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Semen parameters in a fertile versus subfertile population: a need for change in the interpretation of semen testing

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This prospectively designed study was conducted to compare a fertile and a subfertile population so as to define normal values for different semen parameters. Semen analyses were performed according to the World Health Organization (WHO) guidelines, except for sperm morphology (strict criteria). In the fertile population (n = 144), all patients had recently achieved pregnancy, within 12 months of unprotected coitus. As subfertile controls we examined semen samples from 143 consecutive men attending our infertility clinic during the same study period. Couples with tubal factor infertility and/or ovulatory disorders were excluded from our study. Using receiver operating characteristic (ROC) curve analysis we determined the diagnostic potential and cut-off values for single and combined sperm parameters. Sperm morphology scored best, with a value of 78% (area under the ROC curve). Summary statistics showed a shift towards abnormality for most semen parameters in the subfertile population. Using the 10th percentile of the fertile population as the cut-off value, the following results were obtained: 14.3×10^6/ml for sperm concentration, 28% for progressive motility and 5% for sperm morphology. Using ROC analysis, cut-off values were 34×10^6/ml, 45% and 10% respectively. Cut-off values for normality were different from those described in the WHO guidelines. Routine bacterial and non-bacterial cultures turned out to be of little prognostic value.

Key words: human/infection/morphology/semen analysis/spermatozoa

Introduction

Some 15% of couples are subfertile, and in half of these a male factor is involved (Howards, 1995). Because human fertility is linked to sperm quality, the establishment of reference values for normality is mandatory. MacLeod and Gold (1951a,b) first reported the differences in sperm characteristics between fertile and infertile men. Since then, a number of retrospective studies have shown that sperm parameters are, on average, superior in fertile males, but there is a substantial shortage of prospective studies comparing semen characteristics between proven fertile and subfertile populations. Using donors in a semen donor insemination programme as the reference population for normality is surely not the best option because not all donors have established their fertility and, even if they did so, this population is positively biased for fertility.

Conventional techniques for the evaluation of a spermiogram have been standardized by the World Health Organization (WHO, 1987, 1993). Because all methods for the evaluation of sperm parameters are of a subjective nature, the conclusions after the interpretation of one or more semen analyses are sometimes incorrect, which might lead to unnecessary treatment procedures (Comhaire *et al.*, 1992; Ombelet *et al.*, 1995).

Although WHO methods have been of paramount importance in the management of infertile couples, serious concerns exist about the true value of WHO semen analysis results and cut-off values (Hull *et al.*, 1985; Helmerhorst *et al.*, 1995).

Our aim was to perform a prospectively designed, controlled study using defined methods to compare a fertile versus subfertile population in the Limburg area of Belgium. Although it might appear to be easy to set up such a study, recruiting semen from fertile (recently pregnant) couples is in fact very difficult. This also explains why studies comparing fertile and subfertile populations are so scarce in the literature, although the WHO (1987, 1993) recommends all andrology laboratories to perform such a reference population study.

In the original design a multicentric study, involving centres from Europe, the USA, South Africa and Brazil, was foreseen, with all centres adhering to the same protocol. Unfortunately, for various reasons (e.g. request for payment of the participants, lack of collaboration between obstetric care units and fertility centres, and suspicions that results would be used for paternity control), most of the participating laboratories experienced major difficulties in recruiting an unbiased group of fertile men. It became evident that this type of recruitment would itself lead to important bias, and therefore the study was limited to our Flemish population.

Materials and methods

Patients and study design

During an 8 month period (from November 1994 to June 1995 inclusive) and at a gestational age of 18–20 weeks, 155 consecutive pregnant patients were asked to bring a semen sample of their partner to our laboratory. All patients became pregnant within 1 year of

unprotected coitus and did not have a history of previous subfertility or habitual abortion. All men volunteered without payment and after oral consent. As subfertile controls, during the same period we examined 143 consecutive couples attending our infertility clinic for the first time with a history of subfertility for at least 13 months.

Because all deliveries in the Genk region occurred in our maternity unit and because our fertility clinic was the only one in the same region during the study period, the socio-economic status of the patients was well matched in the fertile and subfertile groups.

A total of 144 fertile men and 143 subfertile patients agreed to volunteer, and semen was collected by masturbation into a sterile polystyrene jar, after a recommended period of sexual abstinence of 2–3 days. All samples were examined in a blinded fashion within 1 h of sperm collection. Semen samples were analysed at room temperature and after complete liquefaction according to the WHO (1987, 1993) guidelines.

The volume of the semen samples was determined using sterile disposable 5 ml pipettes. Sperm motility was assessed on a freshly prepared wet preparation before cooling or drying. We always evaluated a fixed volume of semen (15 ml) delivered onto a clean glass slide and covered with a 24×32 mm coverslip. The preparations were examined at a magnification of ×400 using a phase-contrast microscope (Olympus Corporation, New York, USA). Progression of individual spermatozoa was assessed separately, and forward progression was rated on a semisubjective scale. Grade A motility was defined as a rapid progressive motility; grade B was classified as slow or sluggish linear or non-linear motility. For sperm concentration, the Makler counting chamber (Sefi Medical Instruments, Haifa, Israel) was used according to the guidelines of the manufacturer. Sperm morphology was assessed on a thin and well-spread smear, which had been air dried, fixed and stained according to the Papanicolaou method (WHO, 1987). To evaluate sperm morphology we used the strict criteria described first by Kruger *et al.* (1986). At least 200–500 spermatozoa situated in >10 different fields were evaluated, and the percentages of sperm cells with complete normal forms according to these criteria were noted.

All semen samples were evaluated by the same two technicians, and studies examining intra- and interobserver variability on different occasions between these two observers showed good within- and between-observer variability (Pearson coefficients of correlation 0.89–0.92), not only for sperm motility but also for sperm concentration and morphology (Bosmans *et al.*, 1991).

A hypo-osmotic swelling test (HOST) was performed on all semen samples as described by Jeyendran *et al.* (1984). Motile count (total count×motility), normal count (total count×morphology) and normal motile count (total count×motility×morphology) were also calculated and included in the evaluation.

After classification of the samples into eight different categories according to their seminal report [normal (Nl), oligozoospermia (O; $<20\times10^6$/ml), asthenozoospermia (A; <50% progressive motility), teratozoospermia (T; <10% normal forms) and different combinations of semen abnormalities (OA, OT, AT, OAT)], the prevalence of these seminal abnormalities was compared between the fertile and subfertile populations using χ^2 statistics.

When the total number of round cells was $>1\times10^6$/ml, a specific leukocyte staining method (LeucoScreen; Ferti Pro M.V., Aalter, Belgium) was used to indicate the presence and number of leukocytes.

All specimens were examined for infections (*Ureaplasma urealyticum*, *Mycoplasma hominis*, *Chlamydia trachomatis* and bacterial cultures). Microbiological cultures were routinely performed by inoculating semen on several culture media. Agar plates were incubated at 35°C for 18–35 h. Sheep blood agar was incubated at 5% CO_2, while more selective media (McConkey, Chapman, BioMérieux Benelux, Brussels, Belgium) were incubated at 37°C in a normal atmosphere. Sensitivity to antibiotics was performed on a Mueller Hinton agar using the BBL-Susceptibility test discs (Becton-Dickinson, Franklin Lakes, NY, USA).

In our search for *U.urealyticum* and *M.hominis*, the hydrolysis of ureum and arginine was noted after 2 days of incubation in a microaerophylic environment. For *C.trachomatis* DNA detection, we used a quantitative polymerase chain reaction described previously (Beliën *et al.*, 1995).

Because the age of both partners may influence the fertilizing potential of semen samples, the age distribution among males and females in both the fertile and subfertile subgroups was examined.

Statistical analysis

Using the MedCalc programme (MedCalc Software, Mariakerke, Belgium), basic statistics [mean, median, range and fifth and 10th percentiles (P_5 and P_{10} respectively)] were calculated and compared using Student's *t*-test for means of unpaired samples including most semen parameters: volume, concentration, total count, total motility, grade A motility, morphology, HOST, motile count, normal count and normal motile count. A similar approach was used to compare the age distribution among males and females. The frequency distribution of microbiological infections between both groups was compared using χ^2 statistics.

Using the receiver operating characteristic (ROC) curve analysis, the diagnostic accuracy and the ability for correct classification of subjects into fertile and subfertile subgroups were documented for the different semen parameters. Each parameter was analysed in terms of diagnostic sensitivity, specificity, positive and negative predictive value and positive and negative likelihood ratio, assuming a 15% prevalence of the disease. Decision thresholds for all parameters were calculated for optimal sensitivity and specificity. The area under the ROC curve was expressed as a percentage and was plotted to compare the overall diagnostic performance of the different semen parameters.

After classification of the semen characteristics into Nl, O, A or T, or into a combination of defective sperm parameters according to threshold values based on the ROC analysis, χ^2 analyses were performed to test for significant frequency differences between semen analysis patterns in fertile and subfertile men.

Finally, using the MedCalc ROC curve analysis module, the most relevant diagnostic semen factors were compared for significant differences between areas, i.e. their diagnostic performances.

Results

Azoospermia was observed in seven (4.9%) patients from the subfertile group and in none from the fertile group.

The mean volume of seminal fluid was identical in both groups (3.1 ml). Differences between fertile and subfertile groups were most significant for sperm morphology (mean 12.0 versus 6.6%) and normal motile count (mean 11.1 versus 4.6×10^6) ($P < 0.0001$), as shown in Table I.

The mean number of leukocytes was 0.16×10^6/ml in the fertile population versus 0.15×10^6/ml in the subfertile group (difference not significant). Leukocytospermia was diagnosed in two and three cases in the fertile and subfertile populations respectively. Considering the number of round cells (immature germ cells + leukocytes), comparable mean values were found in both groups (0.64 versus 0.67×10^6/ml).

The incidence of *Mycoplasma* infections was not statistically

Table I. Comparative analysis of 10 (single or combined) semen parameters in a fertile versus subfertile population

	Fertile population (n = 144)					Subfertile population (n = 136)					P value (t-test)
	Mean	Median	Range	P_5	P_{10}	Mean	Median	Range	P_5	P_{10}	
Volume (ml)	3.1	2.8	0.5–12.7	1.0	1.3	3.1	3.1	0.5–7.1	0.9	1.2	NS
Concentration ($\times10^6$/ml)	53.1	47.5	1.0–215.0	7.0	14.3	32.9	26.5	0.1–141.0	1.4	3.0	<0.001
Total count ($\times10^6$)	149.5	124.9	1.7–545.3	15.3	29.6	103.9	69.1	0.4–550.8	4.0	7.4	<0.05
Total motility (%)	53.4	57.5	0–85	18.1	28.0	45.8	48.5	0–85.0	11.0	15.0	<0.01
Grade A motility (%)	16.9	14.5	0–57	2.0	3.0	12.2	8.5	0–57	0.0	0.0	<0.01
Morphology (%)	12.0	12.0	1–27	4.0	5.0	6.6	7.0	0–20	1.0	1.0	<0.0001
HOST	59.3	62.0	23–89	31.0	41.0	50.7	51.0	6–89	24.6	31.0	<0.0001
Motile count ($\times10^6$)	84.5	68.2	0–376.2	2.2	8.2	55.4	27.8	0–374.5	0.8	1.4	<0.001
Normal count ($\times10^6$)	19.5	14.2	0.1–81.7	0.6	1.8	8.5	3.8	0.1–82.6	0.02	0.1	<0.0001
Normal motile count ($\times10^6$)	11.1	7.5	0–56.4	0.17	0.52	4.6	1.4	0–56.1	0.0003	0.03	<0.0001

HOST = hypo-osmotic swelling test; NS = not significant; motile count = volume×concentration×total motility; normal count = volume×concentration×normal morphology; normal motile count = volume×concentration×total motility×normal morphology; P_5, P_{10} = fifth and 10th percentiles respectively.

Table II. Semen microbiology (bacterial and non-bacterial) in the fertile versus subfertile populations

	Fertile population (n = 144)	Subfertile population (n = 143)	P value (χ^2)
Non-bacterial			
Ureaplasma urealyticum	11 (7.6)	17 (12.5)	NS
Mycoplasma hominis	2 (1.4)	4 (2.9)	NS
U.urealyticum + *M.hominis*	5 (3.4)	7 (5.1)	NS
Chlamydia trachomatis	6 (4.2)	11 (7.7)	NS
Total	24 (16.6)	39 (27.2)	P<0.05
Bacterial			
Proteus mirabilis	1 (0.7)	3 (2.1)	NS
Escherichia coli	5 (3.5)	5 (3.5)	NS
Enterococcus	17 (11.8)	9 (6.3)	NS
Combined	6 (4.1)	2 (1.4)	NS
Total	29 (20.1)	19 (13.2)	NS
Negative	99 (68.7)	103 (72.0)	NS

Values in parentheses are percentages.

different between the groups, although a trend towards an increased risk for infection was observed in the subfertile population (19.6 versus 12.5%). Bacterial cultures were positive in 20.1% of the fertile and in 13.2% of the subfertile population (Table II). The presence of bacterial growth was not related to sperm abnormalities.

However, as far as the presence of *Chlamydia* DNA was concerned, six out of the 144 (4.2%) semen samples from the fertile group were positive, whereas 11 positive samples were found in the 143 subfertile and azoospermic males (7.7%). In the group of azoospermic males, *Chlamydia* DNA was present in three out of seven of the semen samples (42.9%).

Using ROC curve analysis we demonstrated that sperm morphology was the best parameter, showing the highest predictive power (78% under ROC curve), followed by the normal count (74.6%) (Table III and Figure 1). The area under the ROC curve differed significantly between sperm morphology compared with all other semen parameters investigated ($P < 0.01$), except for normal count (Table IV).

The fertile and subfertile populations could only be differentiated if two or three semen parameters were abnormal utilizing the ROC-based cut-off values (double parameter defect: $P < 0.01$, triple parameter defect: $P < 0.0001$) (Table V).

The mean ages of the men and women were comparable between the fertile and subfertile men (30.5 versus 30.6 years, not significant) and women (28.7 versus 29.3 years, not significant).

Discussion

Biological evidence of male sterility is only present in cases of azoospermia or globozoospermia or in the presence of a complete lack of sperm motility with underlying genetic deficiencies such as Kartagener's syndrome. Because such cases of male sterility are uncommon, clinicians expect to obtain a clear indication of a man's fertilizing potential from semen analysis. This also stresses the primary importance of establishing as precisely as possible the predictive potential of single or combined semen parameters. We compared differences in a fertile versus subfertile population for sperm parameters that can be easily examined in most laboratories. We are aware of the basic idea that the assessment of sperm function can help to reclassify subfertile men into several subgroups (Vigil *et al.*, 1994). For sperm concentration, most studies have reported a large and overlapping distribution in the fertile and subfertile populations. The cut-off value of 20×10^6/ml below which male fertility seemed to be diminished was based on a study showing that above this value the time required to become pregnant no longer depended on sperm concentration (MacLeod and Gold, 1951a; Freund and Peterson, 1976). Other (mostly retrospective) studies have advocated lower limits (10 or even 5×10^6/ml; Santomauro *et al.*, 1972; Zukerman *et al.*, 1977; Bostofte *et al.*, 1982). In our study and using the 10th percentile of our fertile population as the lower limit of normality, the cut-off values for sperm concentration and total count were 14.3×10^6/ml and 29.6×10^6 respectively.

Previous studies on sperm motility as a predictor for infertility have been contradictory (Zaini *et al.*, 1985; Jouannet *et al.*, 1988; Comhaire and Vermeulen, 1995). In this study, total motility and total motile count had a rather low discriminating power in predicting subfertility (Table III and Figure 1).

The usefulness of sperm morphology assessments as a predictor of a man's fertilizing potential has often been

Table III. Diagnostic potential of nine (single and combined) semen parameters through receiver operating characteristic (ROC) curve analysis

Semen parameter	Area under the ROC curve	SE	95% Confidence interval	Cut-off value	Sensitivity	Specificity	+LR	–LR	+PV	–PV
Concentration	0.694	0.031	0.636–0.748	34	62.5	73.6	2.37	0.51	29.5	91.8
Total count	0.664	0.032	0.606–0.720	80	58.1	76.4	2.46	0.55	30.3	91.2
Total motility	0.609	0.033	0.550–0.667	45	45.6	73.6	1.73	0.74	23.4	88.5
Grade A motility	0.636	0.033	0.576–0.692	8	50.0	72.2	1.8	0.69	24.1	89.1
Morphology	0.777	0.028	0.724–0.825	10	81.6	61.8	2.14	0.3	27.4	95.0
HOST	0.656	0.033	0.597–0.712	48	47.4	80.6	2.44	0.65	30.1	89.0
Motile count	0.673	0.032	0.614–0.777	36.36	58.8	76.4	2.49	0.54	30.5	91.3
Normal count	0.746	0.029	0.691–0.796	5.64	62.5	81.2	3.33	0.46	33.7	92.4
Normal motile count	0.741	0.029	0.686–0.791	3.24	64.7	75.7	2.66	0.47	29.1	92.3

Cut-off value denotes that with optimal sensitivity and optimal specificity. +LR = positive likelihood ratio; –LR = negative likelihood ratio; +PV = positive predictive value; –PV = negative predictive value. For abbreviations and an explanation of the semen parameters, see Table I.

Table IV. Ranking and statistical comparison (*P* value) of sperm parameters according to receiver operating characteristic (ROC) curve analysis

Area under ROC	Morphology 0.770	Normal count 0.746	Normal motile count 0.741	Motile count 0.673	Total count 0.664	HOST 0.656
Morphology	–	NS	<0.01	<0.01	<0.01	<0.01
Normal count		–	<0.001	<0.001	<0.001	<0.05
Normal motile count			–	<0.001	<0.001	<0.05
Motile count				–	NS	NS
Total count					–	NS

HOST = hypo-osmotic swelling test; NS = not significant.

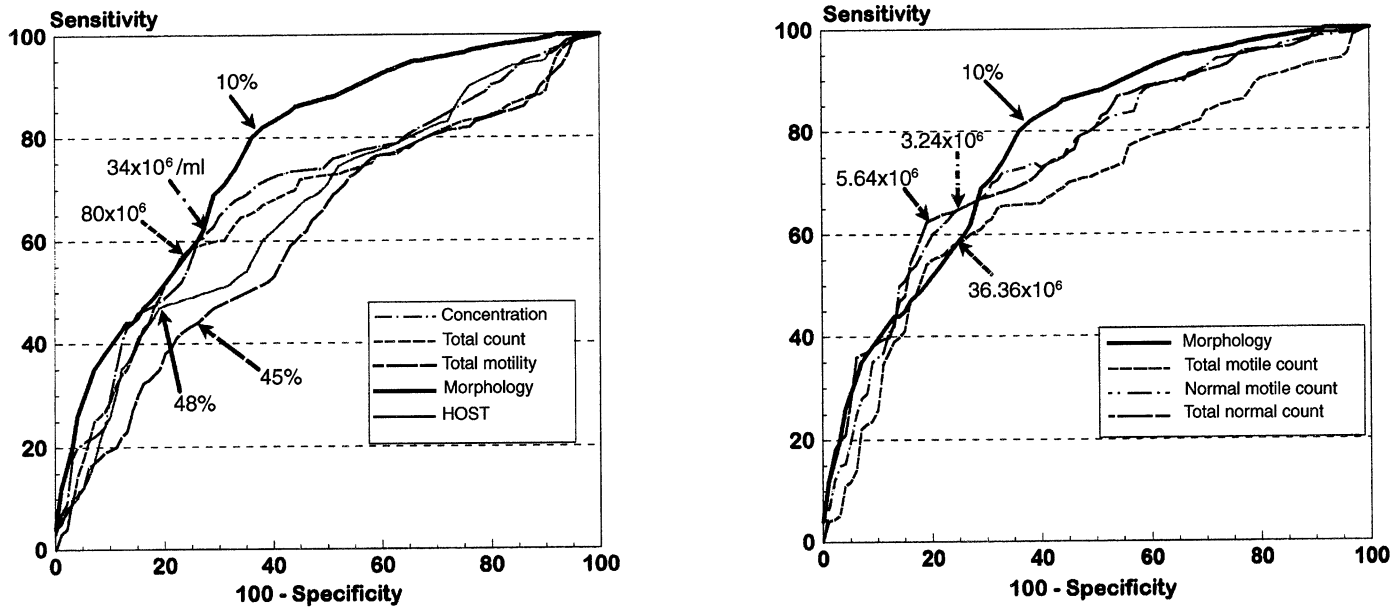

Figure 1. Receiver operating characteristic (ROC) curve analysis of nine semen parameters showing their cut-off values. HOST = hypo-osmotic swelling test; motile count = volume×concentration×total motility; normal count = volume×concentration×normal morphology (%); normal motile count = volume×concentration×total motility×normal morphology.

challenged due to different classification systems, various slide preparation techniques and problems with reproducibility because of observer variations (Ombelet *et al.*, 1995). The strict criteria were originally based on the morphology of post-coital spermatozoa found at the level of the cervical os, which comprise an apparently homogeneous population. The rationale of the strict criteria is the concept of using these spermatozoa as the reference population for sperm normality. There is also a considerable body of clinical data, mostly from in-vitro fertilization studies but also from intrauterine insemination programme reports, to support the use of strict criteria (Kruger *et al.*, 1987, 1988; Oehninger *et al.*, 1988, 1992; Enginsu *et al.*, 1991; Grow *et al.*, 1994; Ombelet *et al.*, 1994, 1996; Toner *et al.*, 1994).

Recently, the WHO changed its cut-off value for normality (from 50 to 30%). Surprisingly, this change was not based on any biological data – yet another reason to investigate the power of sperm morphology to predict subfertility *in vivo*.

In our study, sperm morphology was the most significant indicator for subfertility, with a cut-off value of 10% according

Table V. The prevalence of fertility and subfertility after classification of the males in different groups and using the cut-off values for normality [based on (i) the World Health Organization criteria for concentration/total motility, and (ii) the ROC curve cut-off for morphology [<10%]

	Fertile	Subfertile	*P* value
Azoospermia	0	7 (4.9)	<0.0001
Normal	73 (50.7)	15 (10.5)	<0.0001
Oligozoospermia (O)	11 (7.6)	11 (7.7)	NS
Asthenozoospermia (A)	10 (6.9)	4 (2.8)	NS
Teratozoospermia (T)	18 (12.5)	26 (18.0)	NS
Single parameter	39 (27)	41 (28.5)	NS
OA	5 (3.5)	6 (4.2)	NS
OT	13 (9.0)	35 (24.5)	<0.001
AT	6 (4.1)	5 (3.5)	NS
Double parameter	24 (16.6)	46 (32.2)	<0.01
OAT			
Triple parameter	8 (5.5)	34 (23.8)	<0.0001

Values in parentheses are percentages. NS = not significant.

to ROC analysis and 5% using the 10th percentile of the fertile population. By optimizing the diagnostic potential of all semen parameters studied through ROC analysis, sperm morphology obtained the highest predictive score of 78% (Table III and Figure 1).

Although we assessed only the smears according to strict criteria, the importance of other classification systems highlighting the association of sperm abnormalities with underlying clinical disturbances (David *et al.*, 1975; Hofmann *et al.*, 1982; Hofmann and Haider, 1985) is acknowledged. Examining the predictive value of sperm morphology according to different criteria will be the subject of another study.

The HOST was introduced to evaluate the functional integrity of sperm membranes (Jeyendran *et al.*, 1984). It is a simple test claimed to measure aspects of sperm behaviour that could be involved in the fertilizing capacity of spermatozoa (Jeyendran *et al.*, 1984; Check *et al.*, 1988; Okada *et al.*, 1990). Studies in favour of this test have not been confirmed in many other reports (Chan *et al.*, 1985, 1988; Wang *et al.*, 1988; Colpi *et al.*, 1990). In this study we observed a significant difference in mean value between the two groups (Table I). Using ROC analysis, the HOST achieved an intermediate score in predicting subfertility (Table III and Figure 1).

Figure 1 demonstrates the cut-off values using ROC curve analysis. These values represent points of optimal specificity and sensitivity. Using these values one might differentiate the patients into different groups with single or combined factors. Another approach may be to use the cut-off values presented by the WHO for sperm concentration and total motility. After classifying the males into different groups according to these cut-off values, the prevalence of semen abnormalities only differed between the two groups if more than one sperm parameter was involved. This finding was independent of which cut-off values were used (Table V).

Asthenozoospermia was more frequent in the fertile group. According to these results, the power of sperm motility as a useful parameter in the prediction of subfertility seems to need re-evaluation. It is possible that computer-assisted sperm analysis systems based on sperm motility may alter this picture, but this is surely unproved at present.

Our results support the major finding that differences between the fertile and subfertile populations become clear only if two or all three semen parameters are involved (Table V).

The quantification of leukocytes is another integral part of semen analysis, although the relationship between leukocyte populations in the ejaculate and male fertility status remains controversial (El-Demiry *et al.*, 1986; Wolff *et al.*, 1990; Fedder *et al.*, 1993; Tomlinson *et al.*, 1993). According to the WHO (1987, 1993) guidelines, a concentration $>1\times10^6$ leukocytes/ml of semen is considered to be abnormal, but no study has evaluated whether this threshold is actually predictive in the estimation of one's chance of achieving pregnancy. One study even mentioned the possibility of a positive role for seminal leukocytes by the removal of morphologically abnormal sperm forms (Tomlinson *et al.*, 1992). The current belief is that leukocytes can be extremely damaging to sperm function only in the absence of the protective antioxidant properties of seminal plasma (Jones *et al.*, 1979; Aitken *et al.*, 1994).

According to our data, measurements of seminal leukocytes in routine semen analysis appear to be of little prognostic value, and leukocytospermia was not found to be associated with sperm abnormalities.

The existence of pathogenic bacteria in seminal plasma is considered to be a sign of an active infection in the male reproductive tract (Dahlberg, 1976). Because the male urethra is colonized by a variety of micro-organisms, contamination is very difficult to avoid when the sample is provided by masturbation. Our study did not support the findings of others that, on average, twice as many positive bacterial cultures are obtained from subfertile than from fertile men (McGowan *et al.*, 1981; Toth and Lesser, 1981; Megory *et al.*, 1987). On the contrary, we found more positive cultures in the fertile group, although the difference was not statistically significant. The reason for this observation remains unclear, but confirms other reports (Comhaire *et al.*, 1980; Gregoriou *et al.*, 1989). We found a higher frequency of *Chlamydia*-specific DNA in semen from the infertile group, but this difference was not statistically significant. However, after dividing the infertile group into a subfertile and an azoospermic population, we found a surprisingly high incidence (42.9%) of *Chlamydia* DNA in the azoospermic group, whereas only eight out of 136 (5.8%) subfertile men were positive, representing a highly significant difference ($P < 0.01$).

This finding may indicate that the chronic progression of *Chlamydia* infection in the male reproductive tract may lead to azoospermia, but more extensive controlled studies will be necessary to document the impact of *Chlamydia* infections on the pathogenesis of obstructive azoospermia.

In conclusion, our prospective data revealed the importance of sperm morphology using strict criteria as a valuable predictor of a man's fertilizing potential. The present data apply to in-vivo reproduction, and our cut-off values for normality differ substantially from those proposed by the WHO. It would be interesting if similar studies could be organized in different countries and continents in the near future. Our observations indicate a limited clinical value of single sperm parameter

defects and larger series of men might find cut-off values for normality in isolated oligo-, astheno- and/or teratozoospermia.

The search for bacteriospermia and leukocytospermia in a routine semen examination was not supported by this study and is probably only useful in selected cases of chronic prostatovesiculitis or azoospermia.

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