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Scand J Work Environ Health 1999;25(1):23-25

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This article in PubMed: www.ncbi.nlm.nih.gov/pubmed/10235402

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Scand J Work Environ Health 1999;25 suppl 1:23—25

Quality assurance of semen analysis in multicenter studies

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Giwercman A, Spano M, Lähdetie J, Bonde JPE, Asclepios. Quality assurance of semen analysis in multicenter studies. *Scand J Work Environ Health* 1999;25 suppl 1:23—25.

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Semen analysis is a cornerstone of the laboratory assessment of male reproductive function. Apart from the classical human semen parameters — concentration, motility and morphology — other markers have also been used to get a better evaluation of spermatogenesis and the function of accessory sex organs. They include a variety of biochemical parameters and measures of structural and numerical abnormalities of genomic material. However, quality assurance is only seldom implemented in laboratories performing semen analysis. There are few reports on intra- and interlaboratory variation in the assessment of sperm parameters, and these studies indicate a high level of variation, mainly in the evaluation of sperm motility and morphology, but also in the counting of sperm concentration (1, 2).

When the results of semen analysis are assessed, it should be kept in mind that, apart from the potential considerable variation in the analytical procedure, there is also significant intra- and interindividual variation. Therefore, when epidemiologic studies are carried out, it is important to have data on the variation related to laboratory procedure in order to be able to evaluate the degree to which geographic or time-dependent differences are due to a true biological phenomenon or can rather be ascribed to the laboratory procedure or the sampling protocol. Furthermore, occupational field studies can imply some specific problems, since the workplace is often rather distant from a specialized laboratory that carries out semen analysis. Therefore, either a mobile laboratory unit needs to be established, or one has to rely on local laboratory facilities without expertise in semen quality assessment. It remains to be investigated whether short training periods are enough to establish a satisfactory level of quality among laboratory staff not experienced in semen analysis.

As part of a concerted action of the European Union on occupational hazards to male reproductive function (Asclepios), a quality assurance program was established to evaluate the laboratory variation in the assessment of sperm concentration, the most commonly used semen parameter. In addition, some preliminary data are given on laboratory variation for 2 end points of potential value in studies of environmentally induced damage of the sperm, that is, sperm chromatin structure assay (SCSA)

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5 The Asclepios project on occupational hazards to male reproductive capability is a biomedical research project of the European Union that was carried out in 14 European centers in 1993—1998. The project was coordinated by The Steno Institute of Public Health, University of Aarhus Denmark, and it included the following researchers: Belgium, Gent (P Kiss, A Mahmoud, M Vanhoorne, H Verstraelen); Denmark, Aarhus (A Abell, JP Bonde, SB Larsen, G Danscher, E Ernst, H Kolstad), Copenhagen (A Giwercman); England, London (A Dale, M Joffe, N Shah); Finland, Helsinki (M-L Lindbohm, H Taskinen, M Sallmen), Turku (J Lähdetie); France, Paris (P Jouannet, P Thonneau), Strasbourg (A Clavert); Germany, Erlangen (KH Schaller, W Zschiesche); Italy, Brescia (P Apostoli, S Porru), Milano (L Bisanti), Pietrasanta (L Lastrucci), Rome (M Spanò); The Netherlands, Nijmegen (N Roeleveld, H Thuis, GA Zielhuis), Zeist (W de Kort); Poland, Lodz (K Sitarek).

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(which measures the stability of sperm chromatin structure) and fluorescence in situ hybridization (FISH) (used to estimate numeral chromosome abnormalities.

Material and methods

All quality assurance samples were distributed from the University Department of Growth and Reproduction, Rigshospitalet, Copenhagen. They were obtained from healthy volunteers. For the conventional semen analysis only sperm concentration was included since the interlaboratory variation for motility and morphology assessment had been found previously to be very high (3).

Definitions. Sperm concentration assessment was performed in 4 laboratories. Therefore both inter- and intralaboratory variation was assessed. To assess intralaboratory variation, coefficients of variation (CV) for inter- and intraassay variation were calculated.

SCSA and FISH analyses were both performed in only 1 laboratory and, therefore, only intralaboratory variation was assessed. For SCSA both intra- and interassay CV values were calculated, whereas for the FISH analysis the interassay variation indicates variation between assays consisting of one or more laboratory procedures resulting in the analysis of 10 000 sperm and, for the same reason, intraassay variation cannot be measured.

Sperm concentration. Four laboratories participated (Brescia, Italy; Gent, Belgium; Versilia, Italy; Århus, Denmark) in the assessment of sperm concentration. Before receiving the quality assurance samples, representatives from each of the 4 laboratories participated in a 2-day training course on the assessment of sperm density according to criteria of the World Health Organization. A total of 8 semen samples from 8 persons were included.

Sperm chromatin structure assay. Altogether 8 ejaculates from 8 men were prepared for the SCSA quality assurance study. The samples were manipulated according to the procedure described by Evenson et al (5) with slight modifications, and the technique has been described elsewhere in these proceedings.

Fluorescence in situ hybridization. The assessment of sperm aneuploidy by FISH was performed at the Department of Medical Genetics, University of Turku, Finland, as described earlier (4). Six ejaculates from 6 men were used.

Data analysis. All the samples were coded, and the code was not broken before the results were sent to the institution coordinating the quality assurance program. Thus all the measurements were performed blindly. The CV was calculated as the ratio between the standard deviation and the mean. In addition, the 95% confidence interval (95% CI) was computed.

Results

Sperm concentration. The results of the sperm concentration assessments are summarized in table 1. The results of this quality assurance study are compared with that of similar studies in table 2.

Sperm chromatin structure assay. For the βT-mean, the intraassay CV varied between 1.0% and 9.1%, and the corresponding values for the interassay CV was 5.2% and 8.6%. The ranges for the βT SD were 1.3—5.8% and 10.2—16.6%, and for βT-COMP they were 0.8—16.8% and 9.3—22.3%.

Fluorescence in situ hybridization. For sperm chromosome aneuploidy (FISH) a considerable interassay variation was found. Sperm with hyperploidy, either disomy-1, disomy-7, or diploidy are rare events, occurring in frequencies of 0—30 per 10 000 sperms. For disomy-1 and disomy-7 the mean CV was 36.1% and 55.6%, respectively. For diploidy the CV was 29.7%, and for the sum of all 3 hyperploidies, total hyperploidy, the CV was 27.3%

Discussion

A quality assurance program was performed as part of a multicenter study on occupational hazards to male reproduction. Based on a total of 35 semen samples originating from 8 different ejaculates, a mean interlaboratory CV of 23% was found for the assessment of sperm concentration. This value is slightly lower than the mean of 37.5% found in a previous quality assurance study including 10 German andrological laboratories (1, 2). In this study the mean intralaboratory CV was 10%, which corresponds to the level of the intraassay CV for 3 (II,

Table 1. Quality assurance data on sperm concentration assessment. (CV = coefficient of variation)

Sperm concentration range (million/ml)	Center 1		Center 2		Center 3		Center 4	
	Intraassay CV (%)	Interassay CV (%)	Intraassay CV (%)	Interassay CV (%)	Intraassay CV (%)	Interassay CV (%)	Intraassay CV (%)	Interassay CV (%)
1.9—64.5	30.1	29.2	12.5	10.3	12.0	9.6	11.0	22.0

III, IV) of the laboratories and the interassay CV for 2 (II, III) of the them. A factor which may have contributed to the lower interlaboratory variation may be the fact that the persons performing the sperm concentration assessment participated in a training course and used a common written protocol. In a recent study, Jørgensen and his co-workers found a CV of 15% for interlaboratory variation in a sperm concentration assessment when staff from 4 experienced semen laboratories used their own methodology but investigated the samples at the same place. This figure is somewhat lower than the variation found by us, but the CV increased from 15% to about 29% when the same laboratories participated in a quality assurance program in which the samples were sent to them in a manner similar to the one used in the present study (Giwercman et al, unpublished). Thus our data indicate that occupational field studies of sperm concentration can be performed by — in respect to semen analysis — inexperienced staff after a relatively short training course.

SCSA and FISH are two relatively new and promising methods for evaluating environmental influences on sperm DNA. This study also gave an indication of the magnitude of the CV for these analyses. In the case of SCSA, the interassay variability was somewhat higher than the intraassay variation for each sample, but the CV was low when compared with that of assays for other known semen parameters. No similar studies assessing laboratory variation in the SCSA procedure have been published, but our figures are similar to the observations on intraindividual variation reported by Evenson et al (5). Nevertheless, these values are low, and, when coupled with a remarkable reproducibility of SCSA assessment for each person along time, they indicate that SCSA is amenable to application in longitudinal toxicologic studies.

The analysis of chromosomal aneuploidy in sperm by FISH is a novel method for studying the effects of environmental and occupational exposures and other factors of the genetic constitution of spermatozoa. The method has not yet been standardized, and rather large differences between the results of different laboratories applying slightly different methodologies, probes for different chromosomes, variable scoring criteria, and the like are known to exist. After a review of the results obtained, we can conclude that a reference slide should be regularly included in the FISH assays to observe scorer differences and possible trends in the laboratory (2, 6, 7).

In conclusion, in a quality assurance study, we assessed intra- and intralaboratory variation in the assessment of sperm concentration and inter- and intraassay variation for 2 relatively new methods of evaluating sperm DNA — SCSA and FISH. These results may be of value in the design and interpretation of studies on the toxicologic and environmental impact on male reproductive function.

Table 2. Comparison of different studies on inter- and intralaboratory variation in sperm concentration assessment. (ND = not done, CV = coefficient of variation)

Study	Laboratories (N)	Intralaboratory CV (%)	Interlaboratory CV (%)
Neuwinger et al, 1990 (1)	10	10	37.5
Jørgensen et al, 1997 (3)	4[a]	ND	15
Giwercman et al (unpublished)	4[a]	ND	29
Asclepios	4	16	23

[a] The same 4 laboratories were included in both studies. In the study by Jørgensen et al, staff members from all 4 laboratories worked at the same place using their own techniques and equipment. Giwercman et al (unpublished) refers to a subsequent quality assurance program in which the laboratories received semen samples by mail, as in the Asclepios study.

Acknowledgments

This study was economically supported by the EU-Biomed II program (grant BMH1-CT93-1186) and the Finnish Work Environment Fund.

Ms Mari Ajosenpää-Saari is acknowledged for her help with the FISH experiments, and Ms Majken Simonsen for her preparation and shipment of the semen samples.

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