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Perspectives

Semen analysis from an epidemiologic perspective

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Abstract

The fifth edition of the World Health Organization (WHO) manual for semen analysis includes for the first time reference values for human semen characteristics. This paper considers whether such values will help to resolve the intensely debated data indicating temporal and geographical shifts in sperm counts and hypotheses that anthropogenic activities that result in the release of chemicals into the environment are detrimental to male reproductive health. The reasons that these reference values will not fulfil these purposes are also explained. Although established reference values for semen characteristics are of limited value in analytical epidemiologic research, the WHO guidelines are of utmost importance for supporting the development of appropriate research protocols. Moreover, in spite of its limitations, semen analysis is still a useful research tool in epidemiological research, and no superior alternatives are on the horizon.

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The fifth edition of the World Health Organization (WHO) manual for semen analysis includes for the first time reference values for human semen characteristics. An important issue to consider is whether such values help to resolve the intensely debated data indicating that there have been temporal and geographical shifts in sperm counts and other semen characteristics [1–6], and hypotheses that anthropogenic activities that release chemicals into the environment are detrimental to male reproductive health [7, 8]. The answer to this question is a definite 'no', for reasons that are outlined below.

The cornerstone of modern epidemiologic studies that seek to unravel deleterious effects of specific exposures is the counterfactual principle [9]. How would semen characteristics look in a group of men had they not been exposed to a specific variable of interest but had otherwise lived, worked and behaved in exactly the same way? As time cannot be turned back, we choose a group of men who were as similar as possible to the exposed men for comparison. In principle, the men selected for reference should be in all respects identical to the exposed men, with the single exception that they are not exposed to the substances or noxious agents that are being investigated. For that reason, general reference values determined from a more or less well-defined sample population can never substitute for the reference group that needs to be defined and investigated in the context of a particular study. The carefully selected reference group can also be used for comparisons between exposed and non-exposed men with respect to individual characteristics such as fetal development, birth, education, socio-economic class, life style, health and reproductive experience. Perhaps more importantly, the recruitment procedures and collection, preparation and processing of biological samples, including semen, should be the same for the exposed and reference groups [10]. The proportion of men who are willing to provide a semen sample in population-based research is highly variable, ranging between 10% and 80% [11–14]. It is important for epidemiological research that the reasons given for not signing up for a semen study is similar between exposed and reference groups—a basic

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request that cannot be achieved by a general population reference. Subfertile men are commonly overrepresented among men who contribute to cross-sectional semen studies. This may not be a major problem if the selective participation is independent of exposure status [15]. Whether or not this is the case is difficult to verify, however, because information about men who decline to participate is often scanty. Therefore, cross-sectional semen studies with limited participation rates always call for cautious interpretation, and the corroboration of findings in other settings is usually crucial. General reference values for semen characteristics will not solve these problems. Longitudinal studies in which semen characteristics are examined before, during and after a particular exposure are attractive alternatives [16], but they are seldom feasible and not appropriate if cumulative long-term effects of exposure are likely. For these and other reasons, from an epidemiologic perspective, there is a pressing need to discover new markers of male fecundity that are more readily accessible. Inhibin-B concentrations in plasma seemed to be a promising alternative from a biological perspective [17], but they were determined to be far less closely related to fecundity than are sperm counts and sperm chromatin integrity [18, 19].

Although it is evident that semen analysis has several limitations for epidemiological studies of male fertility, there are also advantages. First, it permits study of male fecundity independent of actual attempts to obtain a pregnancy, and, in contrast to many biological markers investigated in epidemiological studies, the relationship between sperm count and biological fertility is fairly well established. The same applies to other semen characteristics, such as sperm chromatin integrity. Moreover, it should not be forgotten that carefully conducted and controlled studies of semen quality have in fact contributed substantially to current knowledge on reproductive toxicity of many chemicals in humans. A similar measure of female fecundity is lacking, and much less is known about environmental risks to fertility in women. In spite of these limitations and low participation rates, semen analysis should be considered a useful and valuable tool for epidemiological studies. A functional measure of fertility, such as the time taken to conceive from discontinuation of contraception (time to pregnancy, TTP), is a complementary methodology, not an alternative, and the TTP measure also has several limitations that need to be considered. For the same reasons, reference values for semen characteristics do not help to resolve suggestions of temporal and geographical trends in sperm count that have been debated since the early 1970s [6]. The desire for comparability with respect to recruitment, enrolment, semen collection, preparation and processing is, of course, equally important in this context, but such comparability is almost impossible to ascertain from antecedent data. It is possible, however, to initiate repeated studies of appropriate random samples of men in order to characterize future developments, as has been done in Denmark since the mid-1990s [20]. Interestingly, these prospectively collected data indicate unchanged values over the past 10 years.

Spatial shifts in sperm count and other semen characteristics are easier to ascertain if studies are performed according to uniform protocols and close attention is paid to quality assurance and training issues [5, 21]. Nevertheless, differences in participation rates and several other limitations make it risky to interpret apparent regional differences. For instance, a coordinated study of semen quality among fertile men in four European capitals reported differences in median sperm counts [5], whereas European occupational studies found amazingly similar sperm count distributions among blue-collar workers from Italy, the United Kingdom, Belgium and Denmark (Table 1) [22]. Additionally, population-based environmental semen studies that enrolled spouses of pregnant women in highly diverse populations such as Ukraine, Kharkiv, Greenland and Sweden reported sperm count distributions in precisely the same range [23]. So far, the only geographical difference in sperm count that has been corroborated in several studies is the remarkably higher values of Finnish men compared with men from a number of other European countries, the reasons for which are entirely unknown.

The hypothesis that environmental chemicals can cause major damage to male reproductive health in affluent countries through interference with the hormonal regulation of male sexual organ development in fetal and perinatal life is interesting, but it is difficult to corroborate and almost impossible to reject. Now, more than 15 years after this hypothesis was forwarded by Sharpe and Skakkebaek [7], we still do not know whether environmental chemicals are important contributors to low human fecundity. Epidemiological studies are scarce and circumstantial. However, data are emerging that heavy exposure to maternal tobacco smoking during fetal development has a stronger impact on sperm count than smoking in adulthood, but again, the mechanisms are not known [12]. It is also of interest that extremely low serum concentrations of persistent pollutants, such as polychlorinated biphenyls, have been shown to interfere with sperm motility in more than four different populations [23]—a highly consistent pattern corroborated by experimental evidence. Reference values are of little help in answering these questions, but carefully controlled follow-up studies of sons from cohorts of women who provided blood for biobanking when pregnant may be the most promising way to learn more.

Table 1. Crude sperm concentration in various regions of Europe and Greenland.

Region	*n*	Median sperm concentration (million mL^{-1})	p5–95	References
Men providing samples when wife is pregnant				
Copenhagen	349	61	10–207	[22]
Paris	207	74	15–231	
Edinburgh	251	77	15–222	
Turku	275	82	19–262	
Warsaw	198	64	7–258	[24]
Kharkiv	208	59	9–166	
Greenland	201	53	11–178	
Men providing samples independent of pregnancy or infertility				
Danish conscripts	708	41	3–167	[20]
Danish pregnancy or first-pregnancy planners	418	50	3–202	[18]
UK, blue-collar workers	185	47	2–158	[23]
Belgium, blue-collar workers	160	47	5–118	
Italy, blue-collar workers	140	47	8–230	
Hamburg	334	49	3–186	[21]
Leipzig	457	45	6–174	

Although established reference values for semen characteristics are of limited value in epidemiologic research into regional and temporal shifts of sperm count, and causes of infertility, the WHO guidelines for preparation and analysis of semen characteristics are of utmost importance in supporting the development of standards and appropriate research protocols. This aspect should not be understated.

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