

Data gaps and new methodologies in the assessment of male fecundity in occupational field studies

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In recent years, semen analysis has become the primary research tool for studying the effects of potential toxicants on the human male reproductive system. This technique provides a reliable assessment of spermatogenesis and sperm cell and accessory gland function. However, reproductive toxicants can attack other sites in the male reproductive system. They can, for example, alter the genetic integrity of the sperm cell, disrupt the neuroendocrine system, and inhibit sexual function. Better methodologies and data are needed for these three areas. I have addressed them accordingly.

Few methodologies are available for assessing genetic damage to the sperm cell. Methods being evaluated are the sperm chromosome and deoxyribonucleic acid (DNA) stability assays for use in field studies. Current data suggest that the chromosome assay is too time consuming and unreliable for field application. Some data also indicate that the DNA stability assay appears to be reliable and repeatable and is being evaluated in field studies.

The neuroendocrine system is often not evaluated due to the clinical analysis method, which requires three blood draws 20 min apart. Using statistical power analysis, data of the National Institute for Occupational Safety and Health (NIOSH) indicate that three blood draws provide only a marginal improvement over a single blood draw in detecting population shifts for follicle stimulating hormone (FSH), luteinizing hormone (LH), prolactin, and testosterone. Thus a single blood draw will provide useful occupational field study data for these hormones.

Reliable methodologies are also lacking for the assessment of the effects of potential toxicants on sexual function. While there are clinical methods for assessing male sexual function, few field studies have addressed this issue due to the expense of the analytical equipment and potential inaccuracies in interview data due to the extremely personal subject matter. Further research is needed in this area.

Genetic damage in the spermatozoa

Spermatogenesis is the cytological process that results in the formation of spermatozoa from stem cells. Spermatogenesis can be subdivided into the following three stages: (i) mitotic proliferation, (ii) meiotic division, and (iii) morphological transformation. These processes occur within the seminiferous tubules of the testes of the adult male. Toxicants can disrupt sperm production or cause alterations in the genetics of the sperm produced. Semen analyses (sperm count and sperm morphology) are the primary assays used to determine whether the production of sperm has been affected by a toxicant. There are reports showing toxicant effects on these variables (1, 2). These assays do not detect changes in the genetic make-up of the sperm.

Genetic damage is difficult to detect in human sperm. Animal studies using the dominant lethal assay (3) indicate that paternal exposure can produce an adverse pregnancy outcome. Epidemiologic studies of large populations have demonstrated an increased frequency of spontaneous abortion among women whose husbands were working with specific toxicants or in certain occupations (4, 5). Such studies indicate a need for methods to detect genetic damage in human sperm. Methods being developed by several laboratories, including NIOSH, are DNA stability assessment by flow cytometry (DNA/FC) (6), DNA probes to discern mutations (7), and sperm chromosome karyotyping (8).

The DNA/FC assay can provide information regarding genetic damage to nucleic acids. In the performance of the assay, the sperm DNA is stressed chemically and then stained with acridine orange and analyzed in a flow cytometer (9). Animals exposed to known mutagens have an increase in single-stranded DNA (10-12). The DNA/FC assay has shown that bull fertility is correlated with the percentage of double-stranded DNA (13). The DNA/FC assay is highly repeatable for ejaculates from the same man (14). This procedure has been developed in only a few laboratories at this time and therefore needs to be established in more laboratories before its usefulness can be fully validated.

Methods to evaluate DNA adducts and DNA probes can be useful in the future. DNA adducts, the complex formed between a toxicant or its metabolites and the sperm DNA, may be carried in the sperm chro-

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matin to the site of fertilization. New methods for identifying nucleotides may permit the detection of these adducts in ejaculated sperm. DNA probes are monoclonal antibodies to distinct modifications of the DNA molecule (7). These antibodies serve as specific probes that can allow for the detection of subtle changes in the DNA code.

The evaluation of sperm chromosomes is difficult because the sperm chromatin is tightly packed in the nucleus for transport to the egg. Procedures have been developed to assess these chromosomes by human sperm fertilizing denuded hamster eggs (15), in which the male pronuclei is formed and the chromosomes are visualized. Several clinical studies have evaluated sperm chromosomes (16–18), and larger studies have examined the effects of cancer treatment (19–21) on chromosomal activity. The routine analysis of sperm chromosomes in occupational field studies is not currently feasible due to the exorbitant time needed for the preparation and analysis of each sample.

Neuroendocrine system

The reproductive neuroendocrine axis of a man involves principally the central nervous system, the anterior pituitary gland, and the testes. Lead is a classic example of a toxicant that directly affects the neuroendocrine system in both animals (22–24) and humans (25, 26). The reproductive endocrine status of the male is best established from measuring the hormones in the blood. Since the circulating profile of LH is pulsatile, the status of this hormone for the individual, if measured in blood, should be estimated in serial samples. The pooled results of three samples collected at 20-min intervals will provide a reasonable estimate of mean concentration (27). Circulating levels of FSH are not as variable as those of LH. Thus an analysis of a single blood sample for an individual will provide a more reliable estimate of FSH than of LH.

Approximately 2% of circulating testosterone is free, and the remainder is bound to sex hormone binding globulin (SHBG), albumin, and other serum proteins. The free circulating testosterone is the active component and provides a more accurate marker of physiologically available testosterone than does total circulating testosterone under conditions when the SHBG concentration or binding is altered (27). Circulating testosterone levels, like those of LH, fluctuate considerably over time. Estimates of free and total testosterone can be determined in single blood samples, but are greatly improved if multiple blood samples are assayed and the results pooled.

FSH, LH, testosterone, and prolactin can all be evaluated in a population-based study through the assessment of the hormone levels in a single blood sample from each man (preferably at about the same time of day). In the study of an individual, blood samples should be collected every 10 to 20 min for 1 to 6 h (27). The logistics of an occupational field study make these

conditions difficult to implement and discourage participation in the study. In a longitudinal study of unexposed workers, the endocrine profile was analyzed for each man. The blood collection was between the times of 0800 and 2000. Three blood samples were drawn 20 min apart from 45 men, on three occasions during the course of the study (June, October, and February). Serum concentrations of FSH, LH, testosterone, and prolactin were determined. A component of variance model was used to estimate variability between the 20-min blood draws. Statistical power analysis demonstrated that three blood draws provided only a marginal improvement over a single blood draw in detecting population shifts. In addition, if the prospect of three blood draws reduces subject participation by 10–20%, the increase in power would be negated.

Sexual function

Human sexual function refers to the integrated activities of the testes and secondary sex glands, the endocrine control systems, and the central nervous system-based behavioral and psychological components of reproduction (libido). Erection, ejaculation, and orgasm are distinct, independent, physiological, and psychodynamic events which normally occur sequentially in men.

Priapism and impotence represent erection anomalies. Priapism is an erection which occurs without sexual arousal and without resolution after ejaculation. Impotence (erection failure) can have either a psychogenic or an organic etiology. While organic impotence is characterized by reduced ability to achieve an erection at any time, psychologically impotent men continue to experience spontaneous erections during sleep (28). Burris et al (29) recently reported the application of a nocturnal penile monitor for assessing erection at home. This method can provide a convenient, non-clinical means by which to evaluate the erectile function of exposed workers.

There are no field methods other than reproductive questionnaires which are currently available for assessing ejaculatory and libido problems.

Assessment of occupational exposure-induced anomalies of sexual function is difficult. The researcher must rely on the self reports by the worker regarding his sexual function. This testimony can include inaccurate information to protect his ego or masculine image and thus result in misclassifications. Therefore, little reliable data are available on occupational exposure effects on sexual function. Drugs have been shown to affect each of the three stages of male sexual function (30), indicating the potential for occupational exposures to exert similar effects.

New research methods that are reliable and practical for field study conditions are needed to assess these important areas of reproductive function and the possible role toxic exposure may play.

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