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Semen Parameters in Norwegian Fertile Men

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ABSTRACT: The World Health Organization (WHO) provides guidelines for assessing the various semen variables. A set of reference ranges is given in the WHO *Laboratory Manual for the Examination of Human Semen and Sperm-Cervical Mucus Interaction,* but several studies indicate that the values should be revised. Furthermore, semen parameters obtained at different laboratories are not directly comparable even if the same methods are used. Thus, it is recommended that each laboratory establish its own reference ranges. In this study, semen from 99 men who had recently achieved a pregnancy were analyzed to establish reference ranges for semen variables. The reference values were based on the group with time to pregnancy (TTP) 12 cycles or less (92%) and abstinence time from 2 to 7 days. The 5th and 10th percentiles for sperm concentration were 10.6 and 16.9 $\times$ 10^6/mL, respectively, and 33% (5th percentile) and 43% (10th percentile) for spermatozoa with progressive motility. These values were below the WHO lower limit. The percentages of ideal spermatozoa (percentage with normal morphology according to WHO strict criteria) were 3 (5th percentile) and 4 (10th percentile). Thirty-nine percent reported that their partners became pregnant during the first cycle after they had stopped using contraception. The semen parameters in this group were compared with the others. Overall, the semen parameters were more favorable in the group with TTP = 1 cycle than in the group with TTP > 1. Sperm concentration, progressive motility, and percentage of ideal spermatozoa according to WHO strict criteria were significantly different in the 2 groups. However, when analyzed by multiple logistic regression, only ''total numbers of sperm with progressive motility'' remained in the model (P = .002). This is in accordance with previous studies indicating that a combination of semen characteristics provides a better predictor of male fertility potential than the single parameters. In conclusion, new reference ranges for semen variables deviating from the WHO values are established for our laboratory.

Key words: Semen variables, reference range, World Health Organization, partners to pregnant women.

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Ideally, semen quality should predict the fertility potential for a man. However, the diagnostic value of a semen analysis is debated in light of the difficulties in establishing thresholds able to distinguish between fertile and infertile men (reviewed in Tomlinson et al, 1999; van der Merve et al, 2005). Furthermore, semen parameters are considered in different ways on the basis of the clinical settings: as part of infertility investigation or follow-up of infertility treatment, for selection for appropriate method of assisted reproduction, in reproductive toxicology, or in contraception studies. Prospective studies on the association between semen quality and fertility have shown that sperm concentration or sperm number and sperm morphology have a significant relation to likelihood of pregnancy (Bonde et al, 1998; Zinaman et al, 2000). Several reports describe differences in semen quality between fertile and subfertile groups (Ombelet et al, 1997; Gunalp et al, 2001; Guzick et al, 2001; Menkveld et al, 2001) in an effort to establish clinical cutoff values. They all showed that at least morphology was a good predictor for fertility. In these and other studies the reference ranges for semen variables given in the World Health Organization *Laboratory Manual for the Examination of Human Semen and Sperm-Cervical Mucus Interaction* (WHO, 1992, 1999) were discussed. The WHO manual provides guidelines for assessing the various semen variables; however, it is still difficult to compare the values between laboratories. Furthermore, several studies have indicated geographical differences in semen quality, probably related to environmental factors; however, ethnic or genetic differences cannot be excluded (Fisch and Goluboff, 1996; WHO Task Force on Methods of Regulation of Male Fertility, 1996; Jørgensen et al, 2001, 2002; Swan et al, 2003). A common set of reference values may therefore not be appropriate to use worldwide. In line with this it is stated in the WHO manual (WHO, 1999) that each laboratory should determine its own reference range for each semen variable. The reference ranges given in this manual are ''based on the clinical experience of many investigators who have studied populations of healthy fertile men.'' Regarding sperm concentration, total sperm count in the ejaculate, and sperm motility, the reference range is the same as in the WHO manual from 1992

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(WHO, 1992), but an interval for morphology is not given.

For several years the Andrology Laboratory at Rikshospitalet University Hospital in Oslo used the WHO reference range for comparison of patients' results. It was, however, obvious that the proportion of Norwegian patients falling outside what was defined as normal range was too high, and no appropriate reference range could be used in the morphology evaluation. In this study, semen from partners to pregnant woman was examined to establish the laboratory's own reference intervals.

Materials and Methods

Study Description

Partners to 500 pregnant women were invited to participate in the study. The invitation was sent to the woman along with an appointment for a routine screening ultrasound of the fetus (17–18 weeks for a pregnancy) to minimize uncertainty about paternity. One hundred and two men accepted the invitation by informed consent. The couple filled out a questionnaire about time to pregnancy (TTP) after the couple had stopped using contraceptives and treatment for infertility. Three persons were excluded from the study since the pregnancies were achieved after assisted reproduction. Each man was given a number upon delivering the questionnaire, and any identifying personal data were destroyed. The collected data were stored anonymously.

Semen Analysis

Semen analysis was essentially performed according to WHO (1999) guidelines which are described in detail in the joint European Society of Human Reproduction and Embryology–Nordic Association for Andrology (ESHRE-NAFA) manual (Kvist and Björndahl, 2002). The Andrology Laboratory, which was part of the collaboration that resulted in the ESHRE-NAFA manual, has been active in international standardization of laboratory methods for semen analysis and has been a co-organizer of training courses. Furthermore, the laboratory has participated in an external quality control program run by the ESHRE Special Interest Group in Andrology since 1999.

The participants were asked to deliver a semen sample at the day of the ultrasound screening and instructed to keep the abstinence time from 2 to 7 days. The abstinence time was recorded. All the samples were collected in a room close to the laboratory. For analysis, a spermatozoon without morphological defects as evaluated by the strict WHO criteria (Menkveld et al, 1990; WHO, 1999) is defined as ideal (Kvist and Björndahl, 2002). The teratozoospermia index was based on 4 categories of defects according to WHO (1992) and the ESHRE-NAFA manual (Kvist and Björndahl, 2002). The semen sample was discarded after completion of analysis.

Statistical Analysis

Statistical analysis was done using SPSS statistical software for Windows, version 12 (2003) (SPSS Inc, Chicago, Ill). Percentiles were calculated using the default option in the SPSS program, that is, weighted average. The Mann-Whitney *U* test was used to test whether semen variables differed significantly in the groups defined by TTP = 1 and TTP > 1. Statistical significance was defined as $P < .05$. In addition, multiple regression analysis (logistic and linear) was performed to search for independent predictor variables. A tolerance interval for a percentile was computed in R (http://www.r-project.org/) using bootstrapping.

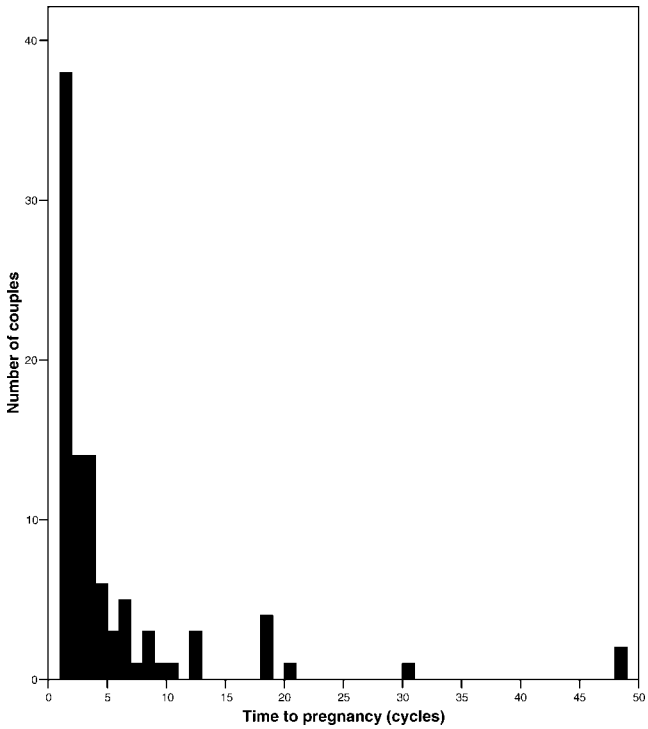

Figure 1. Frequency distribution of time to pregnancy for a group of couples (n = 97) with pregnant women participating in a study of semen parameters in fertile men.

Results

Age, TTP, and Abstinence Time

The mean age of the 99 men was 31 years, and the range spanned from 20 to 45 years. The mean age of their partners was 30 years (range, 21–42), and 86% were less than 35 years old. The self-reported TTP, recorded as the number of cycles, including the cycle of fertilization, is shown in Figure 1. For 2 persons there was no information on TTP. Thirty-nine percent (38 cases) of the 97 couples with TTP recorded conceived during the first cycle, whereas 92% (89 cases) became pregnant within 12 cycles. When a couple has not conceived within a year, it is usually considered to be an infertility problem. The distribution of the abstinence time, recorded in days, is shown in Figure 2. The median was 3 days and the mean, 3.7 days. For 3 persons there were no recordings. Ninety-five percent of the men had an abstinence time in the range 2 to 7 days (Figure 2), which is the recommended abstinence

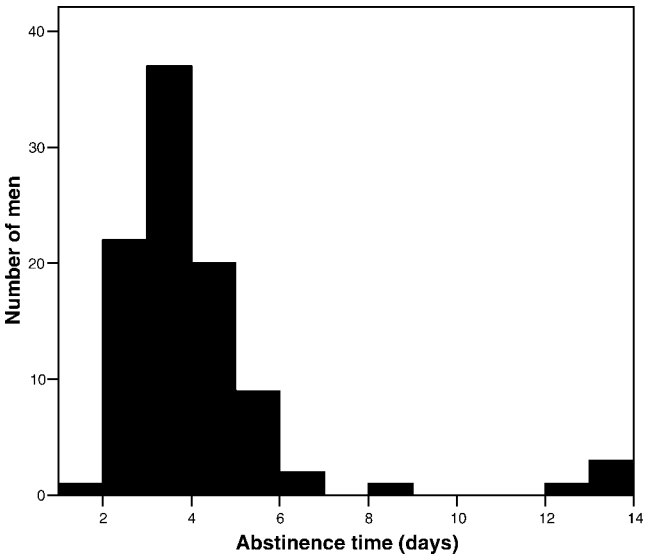

Figure 2. Distribution of abstinence time in a group of fertile men (n = 96).

time interval in the WHO manual (1999). In the following, the semen parameters from the persons with an abstinence time between 2 and 7 days as well as TTP of 12 cycles or less were used as basis for establishing reference ranges.

Semen Parameters

The results of semen analysis from the whole study group are listed in Table 1. The detailed morphology classification is given in Table 2. When the data were restricted to those with abstinence time from 2 to 7 days and TTP $\leq$ 12 cycles, the number of cases was reduced to 82. This group is referred to as a reference group. The results from this group are also shown in Tables 1 and 2. There is only a slight difference between some of the variables in the total and in the reference group. To establish reference ranges for the semen variables, the 2.5th, 5th, and 10th percentiles are listed, as well as the lower limit of the WHO (1999) reference ranges (Table 3). For the various morphological defects, the 97.5th, 95th, and 90th percentiles are shown. It is possible to provide tolerance intervals for the percentiles estimated. To illustrate, we have considered the 5th percentile of the ''sperm concentration.'' We generated 1000 bootstrap samples in R (http://www.r-project.org/), each containing 82 values for sperm concentration. For each sample we calculated the 5th percentile. Ninety percent of these values fall in the interval ranging from 9.0 to 16.5, and this provides a 90% interval for the value 10.6 reported in Table 3.

Association Between Semen Parameters and TTP

The men were divided into 2 groups depending on whether TTP = 1 cycle or TTP $>$ 1 cycle, and the median values of the semen parameters were compared (Table 4). Overall, the parameters were more favorable in the group of men in couples who conceived in the first cycle, compared to the group that conceived after more than 1 cycle. There were significant differences in sperm concentration, total sperm number, progressive motility, and proportion of ideal spermatozoa. No significant difference was seen in volume, rapid progressive motility, and the various sperm defects. We performed multiple logistic regression analysis. Potential predictor variables were those with $P < .1$. In addition we controlled for time of abstinence. However, essentially the same results are obtained basing the analysis on individuals for which abstinence time is restricted to the interval [2,7] (data not shown). We performed backward and forward stepwise logistic regression. In both cases, only the variable ''total number of sperm with progressive motility'' remained in the model ($P = .002$).

Discussion

In this study, semen from men who had recently achieved a pregnancy was analyzed to establish reference values

Table 1. *Semen parameters in partners to pregnant women**

Semen Variable	Mean (SD)		Median (Range)	
	Total Group, n = 99†	Reference Group, n = 82†	Total Group, n = 99†	Reference Group, n = 82†
Volume (mL)	4.2 (2.0)	3.9 (1.6)	3.8 (0.7–13.3)	3.7 (0.7–7.6)
pH	8.3 (0.4)	8.3 (0.4)	8.3 (7.2–10.0)	8.3 (7.2–10.0)
Sperm concentration (10^6/mL)	93.0 (71.4)	94.0 (71.7)	70.0 (0.9–326.0)	70.0 (0.9–326.0)
Total sperm number (10^6/ejaculate)	365.9 (297.9)	355.8 (272.8)	291.6 (2.5–1627)	290.2 (2.5–1380)
Progressive motility (a and b) (%)	53.3 (8.1)	53.6 (8.3)	55.0 (29.0–72.0)	55.0 (29.0–72.0)
Rapid progressive motility (a) (%)	35.0 (11.8)	34.8 (12.0)	36.0 (10.0–67.0)	35.5 (10.0–67.0)
Ideal spermatozoa (%)	13.6 (7.8)	13.9 (7.6)	13.0 (1.0–34.0)	13.0 (2.0–34.0)

* Numbers are given for the total group and for a reference group defined by abstinence time 2–7 days and time to pregnancy $\leq$ 12 cycles. a indicates rapid progressive motility; b, slow or sluggish progressive motility.

† For pH and morphology, n = 98 and 96 in the total group and n = 81 and 80 in the reference group.

Table 2. *Morphological defects in the spermatozoa from partners to pregnant women**

Type of Defect	Mean (SD)		Median (Range)	
	Total Group	Reference Group	Total Group	Reference Group
Head (%)	85.8 (8.0)	85.5 (7.8)	86.0 (66.0–99.0)	86.0 (66.0–98.0)
Neck and midpiece (%)	36.9 (10.7)	36.9 (10.4)	36.0 (12.0–66.0)	36.0 (18.0–66.0)
Tail (%)	6.5 (5.1)	6.3 (4.7)	5.5 (0–34.0)	5.0 (0–34.0)
Cytoplasmic droplets (%)	0.9 (1.0)	0.9 (1.0)	1.0 (0–6.0)	1.0 (0–6.0)
TZI†	1.50 (0.14)	1.50 (0.13)	1.49 (1.17–2.07)	1.48 (1.28–2.07)

* Numbers are given for the total group (n = 96) and for a reference group defined by abstinence time 2–7 days and time to pregnancy ≤ 12 cycles (n = 80).
† TZI indicates terazoospermia index; average number of defects per abnormal spermatozoon.

for semen variables. An arbitrary but common convention is to define the reference interval as the central 95% interval bounded by 2.5th and 97.5th percentiles. However, an asymmetric location of the interval may be more appropriate. This would be the case with most of the semen variables since no pathological conditions are associated with the upper 2.5% outside the 95% central interval. Another size interval could also be of more clinical value. Both the 5th (MacLeod and Gold, 1951; Comhaire et al, 1987) and 10th percentiles have been suggested as cutoff values in previous studies (Ombelet et al, 1997). The results from the Norwegian fertile men in this study show that the values based on the 5th percentile are far below the WHO (1999) lower limit concerning sperm concentration and sperm motility (Table 3). Even the 10th percentile from this study is lower than the WHO value for sperm concentration and motility. There has been an increasing opinion that the WHO reference ranges should be reconsidered (reviewed in van der Merve et al, 2005). However, data from different studies have indicated adjustment in both directions. In a study of first-pregnancy planners (Bonde et al, 1998), the probability of conception increased with increasing sperm concentration up to 40×10^6/mL. This value was therefore considered as a threshold between subfertile and fertile men. In another study recruiting pregnant women and their partners (Slama et al, 2002), an increase in sperm concentration up to 55×10^6/mL was found to influence TTP. Others have compared semen parameters in fertile and infertile men and suggested thresholds for sperm concentration from 9.0×10^6/mL to 34×10^6/mL and for motility from 20% to 52%, depending on the statistical methods used and assumptions made (Ombelet et al, 1997; Gunalp et al, 2001; Guzick et al, 2001; Menkveld et al, 2001). This indicates the difficulties involved in extrapolating cutoff values from one population to another if the selection criteria or study design are different.

The WHO (1999) manual does not give any reference range for normal sperm morphology but states that the value from the WHO (1992) should be adjusted downward when strict criteria are applied. In the Norwegian reference group in this study the 5th percentile for ideal

Table 3. *Selected percentiles for semen parameters in partners to pregnant women**

Semen Variable	Percentiles			WHO Value†
	2.5th	5th	10th	
Volume (mL)	1.2	1.7	2.1	2.0
pH	7.5	7.7	7.9	7.2
Sperm concentration (10^6/mL)	9.1	10.6	16.9	20
Total sperm number (10^6/ejaculate)	15.4	22.3	54.3	40
Progressive motility (a and b) (%)	29.0	33.2	43.0	50
Rapid progressive motility (a) (%)	10.2	15.2	18.0	25
Ideal spermatozoa (%)	2.0	3.0	4.0	‡
Semen variable	Percentiles			WHO Value†
	97.5th	95th	90th	
Head defects (%)	98.0	97.0	96.0	‡
Neck and midpiece defects (%)	62.8	55.0	52.0	‡
Tail defects (%)	16.0	15.0	11.0	‡
Cytoplasmic droplets (%)	5.0	3.0	2.0	‡
TZI	1.75	1.72	1.66	‡

* Abstinence time 2–7 days and time to pregnancy ≤ 12 cycles. a indicates rapid progressive motility; b, slow or sluggish progressive motility.
† Lower limit of World Health Organization (1999) reference range.
‡ No value in the World Health Organization (1999) manual.

Table 4. *Comparison of median values for semen parameters among men in couples with TTP = 1 and TTP > 1**

Semen Variable	TTP = 1 (n = 38)	TTP > 1 (n = 59)†	*P*
Volume (mL)	4.0	3.6	.903
Sperm concentration (10^6/mL)	101.5	64.0	.026
Total sperm number (10^6/ejaculate)	410.1	254.2	.024
Progressive motility (a and b) (%)	55.5	52.0	.022
Rapid progressive motility (a) (%)	38.5	33.0	.058
Total number of sperm with progressive motility (10^6)	238.8	143.5	.013
Ideal spermatozoa (%)	15.0	12.0	.034
Total number of ideal sperm (10^6)	57.0	29.6	.014
Head defects (%)	85.0	89.0	.030
Neck and midpiece defects (%)	35.0	38.0	.488
Tail defects (%)	5.0	7.0	.192
Cytoplasmic droplets (%)	1.0	1.0	.611

* TTP indicates time to pregnancy; a, rapid progressive motility; b, slow or sluggish progressive motility.
† n = 56 for the morphology evaluation.

sperm morphology is 3%. Although this value is not directly comparable to those from other studies, the present result for sperm morphology is comparable with other studies in which strict criteria were used. By evaluation of semen sample from fertile men, the 5th and 10th percentiles have been reported to be 4% and 5%, respectively (Ombelet et al, 1997), and the 10th percentile, 2% (Menkveld et al, 2001). As for the percentage of normal sperm, reference values for the various sperm defects are not given in the WHO (1999) manual. Calculation of teratozoospermia index (TZI) in this manual is an optional test and is limited to head, neck and midpiece, and tail defects, whereas in this study TZI also includes cytoplasmic droplets as described in the EHSRE-NAFA manual (Kvist and Björndahl, 2002). A multiple anomalies index (MAI) has been shown to be associated with the probability of conception among couples with infertility problems (Jouannet et al, 1988). Furthermore, a study of TTP and semen parameters to partners to pregnant women showed that MAI was strongly related to the probability of conception (Slama et al, 2002). However, in these studies MAI was the mean of more than 4 defects per abnormal spermatozoon and not directly comparable to TZI in this study. As far as we know, only 1 study (Menkveld et al, 2001) reports cutoff values for TZI based on strict criteria and 4 defects as in the present study. The median value in the fertile population was 1.54 (Menkveld et al, 2001) compared to 1.49 in the total group in our study and 1.48 in the reference group (Table 2). The cutoff value based on receiver operating characteristic curve analysis between fertile and subfertile populations was 1.64 or 2.09 if 50% prevalence of subfertility was assumed (Menkveld et al, 2001). The 95th and 90th percentiles in our study were 1.72 and 1.66, respectively (Table 3).

The proportion of couples conceiving at the first cycle (39%) is high, which may be due to a selection of couples with high fertility. It was of interest to examine if the semen parameters in men of these couples differed from the others. Overall, the semen parameters were more favorable in the TTP = 1 group than in the TTP >1 group. There were significant differences in sperm concentration, progressive motility, and proportion of ideal spermatozoa between groups of men categorized into TTP = 1 and TTP > 1. It is possible to compare the groups TTP = 1 and TTP>1 with respect to semen parameters while correcting for potential confounders, age, and abstinence, using multiple linear regression. This analysis leads to only minor alterations in semen parameters estimates and to the same conclusions as far as *P* values are concerned. However, on the basis of multiple logistic regression analysis, only the variable ''total number of sperm with progressive motility'' remained in the model ($P = .002$). Although we compare 2 groups of fertile men, and no subfertile group was studied, this is in accordance with findings that differences between fertile and subfertile populations become more pronounced when semen characteristics are combined than when looking only at single parameters (Bartoov et al, 1993; Ombelet et al, 1997). However, the limited sample size of the 2 fertile groups makes it difficult to draw any conclusion from our results as to which semen parameter is the best predictor of fertility potential.

In conclusion, estimated thresholds for the various semen parameters to discriminate between fertile and subfertile men depend on the statistical methods used and populations studied. As recommended by WHO (1999), each laboratory should establish its own reference ranges for the semen variables, and we estimated 5th and 10th percentiles of semen parameters in a group of men who had recently achieved a pregnancy. Most of these values were below the WHO lower limit. We suggest that the 5th percentiles of the semen parameters be used for comparison of the patients' results, but that also the 10th percentiles be included in the sample record form sent to the referring physician. Table 5 shows the reference ranges

Table 5. *Reference ranges for semen variables established for the Andrology Laboratory in Oslo**

Volume (mL)	≥1.7
pH	≥7.7
Sperm concentration (10^6/mL)	≥11
Total sperm number (10^6/ejaculate)	≥22
Progressive motility (a and b) (%)	≥33
Rapid progressive motility (a) (%)	≥15
Ideal spermatozoa (%)	≥3

* a indicates rapid growth progressive motility; b, slow or sluggish progressive motility.

for semen variables established for the Andrology Laboratory in Oslo on the basis of the 5th percentiles.

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