infertility (prewash TMSC 3 to 10 $\times$ $10^6$) IUI is compared with expectant treatment. In the severe range of male infertility (prewash TMSC below 3 $\times$ $10^6$ and postwash above 3 $\times$ $10^5$) ICSI is compared with IVF. Hopefully the results of this study will shed some new light on this topic.

In conclusion, the TMSC grading appears to be a better way to classify male factor infertility than the WHO classification system. The TMSC classification should be used in prospective RCT to show how the different forms of male infertility are best treated.

## Authors' roles

C.J.C.M.H. planned and designed the study, M.B., J.A.M.H., J.M.J.S. and J.A.M.K. were responsible for the data collection. J.A.M.H. conducted the main part of the analysis, while J.A.M.H., M.C., J.P.B., W.L.D.M.N., J.A.M.K. and C.J.C.M.H. contributed to the interpretation of the analysis. J.A.M.H., W.L.D.M.N. and C.J.C.M.H. drafted the article, while all authors critically revised the manuscript and approved the final version.

## Funding

No external funding was either sought or obtained for this study.

## Conflict of interest

None declared.

## References

American Fertility Society. Revised American Fertility Society classification. *Fertil Steril* 1985;**43**:351–352.

Ayala C, Steinberger E, Smith DP. The influence of semen analysis parameters on the fertility potential of infertile couples. *J Androl* 1996; **17**:718–725.

Badawy A, Elnashar A, Eltotongy M. Effect of sperm morphology and number on success of intrauterine insemination. *Fertil Steril* 2009;**91**: 777–781.

Bensdorp AJ, Tjon-Kon-Fat RI, Koks C, Oosterhuis GJE, Hoek A, Hompes PGA, Broekmans FJ, Verhoeve HR, de Bruin JP, van Golde R *et al.* O-037 Preliminary comparative effectiveness of IVF with single embryo transfer or the IVF in the modified natural cycle and IUI with hyperstimulation; a randomized trial (INeS trial). Abstracts of the 29th Annual Meeting of the ESHRE. *Hum Reprod* 2013;**28**:i71.

Brandes M, Hamilton CJ, van der Steen JO, de Bruin JP, Bots RS, Nelen WL, Kremer JA. Severity of oligo-asteno-teratozoospermia no longer determines overall success rate in male subfertility. *Int J Androl* 2011a; **34**:614–623.

Brandes M, Verzijden JC, Hamilton CJ, de Weys NP, de Bruin JP, Bots RS, Nelen WL, Kremer JA. Is the fertility treatment itself a risk factor for early pregnancy loss? *Reprod Biomed Online* 2011b; **22**:192–199.

Brandes M, Hamilton CJ, van der Steen JO, de Bruin JP, Bots RS, Nelen WL, Kremer JA. Unexplained infertility: overall ongoing pregnancy rate and mode of conception. *Hum Reprod* 2011c;**26**:360–368.

Cissen M, de Bruin JP. Study information: cost-effectiveness of IUI, IVF and ICSI for male subfertility. Male Subfertility Treatment Effectiveness (The MASTER study). http://www.studies-obsgyn.nl/MASTER/page.asp?page_id=1417 (1 February 2015, date last accessed), 2004.

Cooper TG, Noonan E, von Eckardstein S, Auger J, Gordon Baker HW, Behre HM, Haugen TB, Kruger T, Wang C, Mbizvo MT *et al.* World Health Organization reference values for human semen characteristics. *Hum Reprod Update* 2010;**16**:231–245.

Deveneau NE, Sinno O, Krause M, Eastwood D, Sandlow JI, Robb P, Granlund A, Strawn E Jr. Impact of sperm morphology on the likelihood of pregnancy after intrauterine insemination. *Fertil Steril* 2014; **102**:1584–1590.

Eimers JM, te Velde ER, Gerritse R, Vogelzang ET, Looman CW, Habbema JD. The prediction of the chance to conceive in subfertile couples. *Fertil Steril* 1994;**61**:44–52.

Esteves SE, Zini A, Aziz N, Alvarez JG, Sabanegh ES Jr, Agarwal A. Critical appraisal of World Health Organization's new reference values for human semen characteristics and effect on diagnosis and treatment of subfertile men. *Urology* 2012;**79**:16–22.

Hull MG, Glazener CM, Kelly NJ, Conway DI, Foster PA, Hinton RA, Coulson C, Lambert PA, Watt EM, Desai KM. Population study of causes, treatment, and outcome of infertility. *Br Med J* 1985;**291**: 1693–1697.

Hunault CC, Habbema JD, Eijkemans MJ, Collins JA, Evers JL, te Velde ET. Two new prediction rules for spontaneous pregnancy leading to live birth among subfertile couples based on the synthesis of three previuous model. *Hum Reprod* 2004;**19**:2019–2026.

Kurger TF, Acosta AA, Simmons KF, Swanson RJ, Matta JF, Oehninger S. Predictive value of abnormal sperm morphology in in vitro fertilization. *Fertil Steril* 1988;**49**:112–117.

National Institute for Health and Care Excellence (NICE) Clinical Guideline 156, Fertility. Assessment and treatment for people with fertility problems, 2013.

Nederlandse Huisartsen Genootschap (NHG), NVOG, NVU, KLEM, NVKC. De netwerkrichtlijn subfertiliteit, 2011.

NVOG. Richtlijn Orienterend fertiliteitsonderzoek, 2004.

Ombelet W, Bosmans E, Janssen M, Cox A, Vlasselaer J, Gyselaers W, Vandeput H, Gielen J, Pollet H, Maes M *et al.* Semen parameters in a fertile versus subfertile population: a need for change in the interpretation of semen testing. *Hum Reprod* 1997;**12**:987–993.

Opsahl MS, Dixon NG, Robins ER, Cunningham DS. Single vs, multiple semen specimens in screening for male infertility factors. A comparison. J Reprod Med 1996;**41**:313–315.

Polansky FF, Lamb EJ. Do the results of semen analysis predict future fertility? A survival analysis studie. *Fertil Steril* 1988;**49**:1059–1065.

Smith KD, Rodriquez-Rigau LJ, Steinberger E. Relation between indices of semen analysis and pregnancy rate in infertile couples. *Fertil steril* 1977; **28**:1314–1319.

Van der Steeg JW, Steures P, Eijkemans MJC, Habbema JDF, Hompes PGA, Broekmans FJ, van Dessel HJ, Bossuyt PM, van der Veen F, Mol BW *et al.* Pregnancy is predictable: a large-scale prospective external validation of the prediction of spontaneous pregnancy in subfertile couples. *Hum Reprod* 2007;**22**:536–542.

Van der Steeg JW, Steures P, Eijkemans MJC, Habbema JDF, Hompes PGA, Kremer JAM, van der Leeuw-Harmsen L, Bossuyt PMM, Repping S, Silber SJ *et al*. Role of semen analysis in subfertile couples. *Fertil Steril* 2011;**95**:1013–1019.

Van Rumste MM, Custers IM, van Wely M, van Weering HG, Beckers NGScheffer GJ, Broekmans FJ, Hompes PG, Mochtar MH, van der Veen F *et al*. IVF with planned single-embryo transfer versus IUI with ovarian stimulation in couples with unexplained subfertility: an economic analysis. *Reprod Biomed Online* 2014; **28**:336–342.

Van Weert JM, Repping S, van Voorhis BJ, van der Veen F, Bossuyt P, Mol BWM. Performance of the postwash total motile sperm count as a predictor of pregnancy at the time of intrauterine insemination: a meta-analysis. *Fertil Steril* 2004;**82**:612–620.

World Health Organization. *WHO Laboratory Manual for the Examination of Human Semen and Sperm-Cervical Mucus Interaction*, 4th edn. Cambridge: Cambridge University Press, 1999, 128.

World Health Organization. *WHO Laboratory Manual for the Examination of Human Semen and Sperm-Cervical Mucus Interaction*, 5th edn. Cambridge: Cambridge University Press, 2010, 223.