# Role of semen analysis in subfertile couples

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**Objective:** To evaluate the associations between the results of the male partner's semen analysis (classified according to the World Health Organization [WHO] criteria) and fathering a child without any treatment.
**Design:** Prospective multicenter cohort study.
**Setting:** Twenty subfertility centers in The Netherlands.
**Patient(s):** A total of 3,345 consecutive couples presenting for subfertility.
**Intervention(s):** None.
**Main Outcome Measure(s):** Associations between the results of the male partner's semen analysis, classified according to the WHO criteria, and fathering a child without any treatment within a time horizon of 1 year. Subsequently, we redefined semen quality criteria and reevaluated the associations.
**Result(s):** Follow-up data of 3,129 couples (94%) were available, of which 517 (17%) had a healthy pregnancy without treatment. The 1-year pregnancy rate in men with WHO normozoospermia did not differ significantly from that in men with WHO impaired semen (24% vs. 23%). In contrast, we observed lower chances of fathering a child for sperm concentrations $<40 \times 10^6$/mL, total sperm count $<200 \times 10^6$, and sperm morphology <20% normal forms. With a multivariable regression model based on the redefined male semen subfertility criteria we were able to make a finer differentiation between subfertile men, with probabilities of fathering a child ranging from 7% to 41%.
**Conclusion(s):** The current WHO criteria for semen quality do not discriminate between fertile and subfertile men. Our redefined and graded semen criteria have strong predictive value. If interpreted correctly, the fast and inexpensive semen analysis remains the gold standard for defining a man's role in subfertility. (Fertil Steril® 2010; ■:■–■. ©2010 by American Society for Reproductive Medicine.)

**Key Words:** Semen, subfertility, prognosis, pregnancy, WHO criteria

Subfertility or involuntary childlessness is one of life's great catastrophes. It occurs in more than one out of ten couples (1–4). Although female age and duration of subfertility are the most important factors to affect fertility, reduced semen quality is commonly claimed to be the cause of subfertility in about one-half of these couples (5, 6). To detect reduced semen quality, semen analysis is recommended as the initial test in the fertility work-up (7, 8).

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Yet the precise definition of male subfertility based on semen analysis has been hotly contested over the last half a century. In the 1950s, MacLeod et al. proposed the first cut-off values for sperm counts ($>20 \times 10^6$/mL, total sperm count $>100 \times 10^6$). These allowed clinicians to distinguish fertile from subfertile men (9, 10). Because of the fact that many couples in whom the men had sperm counts below these thresholds conceived without treatment, subsequent studies attempted to find more appropriate thresholds (11–20).

The growing need for a consistent interpretation of the results of the semen analysis was acknowledged by the World Health Organization (WHO) in their first manual for the examination of human semen in 1980 (21). It is surprising that the WHO criteria for male factor subfertility have never been prospectively validated. Several studies have attempted to evaluate the WHO criteria, but they used either a case-control design, comparing fertile and subfertile couples, or a cohort design among first pregnancy planners (8, 22–26). In this respect, it is important to note that already in 1988 a study emphasized that identification of prognostic factors for the occurrence of pregnancy would be more useful in clinical practice than comparing semen characteristics between fertile and subfertile populations (27). To this day it is not known whether

the WHO criteria can be used in a subfertile population to identify men likely to father a child and those who are not.

## MATERIALS AND METHODS

This study was designed as a prospective cohort study performed in 20 hospitals in The Netherlands. The local ethics committee of each participating center gave Institutional Review Board approval. All couples were informed about this study, but consent was not necessary, because no intervention was given. Between January 2002 and February 2004, we included consecutive subfertile couples that had not been evaluated previously. All couples underwent a basic subfertility work-up according to the guidelines of the Dutch Society of Obstetrics and Gynecology (28).

### Semen Analysis

Semen analysis was performed after 3 days of sexual abstinence. In some hospitals, semen analysis was repeated, but only results of the first semen analysis were used in this study. Semen samples were collected at the hospital or at the participant's home by masturbation directly into a 50-mL polyethylene jar. Semen samples were analyzed within 1 hour after ejaculation. After liquefaction, semen volume was measured in a graded tube with 0.1-mL accuracy. Sperm concentration was counted and motility assessed in a Makler counting chamber or a Bürger-Türk counting chamber at a magnification of $\times$200. For sperm motility the percentage of rapid progressive motile sperm (WHO type A) was analyzed, instead of the total motility which includes also the motile but not-moving spermatozoa. All semen parameters were classified according to the 1999 WHO criteria (29). The laboratory accuracy of sperm concentration, motility, and morphology assessment was monitored by the Dutch Foundation for Quality Assessment in Clinical Laboratories (www.skml.nl). Each laboratory received a standardized set of ten different semen analyses to compare analytic variation.

### Work-Up of the Female Partner

Age of the female partner was ascertained as well as other factors in the female exam, including assessment of ovulation, basal FSH level testing on cycle day 3, postcoital test (PCT), and assessment of the fallopian tubes according to the guidelines of the Dutch Society of Obstetrics and Gynecology (28).

### Follow-Up

After completion of the fertility work-up, the probability of a spontaneous pregnancy within 1 year, leading to live birth, was calculated based on a prediction model we previously published (www.amc.nl/prognosticmodel) (30, 31). This model includes the variables female age, duration of subfertility, pregnancy history, semen quality, result of the postcoital test, and referral status. Depending on that probability, couples were counseled for expectant management in case of a good chance to conceive without treatment (>40%) or fertility treatment according to the national fertility guidelines in case of a lower chance (32, 33). All couples were followed prospectively from the completion of the fertility work-up until spontaneous pregnancy occurred within 12 months resulting in an ongoing pregnancy. Couples that were treated within 12 months were followed until treatment started.

Couples in whom the female partner was diagnosed with anovulation, severe endometriosis (grade III-IV), or two-sided tubal pathology were excluded from the analysis. Couples in whom the male partner was diagnosed with azoospermia were also excluded.

The primary end point was conception without treatment within 1 year resulting in an ongoing pregnancy. Ongoing pregnancy was defined as the presence of fetal cardiac activity at transvaginal sonography at a gestational age of $\geq$12 weeks. Time to spontaneous pregnancy was considered to be censored whenever treatment started or at the last date of contact during expectant management.

### Data Analyses

For each semen parameter, the median and the 5th and 95th percentiles were calculated.

In the first part of the analysis, the Kaplan-Meier estimate of the 1-year cumulative incidence of spontaneous ongoing pregnancy was calculated for each of the categories according to the 1999 WHO criteria: normozoospermia, oligozoospermia, asthenozoospermia, teratozoospermia, oligoasthenozoospermia, oligoteratozoospermia, asthenoteratozoospermia, and oligoasthenoteratozoospermia.

Differences in the number of pregnancies between men with semen quality below the WHO thresholds and normozoospermic men were calculated and tested using log rank statistics. All men with semen quality below the WHO thresholds were compared with normozoospermic men.

In the second part of the analysis, associations between each semen parameter and fathering a child were reevaluated by means of restricted cubic spline functions and Cox regression analyses (34). The spline functions were used to express the probability of fathering a child as a flexible function of the semen parameter. Nonlinearity of the spline function was tested with analysis of variance statistics. Based on the spline functions, we set new semen thresholds where applicable. The redefined semen thresholds were then evaluated in univariable and multivariable Cox regression analyses, including semen volume, sperm concentration, morphology, and motility.

In all analyses, a $P$ value of .05 was used as to indicate statistical significance. Calculations were performed with SPSS (SPSS, Chicago, IL) and S-plus (MathSoft, Seattle, WA) programs.

## RESULTS

### Participants

We registered 4,538 subfertile couples that had not been evaluated previously and who had completed their fertility work-up. In 286 men (6.3%), semen analysis was not performed, and in 65 men (1.4%) azoospermia was detected. In 638 couples (14%), the female partner was diagnosed with anovulation, and in 204 couples (4.5%) there was double-sided tubal pathology.

### Baseline Characteristics and Pregnancy Outcomes

In 1,307 couples, treatment was started within 12 months (39%), and 1,256 couples did not conceive without treatment within 12 months (38%). Data of 216 couples (6%) could not be obtained, even after contacting the general practitioner.

In total, 566 couples (17%) had a spontaneous pregnancy without treatment within 1 year. Of these couples, 511 had a singleton ongoing pregnancy, 6 a multiple ongoing pregnancy, 46 a miscarriage, and 3 an ectopic pregnancy. In these pregnant couples, mean female age was 31.4, mean male age 34.1, and median duration of subfertility 1.3 years. The median semen volume was 3.0 mL, sperm concentration 49 $\times$ $10^6$/mL, progressive motility 39.5%, percentage normophorms 25%, and total motile count 48 $\times$ $10^6$. Of all the pregnant couples, 60% were primary subfertile, 91.4% were referred by their general practitioner, 39.8% of men had normospermia, and 8.1% of women had one-sided tubal pathology.

### Semen Characteristics

The semen characteristics of all men are summarized in Table 1. A total of 3,345 men had one semen analysis performed; in 1,458 men (44%) a second semen analysis was performed (mean number of samples of 1.4). In 349 men (10%), sperm morphology was not documented.

Out of all the men, 29% had a semen volume below the WHO threshold of 2.0 mL, 31% of men had a concentration below the WHO threshold of 20 $\times$ $10^6$/mL, 38% had a progressive sperm motility below the WHO threshold of 25% progressive motile sperm, 32% had a sperm morphology below the WHO threshold of 15%

**TABLE 1**

**Characteristics of the patients (n = 3,345 men).**

| Continuous variables | Mean | 5th–95th percentile |
|---|---|---|
| Male age (y) | 35.0 | 27–45 |
| Female age (y) | 32.1 | 25–39 |
| Duration of subfertility (y), median | 1.5 | 1.0–4.1 |
| **Semen parameters** | **Median** | **5th–95th percentile** |
| Volume (mL) | 3.0 | 1.0–6.0 |
| Concentration ($10^6$/mL) | 40.0 | 2.0–155 |
| Motility, progressive (%) | 33 | 1–70 |
| Morphology (% normal) according to WHO | 26 | 2–56 |
| TC ($10^6$) | 108 | 5–511 |
| **Categoric variables** | **n** | **%** |
| Subfertility of the couple, primary | 2,379 | 71 |
| Previous pregnancies of man in previous partnership | 372 | 11 |
| Previous pregnancies of woman in previous partnership | 352 | 11 |
| Referral status | | |
| Own initiative | 209 | 209 |
| General practitioner | 2,763 | 2,763 |
| Gynecologist | 280 | 280 |
| Other specialist | 93 | 2.8 |
| Current smoking | | |
| Yes | 895 | 35 |
| No | 1,667 | 65 |
| Current alcohol use | | |
| Yes | 1,536 | 61 |
| No | 997 | 39 |
| Current or past disease | | |
| None | 1,962 | 81 |
| Diabetes type I | 16 | 0.5 |
| Cardiovascular disease | 28 | 1.2 |
| HIV | 5 | 0.2 |
| Liver pathology | 8 | 0.3 |
| Cancer in past | 18 | 0.7 |
| Kidney pathology | 7 | 0.3 |
| Psychologic disease | 15 | 0.6 |
| Asthma | 23 | 1.0 |
| Testes | 135 | 5.5 |
| Orchidopexy | 61 | 2.5 |
| Testicular surgery | 37 | 1.5 |
| Inflammation of testes | 19 | 0.8 |
| Pathology of testes | 18 | 0.7 |
| Other | 192 | 8.0 |

*Note:* HIV = human immunodeficiency virus; TC = total sperm count; WHO = World Health Organization.

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normophorms, and 25% had a total sperm count below the WHO threshold of 40 × $10^6$.

This implies that 41% of men were classified with normozoospermia according to the WHO criteria, and 59% were allocated to one of the categories of poor semen quality (Fig. 1).

## WHO Semen Criteria and Spontaneous Pregnancy Rates

Couples in whom the man had normozoospermia by WHO criteria had a spontaneous pregnancy rate of 24% (95% confidence interval [CI]: 21%–27%). The spontaneous pregnancy rate in couples in whom the man had abnormal sperm was 23% (95% CI 20%–26%), a nonsignificant difference (log rank test: $P$=.74).

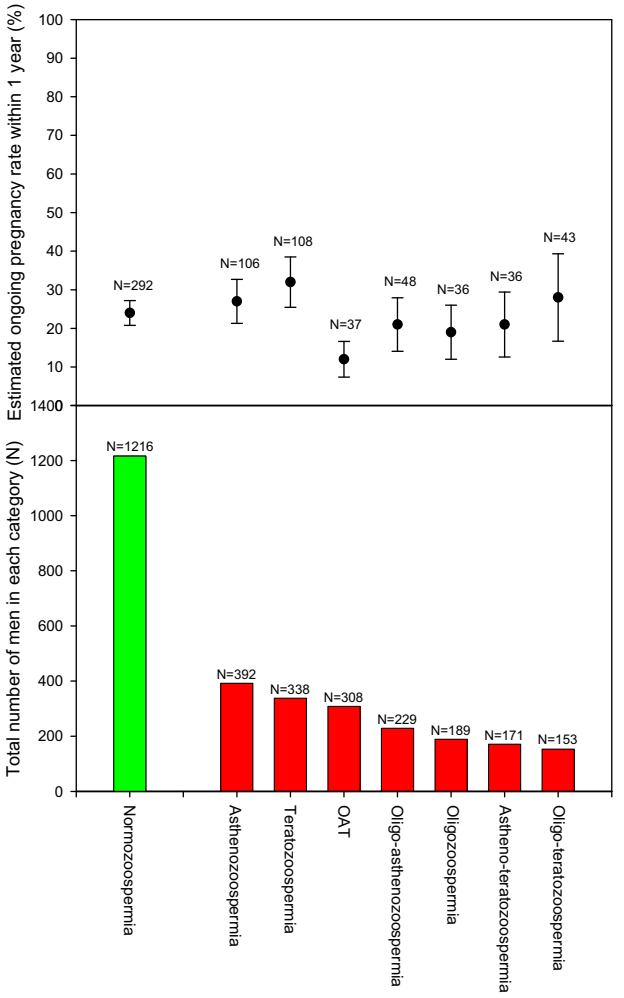
There was no clear pattern between the observed pregnancy rates and the categories of semen impairment. Only oligoasthenoteratozoospermia was associated with a significantly lower cumulative pregnancy rate of 12% (95% CI 7%–17%; Fig. 1).

## Reinterpretation of the Semen Parameters

There was a significant nonlinear association between semen volume and fathering a child ($P$=.02; Fig. 2A). Semen volumes

**FIGURE 1**

Estimates of pregnancy rates and confidence intervals at 12 months for each subcategory of male subfertility based on the Kaplan-Meier estimator. Numbers above the bars are the couples per subgroup. Numbers above the estimated pregnancy rates are the estimated number of couples pregnant after 1 year.

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<2.0 mL were associated with a lower probability of fathering a child (per mL <2.0 mL ~25%: hazard ratio [HR] 0.75 [95% CI 0.59–1.02]). In men with semen volumes >2.0 mL, the probability of fathering a child did not increase any further.

For sperm concentration we also found a nonlinear association with fathering a child ($P<.01$; Fig. 2B). Sperm concentrations >40 × $10^6$/mL were significantly associated with a lower probability of fathering a child (per 10 × $10^6$/mL <40 × $10^6$/mL ~15%: HR 0.85 [95% CI 0.80–0.90]). In men with sperm concentrations >40 × $10^6$/mL, the probability of fathering a child did not increase any more. Sensitivity analysis confirmed the threshold of 40 × $10^6$/mL. In case of a lower threshold (e.g., threshold set at 10 × $10^6$/mL) or higher (e.g., threshold set at 90 × $10^6$/mL), no significant associations with chance to father a child were found.

Progressive sperm motility demonstrated a linear association with pregnancy rates over the whole range (for each 10% <100% progressive motile sperms ~11%: HR 0.89 [95% CI 0.85–0.93]; Fig. 2C). The $P$ value for nonlinearity was >.05. Owing to the linear association, sperm motility did not show a specific threshold.

For sperm morphology, we observed a nonlinear association with fathering a child ($P<.01$). Men with a sperm morphology <20% normal forms had a lower probability of fathering a child (for each 10% <20% normal forms ~34%: HR 0.66 [95% CI 0.55–0.79]).

Finally, there was a significant nonlinear relation between total sperm count and fathering a child ($P<.01$; Fig. 2E). Total sperm counts <200 × $10^6$ were significantly associated with a lower probability of fathering a child (for each 10 × $10^6$ <200 × $10^6$ sperms ~3%: HR 0.97 [95% CI 0.96–0.98]).

The new model based on these semen parameters (multivariable analysis; Table 2) was able to estimate the probabilities of fathering a child within 1 year as between 7% for men with the poorest semen quality and 41% for men with the best semen quality. In contrast, based on the WHO criteria, men with WHO-abnormal semen had an estimated pregnancy rate of 23% versus 24% for men with WHO-normal semen.

## DISCUSSION

This large prospective study of the relationship between semen quality and fathering a child without treatment in subfertile couples observed that the WHO criteria could not distinguish men who were likely to father a child from men who were not. This study also demonstrated that semen quality does matter, because our multivariable model, based on redefined semen criteria, was able to discriminate between men with lower and higher probabilities of fathering a child.

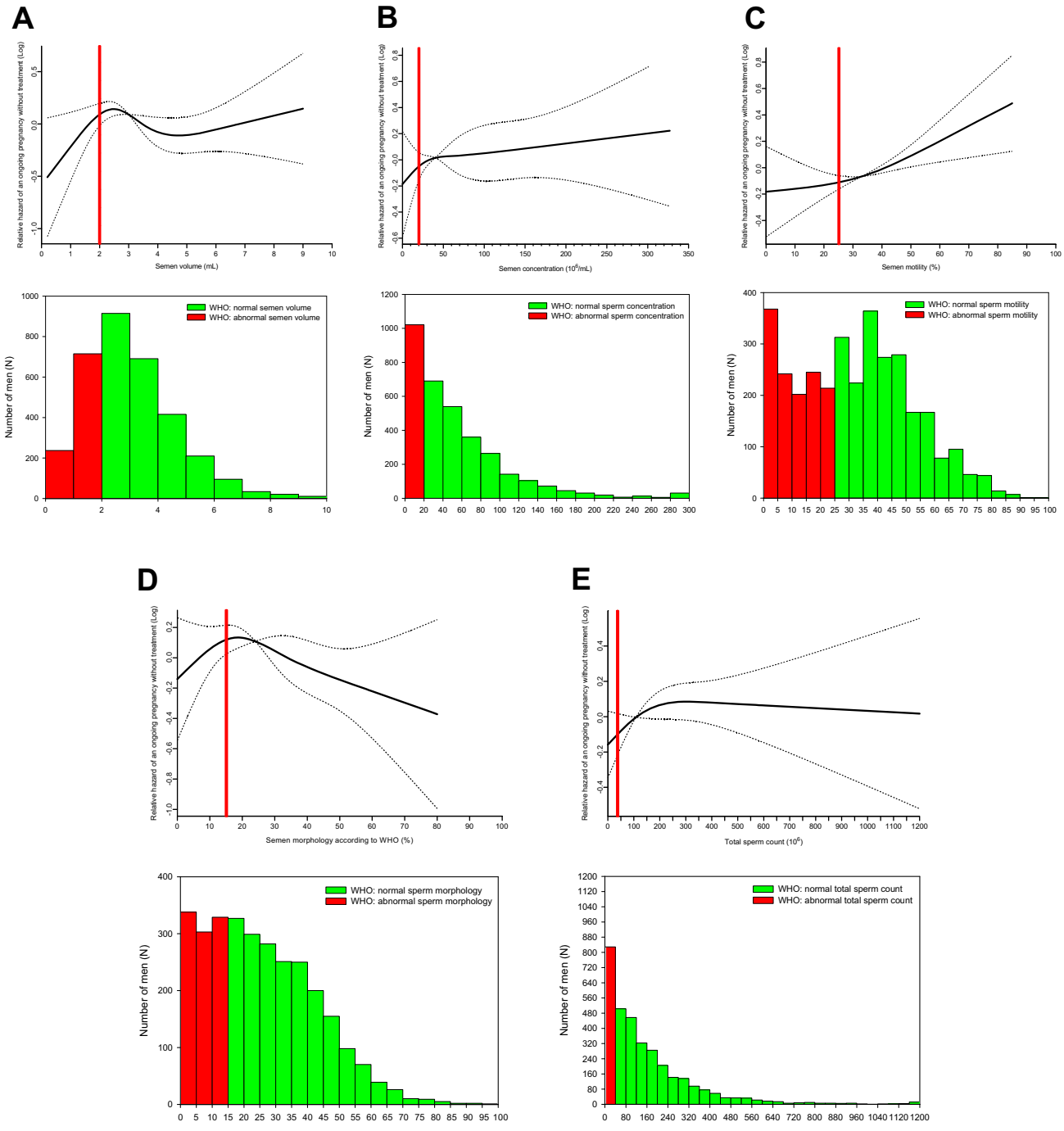
A strength of this study is that it assessed the prognostic value of semen in an unselected subfertile population. Our study cohort is probably representative for subfertility cohorts elsewhere, because 59% of all men had abnormal semen parameters according to the WHO criteria, which is in line with the observation that male factor subfertility plays a role in about one-half of all subfertile couples (5, 29).

A methodologic strength of this study is that the associations between semen parameters and the chance of fathering a child were assessed in a continuous way, rather than dichotomizing them into normal versus abnormal. One earlier study of 430 first-pregnancy planners used a similar method for assessment of semen parameters (23). In both our study and the study by Bonde et al. (23), semen volume, sperm motility, and sperm concentration showed similar findings. It is clear from both studies that standard norms of semen quality based on crude cut-offs, defining normal versus abnormal, are of no use in identifying couples which are likely to conceive without medical assistance and those who are not.

One limitation may be the fact that our study focused on the first semen sample, and did not take results of a second or third sample into account. In clinical practice, most hospitals only performed one semen analysis if the first analysis showed normal semen. As a consequence in this study, most men with a second semen analysis had a poor first semen analysis. Using the second analysis would have introduced selection bias. We are analyzing the additional value of a standard second semen analyses regarding the predictive value of getting pregnant, but only in a selection of hospitals that routinely perform two semen analyses, regardless of the result of the first semen analysis. Apart from that, differences

## FIGURE 2

Semen parameters and the probability of fathering a child. Spline functions: red vertical lines indicate the reference values for sperm quality according to the WHO manual of 1999; dotted lines represent 95% confidence intervals.

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between the 20 laboratories may have caused bias, but adjustment by stratifying the analysis by laboratory did not alter the results (data not shown).

Another potential limitation of this study might have been informative censoring during follow-up. In case of informative censoring, the effects of variables other than semen quality on the probability of pregnancy are not constant over time. In other words, women with men with poor semen quality would be treated with intracytoplasmic sperm injection, in vitro fertilization, or intrauterine insemination sooner than women with men with normal semen

**TABLE 2**

**Pregnancy and male subfertility, based on redefined semen parameters (n = 3,345).**

| Semen parameter | n | % | Univariable analysis HR | 95% CI | *P* value | Multivariable analysis HR | 95% CI | *P* value |
|---|---|---|---|---|---|---|---|---|
| Semen volume (mL), continuous per mL | | | | | | | | |
| ≤2.0 | 952 | 29 | 0.78 | (0.59–1.02) | .07 | 0.70 | (0.50–0.98) | .04 |
| >2.0 | 2,393 | 71 | 1 | | | | | |
| Sperm concentration ($10^6$/mL), continuous per $10 \times 10^6$ less | | | | | | | | |
| ≤40 | 1,711 | 51 | 0.85 | (0.80–0.90) | <.01 | 0.88 | (0.77–1.0) | .05 |
| >40 | 1,634 | 49 | 1 | | | | | |
| Sperm motility (%), continuous per 10% less | 3,345 | 100 | 0.89 | (0.85–0.93) | <.01 | 0.94 | (0.89–0.99) | .01 |
| Sperm morphology (% normal), continuous per 10% less (n = 2,996) | | | | | | | | |
| ≤20 | 1,297 | 43 | 0.66 | (0.55–0.79) | <.01 | 0.78 | (0.64–0.95) | .01 |
| >20 | 1,699 | 57 | 1 | | | | | |
| Total sperm count ($10^6$), continuous per $10 \times 10^6$ less | | | | | | | | |
| ≤200 | 2,406 | 72 | 0.97 | (0.96–0.98) | <.01 | * | | |
| >200 | 939 | 28 | 1 | | | | | |

*Note:* The probability (*P*) of fathering a child within one year can be calculated from the multivariable model with the formula: $P = 0.77^{\exp[(-0.36 \times \text{semen volume}) + (-0.13 \times \text{sperm concentration}) + (-0.07 \times \text{sperm motility}) + (-0.25 \times \text{sperm morphology})]}$. CI = confidence interval; HR = hazard ratio.

* Variable not analyzed in the multivariable analysis.

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quality, owing to other factors than the semen quality. Except for a small difference in duration of subfertility, we did not observe differences in the other variables for these two groups. Although median sperm concentration (38 vs. 42 × $10^6$/mL), motility (32% vs. 37%), and total motile sperm count (32 vs. 40 × $10^6$) were slightly lower in the treatment group than in the group that had expectant management for 12 months, this did not lead to informative bias. In that case, the proportional hazards assumption would not have been met and the results of the Cox analysis may have been biased, owing to the fact that the HR between men with high count and men with low count would change with length of follow-up time. However, we found that the relationship between the HR of motility versus getting pregnant was stable over time, an indication that there was no informative censoring due to semen quality (data not shown).

Earlier studies on the significance of semen parameters conducted either case-control studies of subfertile and fertile couples (8, 22, 25, 26) or cohort studies of healthy fertile couples (23, 24, 35). All of those studies concluded that higher cut-off values than what are currently suggested by WHO are relevant.

Some of those studies derived their own cut-off values (22, 23, 25). For two reasons, they can not be translated to subfertile men. First, the clinical issue is not one of distinguishing men that conceive within 12 months from men that do not, because the first group of men already got pregnant at the time a subfertile couple visits the fertility clinic. Second, semen thresholds derived from fertile men, who fathered a child within 1 year, as used in the current WHO criteria, overestimate the diagnostic accuracy of these semen analyses in a subfertile population (36).

In the decision for fertility treatment, we propose not to focus on semen parameters separately, but to integrate the test results of all four semen parameters. In this way, the chance of fathering a child can be calculated. One can decide that if, for example, the probability of fathering a child within 1 year drops below 25%–30%, fertility treatment is indicated (Table 2, footnote).

In conclusion, there is now extensive evidence that the current WHO criteria for semen quality do not discriminate between fertile and subfertile men in a subfertile population. We found a strong continuous correlation between semen parameters and the probability of natural conception once interpreted with redefined semen parameters. A nihilistic conclusion about the predictive value of the simple semen analysis is not warranted. The inexpensive standard semen analysis should remain the gold standard for determining a man's role in subfertility, albeit according to our redefined interpretation of semen parameters.

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