

SITES OF TOXICANT ACTION OF MALE REPRODUCTIVE TOXICANTS

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Toxicants can attack the male reproductive system at one of several sites or at multiple sites. These sites are discussed individually and include the neuroendocrine system, the testis, accessory sex glands, and sexual function. Methods for assessing each of these sites for toxicant effects are described. This does not necessarily indicate that an absolute one-to-one relationship exists between a particular measurement and the associated site of action. Examples of exposures which have had a detrimental effect on each site are provided.

The methods described are from a human male reproductive profile for assessing the effects of toxicants on male reproductive health. The same profile can be used for both individual and population based 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.

Examples from pertinent veterinary animals will be provided where possible.

Neuroendocrine System

The endocrine system, in concert with the nervous system, coordinates function 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 hormone levels in the blood, 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 concentration¹. Alternatively, an integral of the 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 suffice².

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 from 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 in humans 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 altered¹. Circulating testosterone levels, like those for LH, fluctuate considerably over time. Concentrations of free and total testosterone can be determined in single blood samples, but estimates 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 testosterone³. Quantifying testosterone in saliva affords a convenient alternative to blood sampling while providing a measure of the unbound, biologically active component of circulating testosterone⁴. 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, reduced the amount of sulphated steroids that were excreted into the urine⁵.

Blood levels for both gonadotropins (FSH & LH) become elevated during sleep as the male enters puberty, while testosterone levels maintain this diurnal pattern through adulthood in men⁶. 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 in these workers⁷.

Semen Analysis

Semen analysis provides a useful profile of the function of the male reproductive system. Semen collection is well documented and routine in most farm animals. Easy methods are also available for collection of semen in the dog⁸ and rabbit⁹; but are challenging in the domestic cat¹⁰.

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 the 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 to 20 :m 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 of sperm velocity is to dilute all samples 1 part semen in 1 part iso-osmotic buffer. 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. Using CASA in the assessment of various animals is being established.¹¹ Methods for assessing dog^{9,12,13,14}, cat¹⁵, bull^{16,17}, and horse¹⁸ have been reported.

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^{19,20,21,22}. With recent advances of computerized image analyses, several methods of sperm morphometry have been introduced^{23,24,25,26,27,28,29}. 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 worker³⁰. There are numerous reports of the importance and usefulness of morphometrics in the analyses of sperm from the dog³¹, ram³², stallion^{33,34,35,36}, and bull^{37,38,39}.

Semen Parameter and Toxicant Site

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 testis⁴⁰, while abnormal morphology is probably a result of abnormal spermiogenesis. Azoospermia is probably the most

severe observation as it is often an indication that type A spermatogonia have been lost and recovery is unlikely.

Some toxicants have been shown to exhibit an effect at the testis/spermatogenesis/spermiogenesis site. Exposure to nematocide 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 men⁴¹. 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 outcome⁴². Another example of a toxicant targeting the testis was the study of workers exposed to pesticide ethylene dibromide (EDB). These workers had more sperm with tapered heads and fewer sperm per ejaculate than did controls⁴³. Bulls exposed to EDB also had reduced sperm concentrations and an increase in abnormal sperm morphology (pyriform heads and tail defects).⁴⁴

Genetic damage is difficult to detect in sperm. Epidemiological studies of large populations have demonstrated increased frequency of adverse pregnancies in women whose husbands were working in various occupations⁴⁵. Such studies indicate a need for methods to detect genetic damage in human sperm⁴⁶. The sperm chromatin structure assay^{47,48},⁴⁹,⁵⁰ has been shown to be related to fertility^{51,52,53,54} in animals and genetic damage in men exposed to air pollution⁵⁵. Some other promising methods for assessing sperm genetics are the karyotyping of sperm chromosomes^{56,57},⁵⁸, the labelling of diploidy using fluorescent in situ hybridization (FISH)^{59,60},⁶¹, single cell gel electrophoresis (COMET)^{62,63}, and terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling (TUNEL)⁶⁴,⁶⁵.

Accessory Sex Glands

Seminal plasma is not essential for fertilization. Thus, the artificial insemination of sperm collected from the epididymis can result in conception. On the other hand, seminal plasma contributes importantly to the normal coitus-fertilization scenario. Seminal plasma serves as a vehicle for sperm transport, a buffer from the 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 viability⁶⁶, as measured by stain exclusion or by hypoosmotic swelling (HOS)⁶⁷, or alterations in sperm motility parameters would suggest an effect on the accessory sex glands.

Biochemical analysis of seminal plasma provides insights into the function of the 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 spectrophotometry⁶⁸, while halogenated hydrocarbons have been measured in seminal fluid by gas chromatography after extraction³⁸ or protein-limiting filtration⁶⁹.

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 plasma⁷⁰ and, thereby, affect the sperm, affect 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 this toxicant reduced sperm velocity and semen volume⁷¹. Chronic exposure to EDB decreased sperm motility and viability, decreased seminal fructose levels, and increased semen pH³⁵. EDB also affected the sperm motility in bulls.⁴⁴ Metabolites of EDB³⁹ and 2,4-dichlorophenoxyacetic (2,4-D) residues⁷² have been found in the semen of some exposed workers. 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 membrane⁷³. Animal studies have shown that when the cancer therapeutic drug cyclophosphamide was administered to males it was transmitted to the female during mating causing a dose dependent preimplantation loss.⁷⁴

Sexual Function

Human male 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 detail on function or mechanisms are desired, several reviews and in-depth reports are available^{75,76,77}.

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 Questionnaire⁷⁸ is a standard questionnaire for assessing sexual function.

Monitors of nocturnal erectile function such as the RigiScan⁷⁹ can be used in the privacy of a study participant's home⁸⁰. Such measurements provide useful physiologic information on erectile capability.^{81,82}

The 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 lead⁸³, stilbene^{84,85}, and cadmium⁸⁶. A recent study also reported erectile function deficits in biking police officers⁸⁷.

Veterinary Examples

There are few examples reported of reproductive toxicants affecting companion or agriculture animals. The lack of examples is more likely due to the absence of studies rather than the lack of effects. The scientific literature has extensive examples of chemicals being toxic to the male reproductive system of laboratory animals (rats, mice, rabbits) in controlled studies. The obvious question is whether there are realistic exposure scenarios which may endanger the reproductive function of companion or agriculture animals.

The herbicide 2,4-dichlorophenoxyacetic acid (2,4-D) is useful to evaluate a potential exposure scenario. 2,4-D is used extensively both in the agricultural setting as well as in lawn care. Dogs have been shown to excrete 2,4-D in their urine after exposure to treated lawns.⁸⁸ These urinary concentrations were similar to the urinary concentrations seen in men applying 2,4-D in an agriculture setting. These men also excreted 2,4-D in their semen.⁷² Dogs from yards which had been treated an average of 4.5 days prior to testing had urinary concentrations greater than 0.1 mg/l. Men spraying 2,4-D in an agricultural setting with urinary concentration of 9 mg/l had significantly more sperm with poor morphology and motility.⁸⁹ There was a recovery of sperm motility after exposure, however, the abnormal sperm morphology persisted.

The extensive use of pesticides and solvents in homes and on farms provides several potential exposure scenarios to pets and livestock which may have an adverse affect on their reproductive health.

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