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Semen parameters in a fertile Swiss population

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Summary

Background: The aim of this prospective study was to analyse the different sperm parameters of fertile Swiss men. To date there are no data regarding the quality of spermatozoa of fertile Swiss men.

Methods: We measured the ejaculates (pH, volume, concentration, motility, viability, morphology) of 34 men using strict inclusion criteria. The partners of these men had to be pregnant at the time of inclusion in the study and these pregnancies had to have been conceived spontaneously within 15 months. A questionnaire elucidated the consumption of alcohol, nicotine and drugs. Semen analysis was performed according to WHO-criteria, with the exception of morphology, which was analysed according to Tygerberg's strict criteria.

Results: The mean age of the males studied was 34 years. The semen analysis revealed the following mean values: pH: 8, volume: 2.6 ml, concentration: 60×10^6/ml, total count: 160×10^6, progressive motility: 42%, rapid progressive motility: 36%, viability: 47% and morphology: 8% normal forms. The consumption of alcohol, nicotine and drugs was low to moderate.

Conclusions: No men fulfilled all criteria of normality in the different sperm parameters examined. The most striking results are that the upper limits of normal morphology and viability seem to be too high. Concentration and rapid progressive motility appear to have a high impact on fertility. The combination of several criteria is probably more predictive than a single parameter.

Key words: infertility evaluation; fertile men; semen quality; sperm norm values; WHO-criteria; Tygerberg's criteria

Introduction

One in six couples seeks medical help because of infertility [1]. In about 30% of the cases both partners are jointly responsible for the childlessness. Additionally in 30% each partner will be the sole cause of the infertility. In 10% no explanation can be found so that the sterility has to be called "idiopathic". Our data show that it is of great importance to examine both partners. Overall, we can assume that in approximately 50% of cases the male factor is the cause of the infertility [2].

Semen analysis is the basic investigation in the exploration of male infertility. The methods used to identify potential normal and fertile semen samples are still contradictory and not exactly defined. The usual analysis of semen specimens includes the determination of semen volume, pH, concentration, viability, motility and morphology. Cornerstones such as size and form of the sperm itself, its acrosome, midpiece and tail have been defined by different organisations such as the WHO. It has been recognized that the evaluation of such parameters is to a certain extent subjective [3, 4, 9].

Indeed alternative tests based on more functional aspects such as sperm penetration, capacitation and acrosome reaction have been developed. In addition, the analysis of DNA integrity is a matter of debate and might play a more important role in the future [5–7]. However these methods are not proven to be more predictive with regard to the endpoint pregnancy. At present, in the routine testing of the infertile couple, these examinations have not been generally established. They are also cost and time intensive.

A further serious concern in the interpretation of sperm parameters is the difficulty in comparing the results of different laboratories. Intra- and inter-laboratory variability has been a matter of debate in several publications [3, 4, 8–11]. In particular the morphological classification of sperm is quite subjective [9, 12]. With the aim to overcome such variabilities the Special Interest Group in Andrology (SIGA) of the European Society of Human Reproduction (ESHRE) carried out training courses throughout the world [13].

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Table 1
Semen variables.

	WHO 1992 [16]	WHO 1999 [19]	Tygerberg [23]
Ejaculate volume	>2 ml	>2 ml	
pH	7.2–8.0	>7.2	
Sperm density	>20 × 10^6/ml	>20 × 10^6/ml	
Total sperm count	>40 × 10^6	>40 × 10^6	
Viability	>75%	>75%	
Progressive motile spermatozoa (grade a, b)	>50%	>50%	
Rapid progressive motile spermatozoa (grade a)	>25%	>25%	
Morphology	>30%	not given	>14%

Two widely used classifications for the analysis of semen exist (table 1). The WHO classification was first published in 1980. It is based on the investigations of MacLeod [14] and Eliasson [15]. Eliasson referred to a value of >60% for normal morphology. The cut-off for sperm with normal morphology according to WHO criteria was 30% in the year 1992 [16]. The clinical significance of the WHO criteria is not undisputed [17, 18]. The 1999 WHO manual does not even include normal values for sperm morphology. For the other values the nomenclature was changed from normal values to reference values [19].

The Tygerberg classification (strict criteria) judges slightest morphological deviations as abnormal. The criteria depicting "normal sperm" have been worked out from the evaluation of sperm taken out of the mucus in the upper endocervical channel following sexual intercourse [20, 21–23]. Surveys have shown that sperm that have been classified as normal using the Tygerberg's criteria will show a better binding potential to the zona pellucida of the oocyte. Furthermore this classification allows a prognosis for the fertilization rate in a program for in vitro fertilization (IVF). Patients with morphology between 5 and 14% have a better fertilization rate of the oocytes than patients with 4% or less normal forms [22]. Also, in vivo, these criteria seem to have a significant prognostic value [24].

There are very few data concerning the quality of sperm from proven fertile men whose partners were pregnant at the time of study inclusion [11, 20, 25] and there is a need for more information on the range of semen analysis from various laboratories throughout the world. It is not only in our experience that it is quite difficult to recruit men whose partners are pregnant. In his publication Ombelet describes a multicentre study involving centres from Europe, the USA, South Africa and Brazil set up to survey the semen parameters of fertile populations. As the recruitment of volunteers was so difficult in the other countries his analysis, in the end, was limited to the Flemish population [20]. Guzick et al. [26] compared fertile and infertile couples. In order to facilitate recruitment in his study, fertility was defined as pregnancy within the previous two years rather than current pregnancy. In the study by Chia et al. [27] women were pregnant at the time of participation of the men in the study. However couples were not asked for the length of the attempted conception interval before success. In the study by Osser at al. [28] in which the semen of fertile Swedish men was investigated, the time to pregnancy was not elucidated. There are many studies analysing semen parameters of sperm donors and infertile men. Since these men have not necessarily fathered a child, it is not correct to set up standards for normal sperm parameter using the results of these ill-defined male populations. Even sperm donors who do have a child might have experienced a urological problem and might be subfertile a few years later, when donating sperm at a fertility centre.

With this prospective study our aim was to investigate the different sperm parameters of men in Switzerland whose partners were pregnant at the time of semen analysis. Until now there is no data regarding the quality of spermatozoa of fertile Swiss men. Our results may contribute to the scarce information we have from other studies evaluating the sperm of fertile men. More information on fertile semen will help to counsel couples seeking advice. The data will help answer the question of which sperm values are unlikely to lead to pregnancy and help to avoid under- and over treatments.

Materials and methods

Design of the study

We studied prospectively the ejaculates from 34 Swiss men whose partners were pregnant at the day of study inclusion. The study participants had been living in Switzerland for a minimum of 10 years. The majority of their female partners were in the first and second trimester of pregnancy. All had conceived spontaneously within 15 months of unprotected intercourse. None had a history of fertility problems.

Initially couples were approached by informing pregnant women in the outpatient clinic about our study. Since the feed-back was limited we later decided to advertise in a newspaper.

All study participants completed a standardized ques-

tionnaire, which included questions regarding the general health, the consumption of alcohol, nicotine and drugs by both partners. Also the exposure to toxins was evaluated. The participants gave written consent to participation in the study.

The ethics committee of Canton Berne in Switzerland (KEK) approved the study protocol.

Sperm collection and its analysis

Sperm collection: The participants collected the ejaculates by masturbation at our fertility centre into a 12 ml polyethylene-tube. We encouraged sampling after 2–5 days of abstinence. The exact days of abstinence were however not documented. The semen samples were marked with an anonymous serial number and were then put immediately into a water-bath at 37 °C, where they stayed during the whole analysing process.

Semen analysis: Analysis was started as soon as the ejaculates had liquefied. All ejaculates liquefied within one hour. The volume was then aspirated into a 10 ml pipette providing 0.1 ml accuracy. The pH was measured with a pH tape (pH 6.5–10.0) and recorded after 20 seconds.

Sperm concentration and motility was determined by CASA (Hamilton Thorne, HTM-IVOS, Version 12, Baumann Medical AG, Wetzikon, Switzerland). The setting for CASA was 30 frames, 50 Hz and a minimal cell-size of three pixels at a minimum contrast of 80 (standardised unit by Hamilton). The microscope setting was an objective magnification of ×10. A standard analysis chamber of 20 micron was filled and put into the Hamilton (pre-warmed at 37 °C). Sperm samples were then analysed by counting the sperm within 7 different areas in duplicate and were categorized. The Hamilton Thorne categories "Rapid Progressive (4)" and "Slow Progressive (3)" were interpreted as motile sperm (WHO categories a and b). The mean value has been calculated from the results of these 7 areas. In cases of concentrations indicating less than 5×10^6/ml or ejaculates polluted with debris, the measurement of sperm concentration was repeated using the Makler chamber. Again the analysis was done in duplicate and the mean value calculated. At a deviation of more than 15% from the CASA result, motility was reanalysed by an Olympus BH-2 microscope (2×100 spermatozoa, phase-contrast at a magnification of ×200).

The measurement of viability was performed with the Eosin-Nigrosin-method. 300 spermatozoa were evaluated under oil-immersion at a magnification of ×1000.

For the determination of the morphology 100 µl of the ejaculate were diluted with 1ml sperm-preparation-medium and then centrifuged for 5 minutes at 300 g. The pellet was then resuspended, 4 smears were prepared and stained with Diff-Quik (Dade Diagnostica, Dudingen, Switzerland) after air-drying. 400 spermatozoa were categorized (1000× magnified, oil immersion) according to Tygerberg's strict criteria [21].

Quality control: In this prospective study a single experienced technician evaluated all ejaculates. This technician participates in the continuous quality control system (CQC) under the supervision of T. Kruger, which consists of a continuous sperm morphology training.

Data analysis: Because of the limited number of 34 examined ejaculates, a descriptive analysis has been used.

Results

Table 2

Sperm analysis of fertile Swiss men (n = 34).

	Mean	Median	Range
Age	34	34	20–60
pH	8	8	7.4–8.3
Volume (ml)	2.6	2.6	0.5–5.8
Concentration (× 10^6/ml)	60	47	1–224
Total count (× 10^6)	160	103	1–636
Total motility (%)	42	44	15–66
Grade a motility (%)	36	36	11–61
Viability (%)	47	49	20–67
Morphology (Tygerberg's criteria)	8	9	1–17

Table 3

Number/Percentage of fertile Swiss men who reach normal criteria (n = 34).

	WHO-criteria [19]	Tygerberg's criteria [23]
pH	34 / 34 (100%)	
Volume	22 / 34 (65%)	
Concentration (×10^6/ml)	29 / 34 (85%)	
Total count (×10^6)	19 / 34 (56%)	
Progressive motility (%)	15 / 34 (44%)	
Grade a motility (%)	24 / 34 (71%)	
Viability (%)	0 / 34 (0%)	
Morphology >14%		1 / 34 (3%)
Morphology 5–14%		26 / 34 (76%)
Morphology <5%		7 / 34 (21%)

The mean age of the participants was 34 years; that of their pregnant partners 31 years. The consumption of alcohol was in general low to moderate. Only five participants smoked, two of them heavily with up to 20 cigarettes per day. Six men did not smoke or drink alcohol at all. Two participants were on medication. One man who was being treated for high blood pressure showed limited sperm values. Another man using an antiallergic agent did not show markedly reduced values. No drug addiction was noted.

Twelve ejaculates were hyposperm (<2 ml). Only two of these were oligosperm.

Five men showed an oligospermia (<20 × 10^6/ml). No participant demonstrated a pH value lower than 7.4. Nineteen men showed a progressive motility of less than 50%. Ten ejaculates presented a rapid progressive motility of less than 25%. Ten semen analyses showed an asthenozoospermia (<50% progressive motile sperm and/or <25% rapid progressive motile sperm). No ejaculate was in the normal range for viability (above 75%). The percentages of normal formed spermatozoa ranged from 1 to 17% according to Tygerberg's strict criteria. Only one participant was in the range above 14%. Three ejaculates presented exactly 14%. For most sperm parameters we observed wide ranges (table 2).

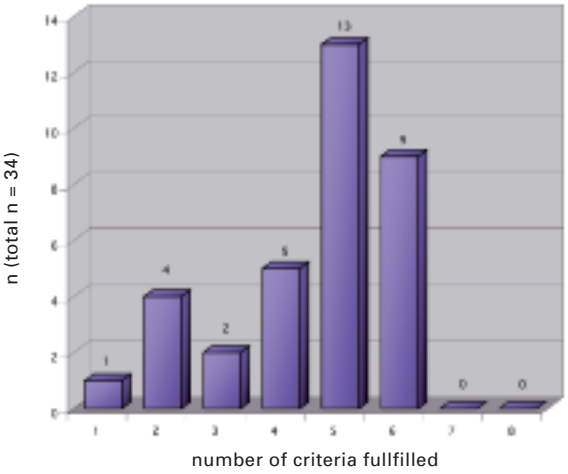

Figure 1

Number of fertile men who reach normal criteria (pH, volume, concentration, total count, total motility, grade a motility, viability, Tygerberg's criteria) (n = 34).

Evaluating the combination of normal values (pH, volume, concentration, total count, total motility, grade a motility, viability, morphology strict criteria), no participant fulfilled all criteria (figure 1, table 3). The most frequent combination was a normal concentration and a normal total count. 26 semen were normal for these two parameters. 21 sperm probes had a normal concentration, a normal count and a normal rapid progressive motility. Adding the criterion normal progressive motility (a+b), only six ejaculates fulfilled all these criteria.

17 semen analyses (50%) showed a morphology with values of 9% or more. Of these 17, only one was not normal for both parameters, concentration and rapid progressive motility. In contrast seven of the 17 probes with a morphology of 8% or less had abnormal results for these two parameters. Only two of all 34 ejaculates examined were abnormal for either concentration or rapid progressive motility (table 4).

Volume was associated with the total count on the one hand and to the concentration on the other hand. Of the 22 semen with normal volume, 21 showed a normal total count. 19 were normal for concentration.

Leucocytospermia was found in three cases and germ cells in two cases.

Table 4

Number of fertile men who reach normal criteria (concentration and/or rapid progressive motility) (n = 34).

	Normal for concentration and rapid progressive motility	Normal for concentration or rapid progressive motility
Morphology ≥9% (n = 17)	16 (47%)	17 (50%)
Morphology ≤8% (n = 17)	10 (29%)	15 (44%)
Total (n = 34)	26 (76%)	32 (94%)

Discussion

In about 50% of all infertile couples we can assume that the male partner is involved in the aetiology of the infertility problem. For this reason the evaluation of sperm quality is fundamental. Occasionally men are judged as subfertile and an IVF with intracytoplasmatic injection of spermatozoa into the oocyte (ICSI) is recommended. However some of the female partners become pregnant during ongoing investigations or even after an unsuccessful embryo transfer. On the other hand couples might be informed that the semen analysis shows no evidence of a fertility problem and the couple waits in vain for positive results.

These situations are often complex, since there are two partners who need medical attention in order to achieve a pregnancy. The medical history of both partners, the age of the women and the duration of the infertility must be taken into account [29]. All this information is important when deciding if the couple should wait for the spontaneous occurrence of a pregnancy, if a treatment should be started and which therapeutic strategy should be chosen. Over- and undertreatment should be avoided as much as possible. Over treatment always carries potential risks and mean not only unnecessary stress but also a financial burden for the couple or the public health care system. In Switzerland only three inseminations are paid for by the health insurance and there is no reimbursement of IVF and ICSI. These two reproductive techniques have to be paid for in full by the couples themselves. Therefore, in the interest of the patients we must be able to rely upon the result of a semen analysis and we must know where the cut-offs are under which the spontaneous occurrence of a pregnancy is unlikely.

Unfortunately there is a large inter-laboratory variability regarding the analysis of semen specimens. In particular the assessment of human sperm morphology is not devoid of methodological problems, making the comparison amongst laboratories difficult [7]. Intra- and inter-laboratory variability has been discussed in several publications [3, 4, 9]. It is accepted that regardless of the morphological classification used, the manual interpretation of sperm quality is to some extent subjective and varies from investigator to investigator [9, 11].

In our prospective study a single experienced technician evaluated all semen material. Our investigator participates in the continuous quality control system (CQC) under the supervision of T. Kruger, which consists of a continuous sperm

Table 5

Comparative analysis of studies that include fertile men who achieved a pregnancy within a maximum of 15 months without the support of fertility treatment.

	Crazzolara et al. (2006) (n = 34)			Menkveld et al. [25] (n = 107)			Haugen et al. [11][1] (n = 82)			Ombelet et al. [20] (n = 144)		
	Mean	Median	Range	Mean	Median	Range	Mean	Median	Range	Mean	Median	Range
Age	34	34	20–60	33.8						30.5		
pH	8	8	7.4–8.3	7.73			8.3	8.3	7.2			
Volume (ml)	2.6	2.6	0.5–5.8	3.56			3.9	3.7	0.7–7.6	3.1	2.8	0.5–12.7
Concentration ($\times 10^6$/ml)	60	47	1–224	81	75	1.3–230	94	70	0.9–326	53.1	47.5	1.0–215.0
Total count ($\times 10^6$)	160	103	1–636				355.8	290.2	2.5–1380	149.5	124.9	1.7–545.3
Total motility (%)	42	44	15–66	53.1	57.5	20–90	53.6	55.0	29–72	53.4	57.5	0–85
Grade a motility (%)	36	36	11–61				34.8	35.5	10–67	16.9	14.5	0–57
Morphology (strict criteria)	8	9	1–17	6.5	5	1–19				12	12	1–27
Morphology (WHO)				40.1	38	9–69	13.9	13.0	2.0–34			

[1] reference group with time to pregnancy <12 cycles

morphology training. We thus have a guarantee of high quality for the analyses performed, a reliable reproducibility and a good comparability with other published data from this group [20, 25].

In table 5 the few existing comparable studies are listed. Ombelet et al. [20] evaluated the semen parameters of 144 men whose partners became pregnant within 12 months and were pregnant at the time of study inclusion. The results were compared to those of a subfertile population. In contrast to Ombelets group Menkveld et al. [25] not only used strict criteria to depict morphology, but also analysed the same sperm according to WHO criteria. In a recent publication Haugen et al. [11] evaluated fertile men, who had achieved a pregnancy within 12 cycles. Their partners were pregnant at the time of study inclusion.

Another large study published in 1998 by Chia et al. [27], determined the semen parameters according to WHO criteria. The length of time until conception was not defined. Two other publications dealt with the regional differences in semen quality of fertile men in Europe and Japan [30, 33]. Again, in these two studies a prolonged waiting time to pregnancy was not an exclusion criteria.

In the present study, a total of 34 specimens were evaluated. With this study design it was impossible to have definite proof that the men examined were responsible for the pregnancy, which could only be known to their partners. However men were partly recruited by informing pregnant women in the outpatient clinic about our study. We assume that women, who knew their partner/husband not to be the biological father, may not have told their partner of the existence of such a study. By advertising we clearly approached men directly.

The mean age of the men was comparable to the above mentioned studies [11, 20, 25]. Twelve ejaculates were hyposperm (<2 ml). We can not exclude that in individual cases the ejaculates have not been collected completely, although the donors were instructed carefully. However, this problem might also arise in other studies. Interestingly only two of these semen were oligosperm. Ombelet et al. [20] and Menkveld et al. [25] did not find a difference in the mean seminal volume comparing fertile and subfertile ejaculates.

For all other parameters (concentration, motility, percentage of normal morphology), Ombelet et al. [20] and Menkveld et al. [25] documented statistically significant differences between fertile and subfertile men. Bonde et al. [32] found that semen volume and motility were of limited value in pregnancy prediction. The percentage of morphological normal sperm and an increasing sperm concentration up to 40 Mio/ml had more influence. Haugen et al. [11] in their study compared the male partners of couples who had conceived in the first cycle after stopping contraception to those that conceived later. They conclude that the variable "total number of sperm with progressive motility" seemed to be particularly important. In our study, a high percentage of ejaculates were normal for concentration (85%) or rapid progressive motility (71%). Only two of 34 semen were not normal for one of these two criteria. In 65% both parameters were normal. In a recently published study [33], sperm motility and concentration were indeed better predictors of fertility potential than sperm morphology.

We assume that the upper limit for viability is too high. We observed no ejaculate with a value above 67%. Unfortunately in no other study with a comparable set-up was this parameter evaluated. Therefore no comparison is possible.

The percentages of normal sperm according to strict criteria ranged from 1 to 17%. 50% of the specimen showed values of 8% or less. Ombelet et al. [20] and Menkveld et al. [25] reported mean values of 12% and 6.5% for fertile men. These data show that the traditional reference value of >/= 14% for normal forms may be too high. Chia

et al. [27] found that >75% of the fertile men had <30% normal spermatozoa according to the WHO criteria of normal morphology. This reference value must also be questioned.

It has to be pointed out that for most sperm parameters wide ranges have been observed in the studies discussed above. This is consistent with our results. The male partner should not be judged to be infertile because of a single parameter being at the very lowest point of a scale.

We observed leucocytospermia in three cases. Ombelet et al. [20] did not find an association between leucocytospermia and sperm abnormalities. According to the WHO guidelines [19] a concentration of ≥1 million leucocytes/ml is considered abnormal.

Guzick et al. [26] concluded that infertile couples are more likely to smoke or to consume alcohol. Chia et al. [27] documented that alcohol consumption and cigarette smoking did not appear to affect sperm quality. However, other studies suggest that cigarette smoking is associated with a reduced semen quality [34, 35]. On average, the consumption of alcohol, cigarettes and medicine was low in our study and no use of proscribed drugs was noted.

To our knowledge this is the first study to investigate the semen quality of presumably fertile Swiss men. It is evident that semen values are more predictive if they rely on data collected from fertile men. It has to be stressed that the recruitment of participants has proven to be very difficult.

From our study we conclude that the combination of several semen criteria appears to be more predictive than a single parameter. The data indicate that concentration and rapid progressive motility probably have a high impact on the fertilisation potential of semen. Considering the results obtained for viability and morphology, the limits defining the normal values seem to be too high. As proposed by the WHO it is preferable for each laboratory to determine its own normal range for each variable. In our opinion this is indeed important in order to be able to interpret results correctly and to be able to counsel the couples appropriately. However we must also be able to compare the results of sperm analysis between different laboratories, since subfertile men who undergo infertility investigations do not necessarily always visit the same laboratory. Therefore, continuous quality control systems should be implemented and laboratory staff should be obliged to undergo regular quality control in order to maintain proficiency. Our data reveal that further investigation and standardization is needed.

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