Man and the workplace: Assessing his reproductive health

By Steven M. Schrader

Over the years, researchers have searched for the elusive marker of male fecundity (the ability to father children). As with most biological entities, the more it is studied the more complex the system becomes. Basically, the sperm cell has a single mission: inject the male genome to the egg cytoplasm. The sperm cell design is sleek and mission oriented. The chromatin is packed tightly in protamines ready for transport. Not unlike one's clothes in a suitcase in the belly of a jetliner ready for a journey, the chromatin is not readily available for inspection or assessment. No protein synthesis or cell replication will take place in the mature sperm so these biochemical processes cannot readily be assessed. The acrosomal cap contains the hyaluronidase and acrosin to digest the cumulus and zona for delivery of the payload. The midpiece contains mitochondria to power the motility engine and other functions of this specialized cell. The tail or flagella must propel the cell through several environments.

Assessment of the ejaculate to determine the fecundity of the individual requires a basic understanding of the spermatozoa and semen function. It appears that semen provides a nutrient rich protective environment to get the sperm to the awaiting cervix of the female. There are over 300 components of semen, the importance of only a few of these is understood. Fructose from the seminal vesicles is sperm's favorite sugar. Zinc from the prostate is important in the eventual acrosome reaction and chromatin dispersion. The buffering basic pH protects the sperm cells from the acidic environment of the vagina. While seminal plasma plays a vital role in the normal reproductive scenario, assisted reproductive technologies (ART) have shown that seminal plasma is not essential for fertilization.

The business of spermatozoa is a numbers game. According to the World Health Organization the normal ejaculate has at least 40 million sperm.[1] Many ejaculates have hundreds of millions. For a normal reproductive scenario (without ART), there are some basic requirements of a sperm. It must be motile, probably progressively motile (defined as swimming a distance of its overall length in one second). It must have an oval shaped head. This is probably for several reasons. First, this head design allows for the progressive motility of being pushed by the tail. Second, an oval shaped head suggests a normal acrosomal cap that will allow penetration of the egg. Third, there is some research to suggest that an abnormal shaped head may mean abnormal chromatin.[2] The numbers game requires millions of sperm. A sperm may be perfect in every way, and yet may not be in the portion of the ejaculate that bathes the cervical os, and thus never gets a chance to demonstrate its progressive motility, outstanding genetics, etc. Millions are left behind at the cervix. Much of the transport through the cervix is dependent on progressive motility. More are lost along the uterus and fallopian tubes. Muscular contractions and beating cilia, along with sperm's own motility, bring some sperm forward while others are lost. This is not a race, as the sperm that get there first (close to the egg) sacrifice their lives, releasing hyaluronidase to disperse the cumulus so that the one of the sperm can park up along side the egg, and release acrosin at just the right time to allow penetration. Once inside, the chromatin has to have been packed right for the development of the male pronuceli. All of this with the great expectation that Ms. Egg has done her part correctly.

From the above description, it is very apparent that there are numerous components and functions which can fail causing fertility failure. Short of a live birth, the chance of having a single biomarker of all aspects of male fecundity combined is impossible. To date, however, there are several biomarkers to assess the key steps above. Andrology labs assess sperm number, sperm motility, and morphology. These have been basic to semen analyses for 50 years, although the methods have improved in both speed and precision. Development of new biomarkers is being attempted to assess other key steps of the life and function of the spermatozoa. The leading edges of biomarker male fecundity research are assessing sperm chromatin and sperm–egg interaction. Obviously there can be millions and millions of sperm that look great and swim perfectly, but if none can produce a viable male pronucleus, it was a worthless journey.

The work of the reproductive toxicologist is to identify chemicals or physical agents which may disrupt this male reproductive function.

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SEMEN ANALYSIS

Semen analysis provides a useful profile of the function of the male reproductive system. Exact instructions should be provided to each man to ensure the semen sample is collected by masturbation after a set time of abstinence (usually 2 days), and delivered to the laboratory within 1 hour from the time of ejaculation. The men should be instructed to maintain the semen at room temperature avoiding any temperature shock to the sperm cells. NIOSH produced a video tape with these instructions which is shown to all participants of a semen study.[10] At the time of semen sample collection, each subject should record the duration of abstinence, time of semen collection, and any information regarding spillage. Providing a label on the jar facilitates the recording of this information.

Semen analyses can be conducted in two phases. The initial evaluation of the sample should be conducted when the sample arrives at the laboratory (or field site), and should consist of recording the temperature, turbidity, color, liquefaction time, volume, and pH of the semen. Modern computer assisted sperm analysis (CASA) systems allow sperm concentration and motility assessment to be measured at the field site laboratory. Slides are prepared and the seminal plasma is frozen for later analysis. Morphologic and morphometric analyses of sperm on the prepared slides are conducted later.

Measurements of sperm motility and velocity should be conducted using a microscope stage warmed to 37°C. An attempt to record 100 motile sperm per sample is desirable if one is interested in the distribution of velocity measurements, but 50 motile sperm will suffice if means are to be compared. If video tapes are being used to calculate the percent motility, one should avoid "hunting" for motile sperm. All fields examined or searched should be included in the calculations. Therefore, recording a certain number of arbitrary fields is advised. Whole semen should be used for measuring sperm motility. If a CASA system is being used for velocity estimates, the number of sperm per field needs to be reduced to minimize cell collisions. Using a 10–20 μm deep chamber, the sperm concentration should be less than 40 million/mL. Diluents (including seminal plasma), however, alter sperm velocity up to a dilution of about 1:1. The current recommendation for CASA for sperm velocity is to dilute *all* samples 1 part semen in 1 part iso-osmotic buffer.[11] If this dilution does not reduce the sperm concentration below 40 million/mL, then an additional dilution in the same buffer should be performed on those concentrated samples. Thus, two recordings should be made: whole semen for percent motility and diluted sperm for sperm velocity.

Sperm morphology should be estimated on air dried, stained semen smears. During the past 30 years, several schemes have been presented for the assessment of normal and abnormal sperm morphologies. Variation in sperm size and shape are not distinct, but rather a continuum. This provides a challenge within, and especially among laboratories to establish a repeatable system for morphological classification.[12–15] With recent advances of computerized image analyses, several methods of sperm morphometry have been introduced.[16–22] These morphometric analysis systems provide objective assessments of individual sperm head size and shape. Comparisons of measurements between different analysis systems should be avoided. Sperm morphometry is now routinely used as part of the assessment of reproductive hazards to the male worker.[23]

SITES OF TOXICANT ACTION

Toxicants can attack the male reproductive system at one of several sites or at multiple sites. This does not necessarily indicate, however, that there exists an absolute one-to-one relationship between a particular measurement and the associated site(s) of action. These sites, and assays associated with their respective functions, are discussed individually. They include the neuroendocrine system, the testes, accessory sex glands, and sexual function. Methods for assessing each of these sites for toxicant effects, i.e., a male reproductive profile for male reproductive health effects, are described. Examples of exposures which have had a detrimental effect on each site are provided. The same profile can be used for both individual and population investigations, but there are some basic differences in methodology. The assessment profile described is that being used by the National Institute for Occupational Safety and Health (NIOSH) to assess populations exposed to potential reproductive toxicants. Differences between assessing the individual *versus* the population will be noted. If individual data (*versus* population comparisons) are to be used, care should be taken to compare the results with the normal range of results of the laboratory conducting the analyses, and not published values. If a cross-sectional population study is being conducted, a concurrent comparison group must be used and the analysts should be blind to exposure status.

Neuroendocrine system

The endocrine system, in concert with the nervous system, coordinates functions of the various components of the reproductive axis, drawing upon external (e.g., sexual cues, temperature) and internal (e.g., checks and balances between endocrine tissue function, metabolic status) inputs. The reproductive endocrine status of the male is best established by measuring the hormones in the circulation, urine, or saliva. The hormones of interest are luteinizing hormone (LH), follicle stimulating hormone (FSH), testosterone, and inhibin B.

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.[3] Alternatively, an integral of an individual pulsatile LH secretion rate may be obtained by measuring this gonadotropin in urine. If a population is being evaluated, a single blood sample per individual may suffice.[4]

Circulating FSH levels are not as variable as those for LH. This is attributable, in part, to a longer circulating half-life for FSH compared to LH. Thus, analysis of a single blood sample for an individual will provide a more reliable estimate of FSH than for LH. FSH can also be measured in urine for the sake of convenience.

Approximately 2% of circulating testosterone is free, whereas the remainder is bound to sex hormone binding globulin (SHBG), albumin, and other serum proteins. The free circulating testosterone is the active component and, therefore, provides a more accurate marker of physiologically available testosterone than does total circulating testosterone under conditions when SHBG concentration or binding is altered.[1] Circulating testosterone levels, like those for LH, fluctuate considerably over time. Estimates of free and total testosterone can be determined in single blood samples, but are greatly improved by assaying multiple blood samples and pooling the results. Alternatively, a single measurement of a testosterone metabolite in urine (e.g., androsterone, etiocholanolone, or testosterone glucuronide) provides a convenient index of total testosterone.[5] Quantifying testosterone in saliva affords a convenient alternative to blood sampling, while providing a measure of the unbound, biologically active component of circulating testosterone.[6] Protein hormones such as the gonadotropins, are not exuded into the saliva.

When measuring steroid hormone metabolites in urine, consideration must be given to the potential that the exposure being studied may alter the metabolism of excreted hormone metabolites. This is especially pertinent since most metabolites are formed by the liver, a target of many toxicants. Lead, for example, has been shown to reduce the amount of sulphated steroids that were excreted into urine.[7]

Blood levels for both gonadotropins become elevated during sleep as the male enters puberty, while testosterone levels maintain this diurnal pattern through adulthood in men.[8] Thus, blood, urine, or saliva samples should be collected at approximately the same time of day to avoid variations due to diurnal secretory patterns.

The neuroendocrine system is obviously sensitive to endocrine active (endocrine disruptor) chemicals. An example of this would be workers exposed to a stilbene (4,4′-diaminostilbene-2,2′-disulfonic acid), a chemical used in the process of making optical whiteners. The chemical is estrogenic and lowered testosterone levels in these workers.[9]

Testes

Sperm count, sperm morphology, and sperm head morphometry all provide indices of the integrity of spermatogenesis and spermiogenesis. Thus, the number of sperm in the ejaculate is directly correlated with the number of germ cells per gram of testis,[24] while abnormal morphology is probably a result of abnormal spermiogenesis. Azoospermia (no sperm) is probably the most severe observation as it is often an indication that type A spermatogonia have been lost and recovery is unlikely. The promising new methods measuring DNA stability and DNA adducts will hopefully provide information about spermatogenesis at the genetic level.

Some toxicants have been shown to exhibit an effect at the testis, spermatogenesis, and/or spermiogenesis site. Exposure to dibromochloropropane (DBCP) reduced sperm concentration in ejaculates to 46 million cells/mL in exposed workers compared to a median of 79 million cell/mL in unexposed men.[25] Upon removing the workers from the exposure, those with reduced sperm counts experienced a partial recovery, while men who had been azoospermic remained sterile. Testicular biopsy revealed that the target of DBCP was the spermatogonia. This substantiates the severity of the effect when stem cells are the target of toxicants. There were no indications that DBCP exposure of men was associated with adverse pregnancy outcome.[26] Another example of a toxicant targeting the testis was the study of workers exposed to ethylene dibromide (EDB). They had more sperm with tapered heads and fewer sperm per ejaculate than did controls.[27]

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Genetic damage is difficult to detect in human sperm. Epidemiological studies of large populations have demonstrated increased frequency of adverse pregnancy outcomes in women whose husbands were working in various occupations such as mechanics and petroleum refinery workers.[28] Such studies indicate a need for methods to detect genetic damage in human sperm.[29] The sperm chromatin structure assay[30–33] has shown genetic damage in men exposed to air pollution.[34] Some other promising methods for assessing sperm genetics are the karyotyping of sperm chromosomes,[35–37] the labeling of diploidy using fluorescent in situ hybridization (FISH),[38–40] single cell gel electrophoresis (COMET),[41] and terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling (TUNEL).[42,43]

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Accessory sex glands

While seminal plasma is not essential for fertilization, it contributes importantly to the normal coitus-fertilization scenario. Seminal plasma serves as a vehicle for sperm transport, a buffer from the hostile acidic vaginal environment, and an initial energy source for the sperm. Cervical mucus prevents passage of seminal plasma into the uterus. Some constituents of seminal plasma, however, are carried into the uterus to the site of fertilization by adhering to the sperm membrane.

The viability and motility of spermatozoa in seminal plasma is typically a reflection of seminal plasma quality. Alterations in sperm viability, as measured by stain exclusion or by hypoosmotic swelling (HOS), or alterations in sperm motility parameters, would suggest an effect on the accessory sex glands that produce seminal plasma.

Biochemical analysis of seminal plasma provides insights into the function of accessory sex glands. Chemicals that are secreted primarily by each of the glands of this system are typically selected to serve as a marker for each respective gland. For example, the epididymis is represented by glycerylphosphorylcholine (GPC), the seminal vesicles by fructose, and the prostate gland by zinc. Note that this type of analysis provides only gross information on glandular function and little or no information on the other secretory constituents. Measuring semen pH and volume provide additional general information on the nature of seminal plasma.

Seminal plasma may be analyzed for the presence of a toxicant or its metabolite. Heavy metals have been detected in seminal plasma using atomic absorption spectrophotometry,[44] while halogenated hydrocarbons have been measured in seminal fluid by gas chromatography after extraction[38] or protein-limiting filtration.[45]

A toxicant, or its metabolite, may act directly on accessory sex glands to alter the quality or quantity of their secretions. Alternatively, the toxicant may enter the seminal plasma[46] and, thereby, affect the sperm, the body of the female partner after intercourse, or be carried to the site of fertilization on the sperm membrane and affect the ova or conceptus.

There are few reports of toxicant effects on the accessory sex glands in humans. Ethylene dibromide is one example of a toxicant that exerts post-testicular effects. Short-term exposure to the toxicant reduced sperm velocity and semen volume.[47] Chronic exposure decreased sperm motility and viability, decreased seminal fructose levels, and increased semen pH.[47] An EDB metabolite was found within the semen of some exposed workers.[45] Other potential toxicants that have been detected in semen include: lead, cadmium, hexachlorobenzene, hexachlorocyclohexane, dieldrin, and polychlorinated biphenyls.[38] Cocaine has been shown to bind to the sperm membrane.[48]

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 psychologic components of reproduction (libido). Erection, ejaculation, and orgasm are three distinct, independent, physiological and psychodynamic events which normally occur concurrently in men. If details on function or mechanisms are desired, several reviews and in-depth reports are available.[49–51]

Assessment of occupational exposure-induced anomalies of sexual function is difficult. The researcher usually must rely on the testimony and recall of the worker regarding his sexual function. This testimony may often be confounded by the bias of the individual to guard his ego or masculine image, to attribute a preexisting libido problem to exposures at work, or natural changes in sexual function due to aging. The International Index of Erectile Function Questionnaire[52] is a standard questionnaire for assessing sexual function.

Monitors of nocturnal erectile function, such as the RigiScan,[53] can be used in the privacy of a study participant's home.[54] Such measurements provide useful physiologic information on erectile capability.[55,56]

Assessment of ejaculate volume may provide information on the integrity of the emission phase of ejaculation. This is, of course, complicated by effects on accessory sex glands secretory capacity. Thus a semen sample of reduced volume, but with a normal ratio of constituents (marker chemicals), supports a diagnosis of an emission phase defect.

Sexual dysfunction problems were reported in men exposed to lead,[57] stilbene,[58,59] and cadmium.[60] A recent study also reported erectile function deficits in biking police officers.[61]

INITIATION OF STUDIES

Many factors influence the decision to study a group of workers. Studies of dibromochloropropane (DBCP) were initiated after informal discussions of infertility problems among wives attending a softball game.[62] A physician noting a cluster of patients from a specific occupation may initiate a study (e.g., the professional truck driver[63]). The petroleum refinery industry exemplifies a profession in which the workers themselves had concerns regarding their reproductive health.[64] Work-related accidents such as the chemical spill of bromide[65] or the nuclear radiation disaster in Chernobyl[66] have led to studies. Corporations may conduct occupational research to validate previous claims as with studies on dinitrotoluene (DNT) and toluenediamine (TDA).[67] In Europe, epidemiologic comparisons of fertility and paternal occupation have been readily made since parents' work records are linked to birth records in these countries. The majority of epidemiological and occupational research, however, has been stimulated by data from animal studies indicating that a compound is a reproductive toxicant. Thus the toxicologist, the physician, the epidemiologist, the worker himself, as well as the labor union and the employer have been and will continue to be "on the lookout" for potential exposures and study populations.

THE LIST OF REPRODUCTIVE TOXICANTS

The most common request to the Reproductive Health Assessment Section of the NIOSH is a request for a list of known reproductive toxicants. The

requestors are quite surprised to find there is not a list of reproductive toxicants affecting either the male or female. There are lists describing positive studies and there are lists of examples of reproductive toxicants;[68] but there is not an all inclusive laundry list of bad chemicals.

The next question is why is there not a list and why are we not making one. The first reason dates back to the late middle ages. Philippus Aureolus Theophrastus Bombastus von Holhenheim-Paracelus (1493–1541) introduced the following concept:

> What is there that is not poison? All things are poison and nothing (is) without poison. Solely the dose determines that a thing is not a poison.
>
> –Paracelsus[69]

This thought is often shortened to the "poison is in the dose." Without discussing the dose of the toxicant (level of exposure) lists can be used, abused, and misunderstood. A trace element like selenium demonstrates this point. Low levels of selenium are needed in a healthy diet. Early research (1935) indicated that selenium was a toxic factor in forage causing disease in cattle and in the early 1950s it was identified as a nutritional essential trace element.[70] Both high and low selenium concentrations in semen have been shown to have a negative effect on the number and motility of human sperm.[71] Does selenium belong on a list reproductive toxicants or not? Again quoting Paracelsus:

> The right dose differentiates a poison from a remedy.
>
> –Paracelsus[69]

The voters in California passed proposition 65 which requires the Governor to publish a list of chemicals that are known to the State of California to cause cancer, birth defects or other reproductive harm. This list must be updated at least once a year. The dose is defined as 1/1,000 of the No Observed Adverse Effect Level. This has been a difficult task.[72] The current Proposition 65 list can be found on the Internet at: http://www.oehha.ca.gov/prop65/prop65_list/Newlist.html.

Groups of chemicals such as heavy metals, pesticides, and solvents containing bromine and/or chlorine may be suspect if a man has significant exposure and is having fertility problems. Industrial accidents and spills may cause acute toxicity when normal work practices are not hazardous (the poison is in the dose).

There are some sources available to determine if there is known toxicity data on chemicals. The first step should be the Material Safety Data Sheet (MSDS) which the employer must supply to employees through the right-to-know laws. The MSDS will often (but not always) describe the known reproductive health effects with references.

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