# Computer-assisted semen analysis parameters as predictors for fertility of men from the general population

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**The predictive value of sperm motility parameters obtained by computer-assisted semen analysis (CASA) was evaluated for the fertility of men from general population. In a prospective study with couples stopping use of contraception in order to try to conceive, CASA was performed on semen samples from 358 men. A recently developed CASA system, Copenhagen Rigshospitalet Image house sperm Motility Analysis System (CRISMAS) was used for assessment of motility parameters. This system has an editing function which allows correction of tracks made by the computer. Probably due to this function, the concentration assessment made by CRISMAS was very close to that made by the technician (median difference <5%) in all concentration ranges. Correlation between CASA parameters and fertility of normal couples (measured as probability of achieving pregancy) was examined by the Cox regression model. In univariate models ln(sperm concentration) [β = 0.331, risk ratio (RR) = 1.392, $P$ = 0.0001], ln(total sperm count) (β = 0.252, RR = 1.286, $P$ = 0.0007) and percentage motile spermatozoa (β = 0.014, RR = 1.014, $P$ = 0.0004) were most significant predictors for fertility. In a multivariate analysis ln(sperm concentration) (β = 0.268, RR = 1.307, $P$ = 0.0016) and percentage motile spermatozoa (β = 0.010, RR = 1.010, $P$ = 0.011) but even more significantly the combined parameter, ln(concentration of motile spermatozoa) (β = 0.329, RR = 1.389, $P$ = 0.0001), were the only parameters of predictive value for fertility of men in the general population. In conclusion, these parameters obtained by CASA measurements can be used for prediction of fertility potential in normal men. This appears to be the first study showing the value of CASA in prediction of fertility in the general male population.**
*Key words:* computer-assisted semen analysis/male fertility/ semen quality/sperm concentration

## Introduction

Standard semen analysis is a rather subjective technique and associated with large inter-laboratory variation (Jørgensen *et al.*, 1997), which makes it virtually impossible to compare sperm motility assessments performed by different laboratories. Furthermore, the World Health Organization classification for sperm motility defines only four categories (WHO, 1992), as a more detailed description of sperm movements is not possible without use of special equipment.

In an attempt to make the assessments of semen quality more objective and detailed, tools for computer-assisted semen analysis (CASA) have been developed. By use of CASA several specific motility parameters describing the movements of spermatozoa in a more detailed manner can be obtained. Furthermore, the classification into motile and immotile spermatozoa can be based on well-defined velocity thresholds.

Parallel with attempts to improve the technical performance of CASA systems, it is important to investigate the biological relevance of CASA parameters in the context of prediction of male fertility potential, a knowledge which is of crucial importance for understanding the biology of fertilization and also for diagnosis and treatment of male infertility.

Several studies have addressed the issue of CASA parameters as predictors of male fertility (Aitken *et al.*, 1984; Barratt *et al.*, 1993; Irvine *et al.*, 1994; Paston *et al.*, 1994; Krause, 1995). However, those studies have often been based on couples recruited from infertility clinics. In such studies the most obvious cases of female infertility, including tubular occlusion or anovulation, can be exlcuded. On the other hand, the percentage of couples with unexplained infertility, which may also be caused by a female factor, is quite significant and men with normal semen parameters may be rather underrepresented. Furthermore, frequently the outcome of different types of artificial reproduction procedures was used as an end-point, making the results of more limited relevance for the naturally achieved pregnancies. The use of such different approaches

and lack of standardization in the field of CASA may explain the fact that virtually all CASA parameters have been reported to be correlated with male fertility potential in at least one study set-up. Taking men from the general population is advantageous because they will span the spectrum of semen profiles from very poor to very good. However, since the female partner is not investigated thoroughly, some men will be misclassified as infertile even though the infertility of the couple is almost entirely down to the female partner. This phenomenon may introduce some 'noise' in the final statistical analysis. Information on the relationship between CASA parameters and the chance of conception in couples from a general population is very sparse.

Recently, it was shown that conventionally assessed sperm concentration is a strong predictor of fertility in normal males (Bonde *et al.*, 1998a). The objective of the present study was to investigate whether CASA motility parameters may add to the information regarding semen quality in relation to fertility of men recruited from the general population.

## Materials and methods

### The follow-up study

The design of the follow-up study has been described in details by the Danish First Pregnancy Planner Study FECUNDA group (Bonde *et al.*, 1998b). Briefly, from 1992 to 1994 trade union members of age 20–35 years, including metal workers, office workers, nurses and day-care workers, were invited to participate in the study by letter. Inclusion criteria were: no previous pregnancies, no prior knowledge of own fertility, living with a person of the opposite sex, and current use of contraception but planning to discontinue in order to conceive. A total of 430 couples were enrolled in the study in Copenhagen (east centre) and Aarhus (west centre). A total of 419 provided a fresh semen sample at enrolment and CASA was performed with these samples. Information on lifestyle factors, including occupation and pathological conditions of female reproductive organs, were collected through questionnaires to allow the results to be adjusted for these factors. Additionally, men and women completed a questionnaire monthly to make sure that important changes in health condition or lifestyle factors were registered. The couples were followed for a maximum of six menstrual cycles or until a pregnancy was achieved.

### Computer-aided semen analysis (CASA)

The CASA system employed in this study was the CRISMAS system, version 1.0 (Image House A/S, Copenhagen, Denmark). It calculated CASA parameters of sperm motility according to the guidelines from WHO (1992). In addition to these specific motility parameters, the computer also calculated sperm concentration, total sperm count, the percentage of motile spermatozoa in the sample, and a classification of the spermatozoa into immotile (curvilinear velocity, VCL $<5$ μm/s), locally motile (VCL $= 5$–$25$ μm/s) and motile (VCL $>25$ μm/s) was made. CRISMAS had an edit function that allowed the user to correct wrong sperm tracks calculated by the computer. It was thus possible to correct misinterpretations such as other particles being recognized as immotile spermatozoa or wrong tracking resulting from paths crossing each other. This property minimized the error rate and made the results more valid. The main components of the CRISMAS system were a Nikon microscope with positive phase contrast optics, a charge-coupled device (CCD) video camera and a personal computer with a digital frame grabber and CRISMAS image processing

software. The measuring time for CRISMAS was $4 \times 2.6$ s and the frame sampling frequency was 25 Hz.

### Experimental conditions

CASA was made on semen samples collected at start of the enrolment, and the analysis is based on samples from 358 men of the 419 men included in the whole study. Thirty samples collected in a pilot study in 1992 were not stored on videotapes for analysis by CRISMAS. Eight samples showed azoospermia ($n = 7$) or severe oligozoospermia ($n = 1$); one sample was too viscous to analyse; 12 samples were not analysed for different but absolutely random causes; 10 samples could not be analysed by CRISMAS because of a low quality of the video recordings of these samples resulting from an insufficient dilution. This could mean a problem of selection bias if the sperm concentration of these samples was higher than the other samples. Attempts were made to solve this problem by including the manually determined sperm concentrations from these 10 samples in the statistical analyses. This did not change the results.

The semen samples were collected and recorded on videotapes for CASA analysis in both Copenhagen and Aarhus, and the same recording protocols were applied in the two centres except of the sample volume pipetted into Makler chamber being 2.5 μl in Copenhagen and 4.5 μl in Århus.

Freshly collected semen samples were allowed to liquefy for 20 min at 37°C. They were diluted appropriately in mixed agglutination reaction (MAR) test buffer (9 mmol/l $KH_2PO_4$, 28 mmol/l $Na_2HPO_4$, 11 mmol/l NaCl) and the diluted sample was pipetted into a Makler chamber, which was placed on a heated microscope stage (37°C). Video recordings were made from four different fields of the chamber using a $\times 20$ magnification objective on the microscope. CASA analysis was based upon capturing sequences of 64 frames per field and counting a minimum of 100 spermatozoa.

### Validation of sperm concentration assessment by CRISMAS

In order to evaluate the accuracy of CRISMAS in distinguishing non-motile spematozoa from other particles, for the 358 samples included in the study, sperm concentration values obtained by CASA were compared to the results obtained by counting in a Bürker–Türk chamber, according to the WHO guidelines (1992). The quality of the standard sperm concentration assessment was regularly checked by internal quality assurance programme showing an inter-technician coefficient of variation of $<15\%$.

### Evaluation of the effect of dilution

For ten randomly selected ejaculates, CASA analysis was performed on undiluted samples as well as samples diluted according to the above-mentioned protocol. The results of the two assessments were compared to each other.

### Statistical methods

CRISMAS calculates CASA parameters for all spermatozoa and for only the subpopulation of motile spermatozoa in a semen sample, respectively. The following statistical analyses were made with CASA parameters for the motile spermatozoa only, because it was found that this sperm population is most relevant in the context of fertilization. When motility parameters for all spermatozoa were utilized, the results of the statistical analyses were virtually the same (data not shown).

The data were analysed in univariate and multivariate discrete Cox regression analyses. The regression model was validated by standard regression techniques. By this regression method the potential of the CASA parameters to predict the chance of pregnancy was examined. All the variables were entered as linear covariates, except for sperm
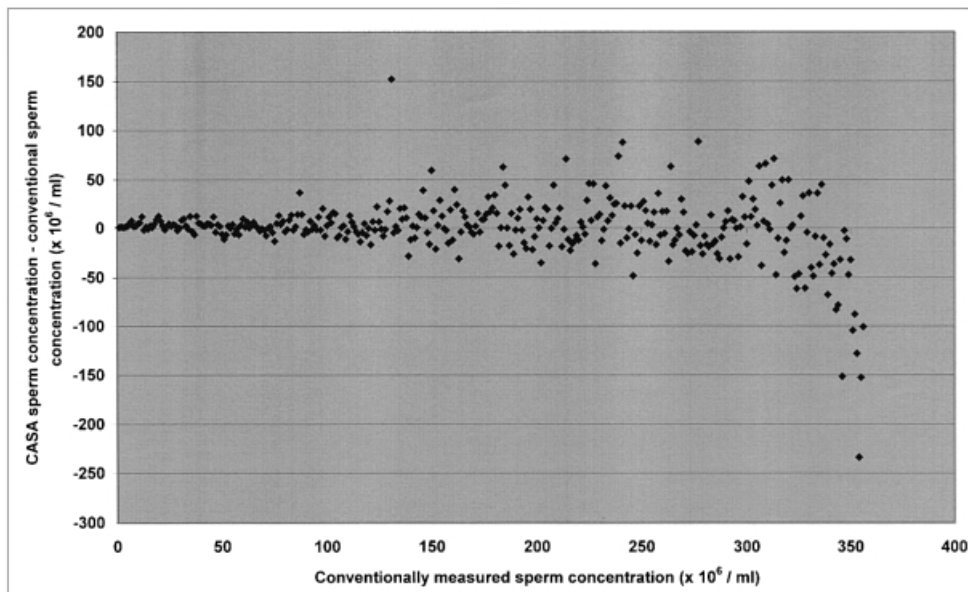
**Figure 1.** Absolute difference between sperm concentration calculated manually and by computer-assisted semen analysis (CASA).

concentration, total number of spermatozoa, concentration of motile spermatozoa and straight line velocity (VSL), which were entered as logarithmic values in order to fulfil the assumption of linearity of the covariates. Model selection was done by backwards model selection techniques and combined with an initial model selection strategy based on subject matter knowledge.

In the multivariate analyses the results were adjusted for a possible confounding effect of several male and female factors. These factors were: abstinence time (number of days since last ejaculation), occupation (1 = metal worker, 2 = office worker, 3 = nurse, 4 = daycare worker), oral contraception as last contraceptive method (1 = yes, 0 = no), female age (years), female smoking (1 = smoking, 0 = not smoking), female body mass index (BMI; kg/m$^2$), if the woman had ever experienced diseases in the internal female reproductive organs (1 = yes, 0 = no) and length of menstrual cycle (days). Because the video recording procedure differed between the two centres the results were also controlled for a confounding effect of 'centre' (1 = Aarhus, 2 = Copenhagen).

Separate Cox regression analyses were done with a subpopulation of spermatozoa having motility characteristics reported to be required for penetration of cervical mucus ('mucus-penetrating spermatozoa'). These analyses were done in order to clarify whether the results were skewed by inclusion of spermatozoa not capable of penetrating cervical mucus and which can be considered as not physiologically relevant for the process of fertilization. Mucus-penetrating spermatozoa have been reported to be characterized by minimum values of VSL, VSL/VAP = straightness and amplitude of lateral head displacement (ALH) of 25 µm/s, 90% and 2.5 µm/s, respectively (Mortimer, 1994).

Mann–Whitney test was applied in order to evaluate the effect of dilution on CASA parameters. The statistical analyses were carried out using the Statistical Analysis System (SAS) for personal computers, version 6.12 for Windows (SAS Institute Inc., Cary, NC, USA).

## Results

### *Sperm concentration*

Figure 1 shows the correlation between sperm concentration of the 358 semen samples measured manually and by CASA.

**Table I.** Comparison of pregnancy rates and manually measured sperm concentrations among 'participants' and 'non-participants' ($n = 419$ semen samples)

| | CASA ($n = 358$) | Non-CASA ($n = 61$) |
|---|---|---|
| No. of pregnancies (%) | 218 (61) | 31 (51) |
| Median (range) of conventionally measured sperm concentration ($\times 10^6$/ml) | 52 (0–262) | 50 (0–408) |

The two groups were not significantly different.
CASA = computer-assisted semen analysis; NS = not significant.

The values obtained by CRISMAS were only slightly higher than the results of a standard evaluation (median: $1.6 \times 10^6$/ml). An analysis of the discrepancy between the two methods revealed a rather small relative difference (median for difference as percentage of average between the two counts: 4.8%), which was particularly remarkable in the oligozoospermic range. The absolute but not the relative discrepancy was increased for higher sperm concentrations.

A comparison of the sperm concentrations (measured manually) among the participants and 'non-participants' (the couples for whom no CASA data exist) showed similar median sperm concentrations in the two groups: $52 \times 10^6$/ml and $50 \times 10^6$/ml, respectively (Table I). A total of 218 couples (61 %) achieved a pregnancy during the follow-up period of six menstrual cycles. The frequency of pregnancies was slightly, but not statistically significantly, lower (51%) among the 'non-participants'. In Table II the range of the CASA parameters of the 358 semen samples analysed in this study is shown.

### *Effect of dilution*

For the population of motile spermatozoa most CASA parameters were not significantly affected by dilution in the MAR test buffer, the only exceptions being beat cross frequency (BCF) and STR.

**Table II.** Range of computer-assisted semen analysis (CASA) parameters in the study population

| CASA parameter | $n^a$ | Median | Range |
|---|---|---|---|
| Sperm concentration ($\times 10^6$/ml) | 356[b] | 54.7 | 0.9–211.0 |
| Total sperm count ($\times 10^6$) | 314 | 156 | 2–1179 |
| Percentage of motile spermatozoa | 356[b] | 59.8 | 0–100.0 |
| VCL (µm/s) | 317 | 64.6 | 26.6–103.1 |
| VSL (µm/s) | 317 | 40.7 | 0.6–72.2 |
| VAP (µm/s) | 317 | 43.8 | 4.2–73.5 |
| LIN (%) | 317 | 61.1 | 1.6–94.0 |
| STR (%) | 317 | 95.8 | 7.4–99.5 |
| ALH (µm) | 317 | 2.0 | 0.9–4.1 |
| WOB (%) | 317 | 65.4 | 8.8–94.5 |
| BCF (beats/s) | 317 | 9.7 | 5.2–20.0 |

[a]$n$ = number of observations.
[b]Forty-one samples were analysed on a previous version of CRISMAS which only measured sperm concentration and motility. Therefore, the number of observations is higher for sperm concentration and percentage of motile spermatozoa than for the other variables.
VCL = curvilinear velocity; VSL = straight line velocity; VAP = average path velocity; LIN = linearity; STR = VSL/VAP (straightness); ALH = amplitude of lateral head; WOB = wobble VAP/VCL; BCF = beat cross frequency.

**Table III.** Results of univariate Cox regression analyses

| Covariate | β-estimate | RR | 95% CI of RR | P |
|---|---|---|---|---|
| ln(sperm concentration) | 0.331 | 1.392 | 1.183–1.638 | 0.0001 |
| ln(total sperm count) | 0.252 | 1.286 | 1.112–1.488 | 0.0007 |
| ln(concentration of motile spermatozoa) | 0.307 | 1.359 | 1.188–1.555 | 0.0001 |
| % motile | 0.014 | 1.014 | 1.006–1.022 | 0.0004 |
| % locally motile | −0.014 | 0.986 | 0.977–0.995 | 0.0018 |
| % immotile | −0.020 | 0.980 | 0.965–0.996 | 0.015 |
| VCL | 0.014 | 1.015 | 1.003–1.026 | 0.0146 |
| VSL | 0.011 | 1.011 | 0.999–1.022 | NS |
| VAP | 0.013 | 1.013 | 1.001–1.026 | 0.0348 |
| LIN | 0.006 | 1.006 | 0.996–1.015 | NS |
| STR | 0.018 | 1.018 | 1.000–1.035 | 0.0459 |
| ALH | 0.236 | 1.266 | 0.983–1.629 | NS |
| WOB | 0.008 | 1.008 | 0.997–1.019 | NS |
| BCF | 0.085 | 1.088 | 1.009–1.174 | 0.0282 |

RR = risk ratio; CI = confidence interval; NS = not significant. For definition of CASA parameters see Table II.

## CASA parameters and fertility

The results of univariate Cox regression analyses are shown in Table III. Ln(sperm concentration), ln (total sperm count), ln(concentration of motile spermatozoa) and percentage of motile spermatozoa were most significantly associated with the probability of achieving a pregnancy, but VCL, average path velocity (VAP), STR and BCF were also of significant predictive value for fertility.

When the covariates were selected into the Cox regression model by a 'backward elimination' procedure, only ln(sperm concentration) and percentage of motile spermatozoa were found to be being independent predictors of the probability of obtaining pregnancy. However, when the two variables were combined with a common parameter, ln(concentration of motile spermatozoa), in a new Cox regression analysis it was found

**Table IVa.** Final Cox regression model including only the selected covariate

| Covariate | β-estimate | RR | 95% CI of RR | P |
|---|---|---|---|---|
| ln(concentration of motile spermatozoa) | 0.307 | 1.359 | 1.188–1.555 | 0.0001 |

**Table IVb.** Adjusted Cox regression model[a]

| Covariate | β-estimate | RR | 95% CI of RR | P |
|---|---|---|---|---|
| ln(concentration of motile spermatozoa) | 0.329 | 1.389 | 1.201–1.607 | 0.0001 |

[a]The model was adjusted for the following factors: abstinence time, centre, occupation, oral contraception, female age, female smoking, female body mass index, urogenital disorders and cycle length.
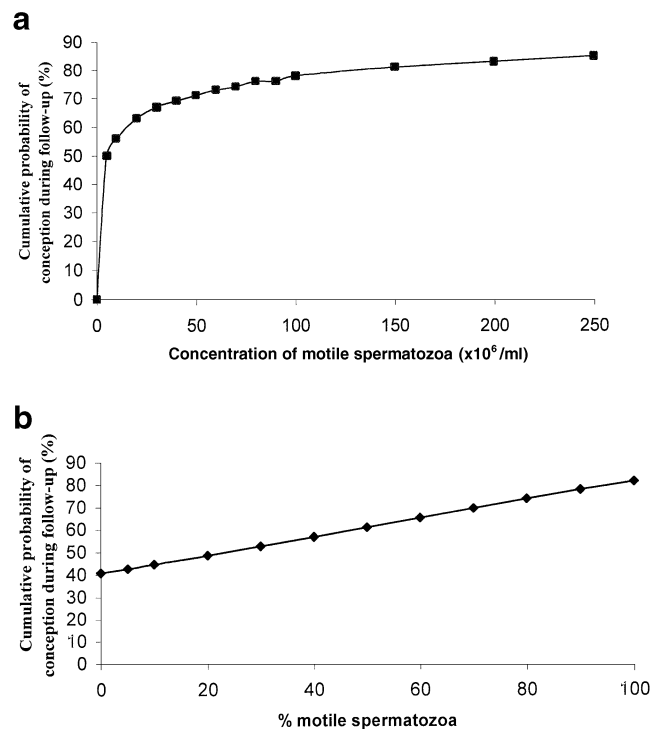RR = risk ratio; CI = confidence interval.

**Figure 2.** The association between sperm characteristics and the probability of conception during follow-up, calculated from univariate Cox regression models. (**a**) The effect of increasing concentration of spermatozoa with curvilinear velocity >25 µm/s on pregnancy rate. The curve indicates that a possible 'threshold' value for motile sperm concentration, separating fertile and subfertile men, lies close to the value of 25–30×$10^6$/ml. (**b**) The effect on increasing percentage of motile spermatozoa on pregnancy rate.

to be the only independent predictor of pregnancy (Table IVa). Adjustment of the model for abstinence time, centre, occupation and several female factors did not change this finding (Table IVb). When the Cox regression analysis was repeated with a 'mucus-penetrating' subpopulation of spermatozoa as described above, the final model still had the same covariates.

In Figure 2a the relationship between concentration of motile spermatozoa and the probability of pregnancy during follow-

up is shown. The data revealed that differences in the concentration of motile spermatozoa in the low range of the scale made the largest differences with regard to male fertility, and a possible 'threshold' value for motile sperm concentration, separating fertile and subfertile men, lies close to the value of $25–30\times10^6$/ml. For the total concentration of spermatozoa this threshold was $\sim50\times10^6$/ml (not shown). A similar analysis with percentage motile spermatozoa showed an almost linear association with the probability of conception (Figure 2b) and no threshold value was found for this parameter.

## Discussion

In this study based on 358 semen samples from a group of men reflecting the general male population, it was found that the concentration of motile spermatozoa, defined as spermatozoa with VCL $>25$ µm/s, was the most significant and independent CASA parameter, in predicting the chance of natural conception. This finding might at first glance appear to be well established knowledge. However, this is the first study in which probability of achieving pregnancy in couples from a general population without previous history of infertility or pregnancy was used as the biological measure of male fertility. Previous studies relating CASA parameters to male fertility were either based on infertile couples or used different types of assisted reproduction, e.g. donor insemination or IVF, as end-points (Aitken *et al.*, 1984; Barratt *et al.*, 1993; Irvine *et al.*, 1994; Paston *et al.*, 1994; Krause, 1995). In an infertility clinic setting, female factor may play an important and unpredictable role for the total fertility outcome. Among first pregnancy planners, which were included in this study, female-infertility probably plays a minor role and, furthermore, in the statistical analysis possible female related confounding factors were taken into account. The problems with extrapolating to general population data from studies using assisted reproduction as end-point are obvious, not only because of the female factor but also as the sperm characteristics necessary for successful fertilization are not necessarily the same *in vitro* as *in vivo*.

The finding of concentration of motile spermatozoa as independent and the most significant parameter predictive for fertility outcome, led to the question of whether CASA is of any aid in assessment of male reproductive function. However, it should be kept in mind that use of CASA assessment is more objective and reproducible than technician-based motility assessment. In a previous analysis, based on the same material (Bonde *et al.*, 1998a), conventionally performed motility assessment was not an independent predictor of fertility. Recently published 'Guidelines on the application of CASA technology in the analysis of spermatozoa' (ESHRE Andrology Special Interest Group, 1998) do not recommend use of such equipment for determination of the proportion of motile spermatozoa, due to lack of reliability in distinguishing between debris and dead spermatozoa. However, the current study showed that by using CRISMAS, median overestimation of concentration was $1.6\times10^6$/ml, which is $<5\%$.

Furthermore, it cannot be excluded that more refined analyses, based on well-defined subpopulations of spermatozoa,

may disclose other motility parameters as predictors of male fertility. In this study a separate analysis was performed on a subtype of spermatozoa possessing characteristics previously reported as necessary for cervical mucus penetration (VSL $>25$ µm/s, STR $>90\%$ and ALH 2.5 µm) (Mortimer, 1994). This analysis did not change the conclusion of the study. However, the studies made by Mortimers group were based on use of Hamilton–Thorne Motility Analyzer–Integrated Visual Optics System (HTMA-IVOS) equipment and due to differences in settings and technical performance the subpopulation characteristics may not be transferable from one instrument to another.

Previous studies have pointed out other motility parameters as being of significance for predicting male fertility. Thus, Barrat *et al.* (1993) found the total number of spermatozoa and VAP to be predictors of chance of pregnancy, whereas Irvine *et al.* (1994) found mean head area and the manually determined percentage of progressively motile spermatozoa as being significant covariates (Irvine *et al.*, 1994). Other parameters as ALH, VSL, VCL and linearity (LIN) have also been reported to be correlated with fertility (Aitken *et al.*, 1984; Barratt *et al.*, 1993; Irvine *et al.*, 1994; Paston *et al.*, 1994; Krause, 1995). However, all these studies were based on patients from infertility clinics, which imply the above-mentioned limitations in extrapolating the data to the general population. The current study also found several of those motility parameters to be significantly correlated with fertility outcome when a univariate Cox regression model was applied. However, when entered in a multivariate regression model only sperm concentration and concentration as well as percentage motile spermatozoa were found to be independent fertility predictors. On the other hand, one cannot exclude that for some of the parameters the lack of significance in the multivariate model is due to a type II error. Thus, the level of statistical significance might be reached if the number of individuals included were increased. Furthermore, it cannot be excluded that a different model may have been produced if a forwards selection strategy was used instead of the backwards selection method used in this study.

It should also be taken into consideration that the most recent recommendations for use of CASA (ESHRE Andrology Special Interest Group, 1998) state that only kinematic data acquired at $\geqslant50$ Hz is reliable. However, according to a recently published study by Mortimer and Swan (1999), the change from 25 to 50 Hz, for non-hyperactivated spermatozoa, has no effect on VSL, VAP, ALH and implies only a slight increase in VCL. It seems, therefore, unlikely that the major conclusions of this study would change if a higher image sampling frequency were used.

In this study a recently developed system (CRISMAS) was used for CASA analysis. It could be questioned whether the results of this study have any relevance for users of other computer systems. However, in a study of 234 infertility couples Krause (1995) also found sperm concentration and percentage motile spermatozoa to be predictors of fertility outcome *in vivo* (Krause, 1995). Interestingly, using the Hamilton–Thorne CASA system in this study the regression coefficients found for the covariates of ln(sperm concentration) and

percentage motile spermatozoa were very similar to those found in the current study (0.268 versus 0.234 and 0.010 versus 0.012, respectively).

VCL >25 μm/s was used as the threshold value for the category of motile spermatozoa, whereas in some other papers this definition is based on the VSL or VCL value. Neither the WHO manual (1992) nor the guidelines from the ESHRE Andrology Special Interest Group (1996, 1998) give any scientific the or recommendations on which of these criteria is most significant from a biological point of view. As already pointed out by others (Lenzi, 1997; ESHRE Andrology Special Interest Group, 1998), there is an urgent need for standardization of CASA systems, particularly as many of the motility parameters are calculated from a smoothed average path and there is no agreement on a common smoothing algorithm.

Samples with a high concentration of spermatozoa were diluted with a MAR test buffer in order to facilitate the CASA analysis. In order to minimize the impact of the dilution procedure, the use of autologous sperm-free seminal plasma as dilutant has been recommended. A comparison of diluted and undiluted samples showed no impact of MAR test buffer on the percentage of motile spermatozoa or motility parameters, except BCF and STR, in the population of motile spermatozoa.

Increasing concentration of spermatozoa as assessed by CRISMAS was positively correlated with fertility outcome up to the level of ~30 $\times 10^6$/ml for motile spermatozoa and slightly higher for the total population of spermatozoa. This finding is in agreement with recently published results of manually determined sperm concentration assessment based on the same population material (Bonde *et al.*, 1998a). An identical threshold value for the manual and CRISMAS assessment might indicate that sperm concentration values obtained by the CASA system used in this study are in good agreement with the traditional sperm counting methods, which are also in the low concentration range. From the biological point of view it seems interesting that increasing sperm concentration, up to a certain value, implies a higher fertility whereas for percentage motile cells such a threshold value does not exist.

One of the limitations of this study is the fact that the analysis is based on only one sample from each of the 358 men. Due to intra-individual variation (WHO, 1992), it is possible that the semen sample delivered when the couple entered the study may not express semen characteristics at the time when the conception took place, which may have occurred up to 6 months after the ejaculate was collected. A recently published study based on the same material addressed this problem in relation to sperm concentration. This analysis has shown that using sperm concentration in a semen sample delivered at the time of beginning of the relevant menstrual cycle did not imply better prediction of cycle-specific fertilization rate, as compared to the ejaculate produced at the beginning of the study (Bonde *et al.*, 1998a). The intra-individual variation in motility parameters is not higher than that found for sperm concentration. Thus, although it is not possible to prove this, the results of the current study might not change significantly even if 'cycle specific' motility parameters were available.

In conclusion, in this study of 358 first pregnancy planners it was found that the concentration of spermatozoa with VCL >25 μm/s was the most significant and independent CASA parameter in predicting male fertility potential. This appears to be the first study showing a correlation of CASA parameters of men from the general population with their fertility *in vivo*.

## Acknowledgements

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