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Male Reproductive Toxicology[a]

STEVEN M. SCHRADER, JAMES S. KESNER

In 1775, an English physician named Percival Pott reported a high incidence of scrotal cancer among chimney sweeps. This observation led to protective regulations in the form of bathing requirements for these workers (1). This response represents one of the earliest occupational health interventions involving the male reproductive system. While male reproductive toxicology remained of interest in the intervening 200 years, this area of occupational medicine was not established until 1977 when Whorton and associates reported the effects of the nematocide, dibromochloropropane (DBCP), on male workers (2). In this case, the animal toxicologists had done their job. In 1961, it had been reported that DBCP reduced testicular weights in rodents (3). Unfortunately, this report went essentially unnoticed until workers became infertile and, in some cases, sterile due to occupational exposure to the pesticide.

Toxicants can attack the male reproductive system at one of several sites or at multiple sites, as illustrated in Figure 1.1. Primary targets include the neuroendocrine system, the testes, post-testicular sites including the accessory sex glands, and sexual function.

The endocrine system, in concert with the nervous system, coordinates function of the various components of the reproductive axis. The testes produce the sperm; yet other processes important for normal fertility, including maturation of the sperm and addition of seminal excretions, occur after the sperm leave the testes. Finally, libido and the associated erection and ejaculation are requisite for normal, unaided male reproductive performance.

With thousands of chemicals in use in the workplace and with new ones added each year, most agents have not been thoroughly tested for male reproductive toxicity. This chapter, however, provides basic information on the known targets of male reproductive toxicants and the methods available to detect adverse effects at early stages.

NEUROENDOCRINE SYSTEM

A detailed description of the reproductive neuroendocrine axis is beyond the scope of this chapter and has been reviewed elsewhere (4–6). The following overview of this axis provides a basis for discussing sites of toxicological insult.

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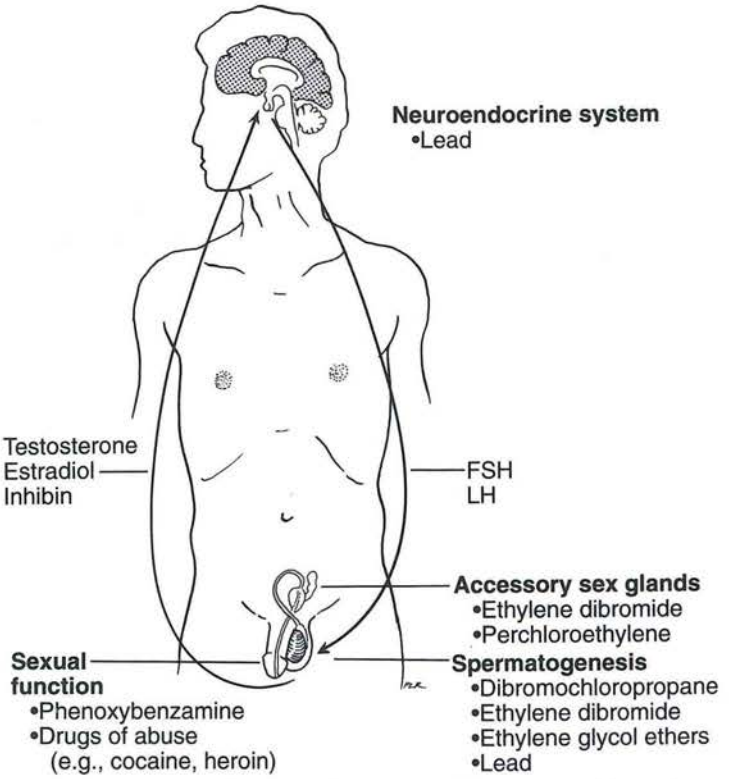

Figure 1.1. Toxicants can act at multiple sites to disrupt normal functioning of the male reproductive system. Primary targets include the *neuroendocrine system,* the *testes,* the *accessory sex glands,* and *sexual function.* Examples of toxicants that act at these various sites are provided.

The reproductive neuroendocrine axis of the male involves principally the central nervous system (CNS), the anterior pituitary gland, and the testes. Inputs from the CNS and from the periphery are integrated by the hypothalamus, which directly regulates gonadotropin secretion by the anterior pituitary gland. The gonadotropins, in turn, act primarily upon the Leydig cells within the interstitium and Sertoli and germ cells within the seminiferous tubules to regulate spermatogenesis and hormone production by the testes.

Hypothalamic-Pituitary Axis

The hypothalamus secretes the neurohormone, gonadotropin-releasing hormone (GnRH), into the hypophyseal portal vasculature for transport to the anterior pituitary gland. The pulsatile secretion of this decapeptide causes the concomitant release of luteinizing hormone (LH) and, with lesser synchrony and one-fifth the potency, of follicle-stimulating hormone (FSH) (6). Substantial evidence supports the existence of a separate FSH-releasing hormone, although none has yet been isolated (7).

Both LH and FSH are glycoprotein dimers comprised of a common α- and specificity-imparting β-protein subunits. These hormones are secreted by the anterior pituitary gland into the circulation. LH acts directly upon the Leydig cells to stimulate synthesis and release of testosterone, whereas FSH stimulates aromatization of testosterone to estradiol by the Sertoli cell. (The testes contribute only 10–30% of the circulating estradiol in the male; the remainder is derived from peripheral aromatization of androgens (8)). Gonadotropic stimulation causes the release of these steroid hormones into the spermatic vein.

Gonadotropin secretion is, in turn, checked by testosterone and estradiol through negative feedback mechanisms (Fig. 1.1). Testosterone regulates hypothalamic GnRH secretion, primarily by reducing the pulse frequency of LH

release. Estradiol, on the other hand, acts upon the pituitary gland to reduce the magnitude of gonadotropin release. Through these endocrine feedback loops, testicular function in general, and testosterone secretion specifically, are maintained at a relatively steady state.

Pituitary-Testicular Axis

Both LH and FSH are necessary for normal spermatogenesis. LH induces high intratesticular concentrations of testosterone. Pituitary FSH and testosterone from the Leydig cells act upon the Sertoli cells within the seminiferous tubule epithelium to initiate spermatogenesis. Sperm production persists after removing either LH (and presumably the high intratesticular testosterone concentrations) or FSH, but it is quantitatively reduced. FSH is required to initiate spermatogenesis at puberty and, to a lesser extent, to reinitiate spermatogenesis that has been arrested (5, 9).

The hormonal synergism that serves to maintain spermatogenesis may entail recruitment by FSH of differentiated spermatogonia to enter meiosis, while testosterone controls specific, subsequent stages of spermatogenesis. FSH and testosterone may also act upon the Sertoli cell to stimulate production of one or more paracrine factors that affect the number of Leydig cells and their testosterone production (9). Testosterone and FSH stimulate protein synthesis by Sertoli cells, including synthesis of androgen-binding protein (ABP), while FSH alone stimulates synthesis of aromatase and inhibin. ABP is secreted primarily into the seminiferous tubular fluid and is transported to the proximal portion of the caput epididymis, possibly serving as a local carrier of androgens (6). Aromatase catalyzes the conversion of testosterone to estradiol in the Sertoli cells and in other peripheral tissues.

Inhibin is a glycoprotein consisting of two dissimilar, disulfide-linked subunits, α and β. Although inhibin preferentially inhibits FSH release, it may also attenuate LH release in the presence of GnRH stimulation (10). FSH and LH stimulate inhibin release with approximately equal potency (11). Interestingly, inhibin is secreted into the spermatic vein as pulses that are synchronous with those of testosterone (12). This observation probably does not reflect direct actions of LH or testosterone on Sertoli cell activity, but rather effects of other Leydig cell products secreted either into the interstitial spaces or the circulation.

Prolactin, which is also secreted by the anterior pituitary gland, acts synergistically with LH and testosterone to promote male reproductive function. Prolactin binds to specific receptors on the Leydig cell and increases the amount of androgen-receptor complex within the nucleus of androgen-responsive tissues (13). Hyperprolactinemia is associated with reductions of testicular and prostate size, semen volume, and circulating concentrations of LH and testosterone (14). Hyperprolactinemia has also been associated with impotence, apparently independent of altering testosterone secretion (15).

Assessment Methods

Overt clinical manifestations of toxic exposure targeting the reproductive neuroendocrine system most commonly reflect alterations in androgen-dependent biological processes. During the physical examination, modifications in any of the following traits may indicate that androgen production has been affected: (*a*) nitrogen retention and muscular development; (*b*) maintenance of the external genitalia and accessory sexual organs; (*c*) maintenance of the enlarged larynx and thickened vocal cords responsible for the male voice; (*d*) beard, axillary, and pubic hair growth and temporal hair recession and balding; (*e*) libido and sexual performance; (*f*) organ-specific proteins in tissues (e.g., liver, kidneys, salivary glands); and (*g*) aggressive behavior (6).

A number of hormonal assays are available to assess the reproductive endocrine status of males who present with evidence of hypogonadism or who are exposed to potential reproductive toxicants. The endocrine profile currently used by the National Institute for Occupational Safety and Health (NIOSH) to evaluate working populations exposed to potential reproductive toxicants includes measurement of LH, FSH, testosterone, and prolactin in appropriate body fluids. With minor methodological modifications, the same profile can be used for clinical purposes.

FSH and LH are generally decreased in conditions that directly affect hypothalamo-

pituitary function and are often increased with primary testicular toxicity, due to loss of feedback inhibition by the sex steroid hormones. Measurement of prolactin levels is important in patients exposed to drugs or chemicals that induce hyperprolactinemia or in men who present with impotence or evidence of a CNS tumor (16). Estradiol assays are also indicated in males with gynecomastia.

Because the circulating profile of LH is pulsatile, serial blood samples are the best way to estimate the status of this hormone for the individual. Pooled results of three samples collected at 20-min intervals provide a reasonable estimate of mean concentration (16). Alternatively, an integral of the pulsatile LH secretion rate can be obtained by measuring this gonadotropin in urine.

FSH levels in blood are not as variable as those for LH; this is attributable, in part, to the longer circulating half-life of FSH. Thus, analysis of a single blood sample from an individual provides a more reliable estimate of FSH than LH. For the sake of convenience, FSH can also be measured in urine. Gonadotropins and other protein hormones are not exuded into the saliva.

Prolactin secretion is variable and affected by several factors including a circadian rhythm (17), eating (18), and physical and psychological stress (19). These variables should be controlled whenever possible. Prolactin has not been found in saliva, nor are specific immunoassays presently available for measuring intact or fragmented prolactin in urine.

Approximately 2% of circulating testosterone is free; 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 testosterone when SHBG concentration or binding is altered (16). (SHBG and ABP are very similar, yet distinct, glycoprotein dimers. While ABP is produced by the testes and is primarily restricted therein, SHBG is of unknown origin and circulates peripherally (20)).

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 (6). Quantifying testosterone in saliva affords a convenient alternative to blood sampling while providing a measure of the unbound, biologically active component of circulating testosterone (21).

Estimates of serum estradiol levels based on a single sample are acceptable but are markedly improved by analyzing two or three samples collected at 20-min intervals (22). Relative to testosterone, estradiol has lower affinity for SHBG, but significantly greater binding to serum albumin (23). As with testosterone, only 2–3% of circulating estradiol is unbound to serum proteins. While measuring this unbound fraction may be a more biologically relevant measure than total estradiol, the minute quantities of free estradiol (≤ 1 pg/ml) challenge the sensitivity limits of existing routine immunoassays. For this same reason, salivary estradiol measurements are also impractical (21). An integral of total estradiol secretion into the blood can be estimated by measuring a metabolite of estradiol in urine, estrone-3-glucuronide (24).

Some toxicants alter hepatic metabolism of sex steroid hormones and may affect the concentration of steroid hormone metabolites in urine. Lead, for example, reduces the amount of sulphated steroids excreted into the urine (25).

Serial blood sampling is obviously impractical for population-based studies. In these cases, a single blood sample can be used to estimate LH, FSH, testosterone, and prolactin levels. 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 (26). Thus, biological samples should be collected at approximately the same time of day to avoid variations due to diurnal secretory patterns.

Examples of Toxicant Effects

Lead is a classic example of a toxicant that affects the neuroendocrine system. In one

study, occupational exposure of males to lead for 1–5 yr reduced serum levels of free and total testosterone and elevated SHBG and LH concentrations in the circulation (27). These results are consistent with the notion that lead acts at the testis to reduce testosterone production, leading to increased production of SHBG (28) and LH. Lesser exposures to lead affected sperm count in the absence of detectable hormonal disturbances (29).

Male workers involved in the manufacture of DBCP had elevated serum levels of LH and FSH and reduced sperm count and fertility. Apparently, these effects are sequelae to DBCP actions upon the Leydig cells, resulting in altered androgen production or action (30).

Compounds may also exert toxicity by virtue of structural similarity to reproductive steroid hormones (30). By binding to the respective endocrine receptor, toxicants may act as hormonal agonists or antagonists to disrupt biological responses. For example, dichlorodiphenyltrichloroethane (DDT) and its metabolites exhibit steroidal properties and may alter male reproductive function by interfering with steroidal hormone functions. Xenobiotics such as polychlorinated biphenyls (PCBs), polybrominated biphenyls, and organochlorine pesticides may also interfere with male reproductive function by exerting estrogenic agonist/antagonist activity (30).

SPERM PRODUCTION, MATURATION, AND TRANSPORT

Spermatogenesis and spermiogenesis are the cytological processes that result in the formation of mature spermatozoa from stem cells. As shown in Figure 1.2, these processes take place in the seminiferous tubules of the sexually mature male. Sperm motility and fertilizing capacity are acquired during transit through the epididymis and vas deferens. A brief overview of sperm production and post-testicular events is presented in this section; extensive reviews can be found elsewhere (31–33).

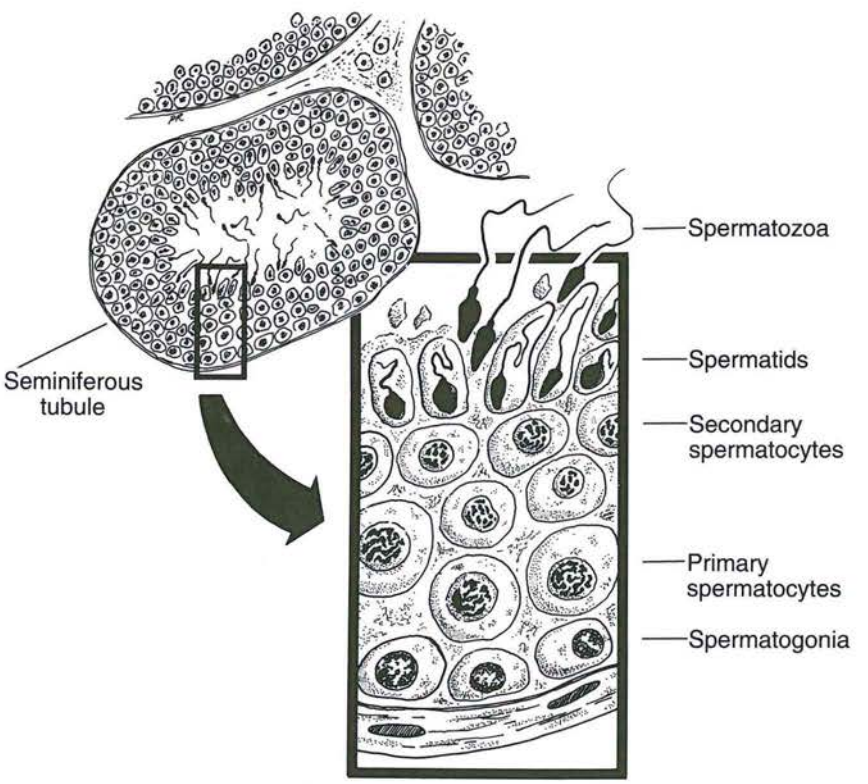

Figure 1.2. Spermatogenesis and spermiogenesis occur within the seminiferous epithelium of the testes.

Spermatogenesis and Spermiogenesis

Unlike the female who receives a fixed endowment of germ cells prenatally, males produce millions of sperm daily in a cyclical, constantly renewing process of cell division. Three major elements comprise sperm cell production: (*a*) mitotic proliferation; (*b*) meiotic division; and (*c*) morphological transformation. In humans, the total duration of spermatogenesis is approximately 74 days (Fig. 1.3).

The seminiferous tubules in the human are 30–70 cm long and 150–300 μm in diameter (34). The basic germ (stem) cells for spermatogenesis are the spermatogonia, which are positioned along the basement membrane of the seminiferous tubules. Spermatogonia are classified as Types A and B. Subclassifications of Type A spermatogonia include Type Ad (dark stain), Type Ap (pale stain), Type Ac (cloudy stain, intermediate to Ad and Ap), and Type Al (elongated shape) (35).

Paniagua et al. (35) have described the function of each subclass of Type A spermatogonia. Type Ad spermatogonia serve as the immediate precursors and divide mitotically to yield Type Al cells. Mitotic division of Type Al cells gives rise to both Type Ad and Type Ap cells. The production of the Ad spermatogonia serves to replenish the supply of precursors, while Type Ap spermatogonia undergo another mitotic division to produce Type Ac spermatogonia. Successive mitotic divisions transform the Type Ac spermatogonia to Type B spermatogonia and then to primary spermatocytes. At this point, the spermatocyte is classified as a resting (preleptotene) spermatocyte.

This series of mitotic divisions increases the number of cells entering meiosis. The resting primary spermatocytes migrate through tight junctions formed by the Sertoli cells to the luminal side of this testis barrier. At this point, DNA synthesis is complete except, possibly, for DNA repair in late zygotene or early pachytene stages of meiosis (36).

Meiosis commences once the primary spermatocytes encounter the lumen of the seminiferous tubule. The primary spermatocytes are activated from the preleptotene stage and differentiate sequentially into leptotene, zygotene, pachytene, and diplotene spermatocytes. During the leptotene stage, the chromatin condenses and becomes filamentous; the homologous chromosomes then move together during the zygotene stage. During the pachytene stage, the chromosome pairs shorten and condense further, the nuclear and cytoplasmic volumes increase, and RNA synthesis accelerates. Chromosomal autosomes may "cross over" during the pachytene stage. The diplotene stage is characterized by continued condensation of the chromosomes and separation of the chromosomal pairs. The nuclear membrane breaks down, and microtubular spindles attach to the chromosomal pairs, causing them to separate. At this point, the first meiotic division is complete and two pseudodiploid secondary

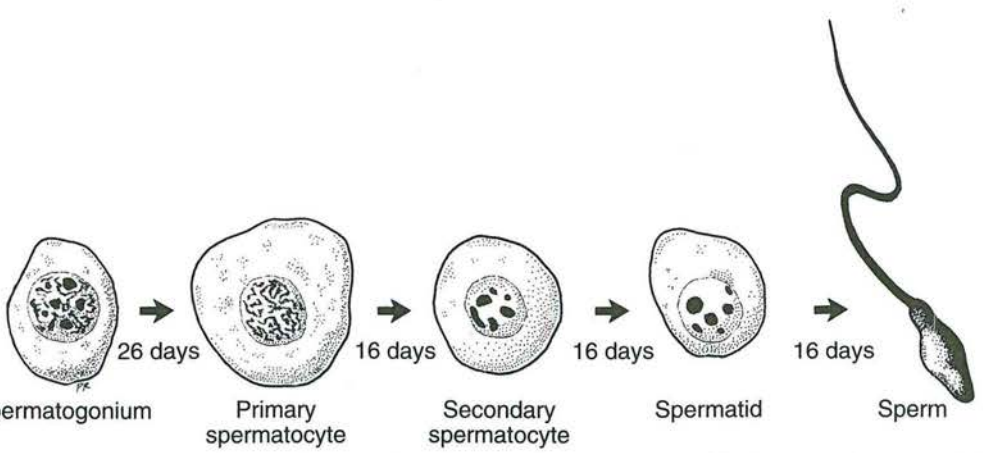

Figure 1.3. The process of spermatogenesis in humans requires approximately 74 days. Data based on Heller CG, Clermont Y. Kinetics of the germinal epithelium in man. Rec Prog Horm Res 1964;20:545–575.

spermatocytes are formed. The secondary spermatocytes undergo the second meiotic division to yield equal numbers of spermatids bearing X- and Y-chromosomes.

Spermiogenesis consists of the ensuing morphological transformation of spermatids to spermatozoa. The DNA and nuclear proteins condense, forming a tight complex that then migrates toward the cell membrane. The golgi form lysosomal granules that coalesce into a proacrosomal granule. This granule also migrates, becomes interposed between the nucleus and the cell membrane, and flattens to form the acrosome. Centrioles move to the "caudal" side of the nucleus to form the axoneme, the axial filament complex that will become the sperm tail. Mitochondria migrate to the anterior portion of this filamentous element to form the midpiece region. The cytoplasm is sequestered into a droplet that forms at the neck of the sperm and moves down the newly formed tail. This process removes the remaining cellular components from the spermatid.

When spermiogenesis is complete, the sperm cell is released by the Sertoli cell into the seminiferous tubule lumen by a process referred to as spermiation. These sperm migrate along the tubule to the rete testis and into the head of the epididymis.

Post-testicular Events

When the sperm leave the seminiferous tubules, they are immature, incapable of fertilization, and unable to swim. The environments and changes the sperm cells undergo until leaving the urethra are described below.

EPIDIDYMIS

Spermatozoa released into the lumen of the seminiferous tubule are suspended in fluid produced primarily by the Sertoli cells. Sperm are concentrated within this fluid and flow continuously from the seminiferous tubules, through the ionic milieu within the rete testis, through the vasa efferentia, and into the epididymis (Fig. 1.4). The epididymis is a single, highly coiled tube (5–6 m long) in which sperm spend 12–21 days.

Within the epididymis, the nature of the suspension fluid changes, and sperm acquire progressive motility and fertilizing capacity. The epididymis absorbs components from the fluid (including ABP and other secretions from the Sertoli cells), thereby concentrating the

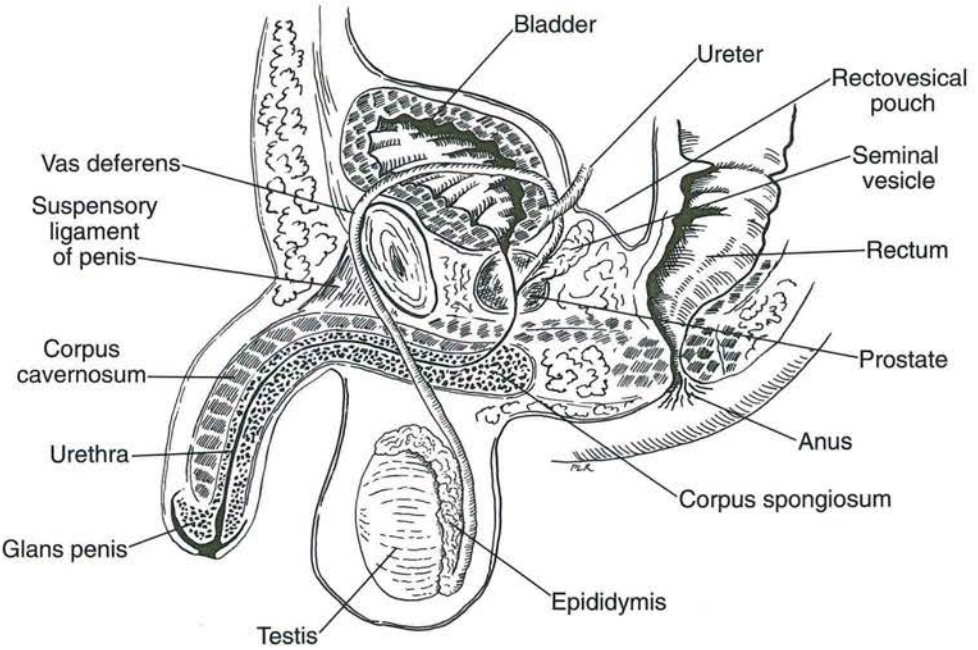

Figure 1.4. The male reproductive organs: lateral view.

spermatozoa. The epididymis also contributes secretions to the suspension fluid, including glycerylphosphorylcholine (GPC) and carnitine. Sperm morphology continues to transform in the epididymis. The cytoplasmic droplet is shed, and the sperm nucleus further condenses.

VAS DEFERENS AND AMPULLA

The vas deferens is approximately 45 cm long and connects the tail of the epididymis with the urethra (Fig 1.4). At the fundus of the bladder, the vas deferens enlarges and forms the ampulla. The vas deferens plays a minor role in secreting seminal plasma fluids. While the epididymis is the principal storage reservoir for sperm until ejaculation, about 30% of the sperm in an ejaculate comes from the vas deferens. Frequent ejaculation accelerates passage of sperm through the epididymis and may increase the number of immature (infertile) sperm in the ejaculate (35).

ACCESSORY SEX GLANDS

Once within the vas deferens, the sperm are transported by the muscular contractions of ejaculation rather than flow of fluid. During ejaculation, fluids are forcibly expelled from the accessory sex glands, giving rise to the seminal plasma. Seminal plasma is not essential for fertilization. Thus, the artificial insemination of sperm collected from the epididymides results 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 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 to the site of fertilization by adhering to the sperm membrane.

The accessory glands do not expel their secretions at the same time. Rather, the bulbourethral (Cowper's) glands first extrude a clear fluid, followed by the prostatic secretions, the sperm-concentrated fluids from the epididymides and ampulla of the vas deferens, and finally, the largest fraction comes primarily from the seminal vesicles. Thus, seminal plasma is not a homogeneous fluid.

Bulbourethral Glands

The bulbourethral glands are paired and are round and lobular in shape. These glands secrete about 0.1 ml of fluid rich in mucoproteins that lubricate the distal urethra.

Prostate Gland

The prostate gland is comprised of two lateral lobes and one median lobe. It is a structural extension of the urinary bladder and consists of smooth muscle encapsulating fibrous glandular tissue. Prostatic fluid is clear, has a pH of about 6.5, and contributes 0.5 ml to the average ejaculate. Prostatic fluid contains high concentrations of citric acid, acid phosphatase, calcium, zinc, spermine, amylase, and inositol.

Seminal Vesicles

The seminal vesicles are two distinct lobular pouches that are 5–10 cm long. The seminal vesicles contribute approximately 2.5 ml to the average ejaculate. The pH of this fluid ranges from neutrality to slightly alkaline. The vesicular fluid contains high concentrations of potassium, phosphorylcholine, fructose, prostaglandins, substrates for coagulation, and most of the proteins within the seminal plasma.

Assessment Methods

SEMEN ANALYSIS

Semen analysis is the most common method used to determine the effects of a toxicant on sperm production. The various indices that are routinely measured in the assessment of occupational exposure are presented in Table 1.1. Sperm count and sperm morphology 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 (37), while abnormal morphology is probably the result of defective spermiogenesis. Alterations in sperm motility or viability suggest post-testicular effects.

Table 1.1.
Semen Profile for Assessing Reproductive Toxicant Effects

Sperm concentration
Sperm viability
Vital stain
Hypo-osmotic swelling
Sperm motility
Percent motile
Curvilinear velocity
Straight-line velocity
Linearity
Lateral head amplitude
Beat cross frequency
Sperm size and shape
Morphology
Morphometry
Semen parameters
pH
Volume
Marker chemicals from glands
Toxicant or metabolite concentrations

Methodological Considerations

The semen sample is collected by masturbation after a set time of abstinence (usually 2 days) and delivered to the laboratory within 1 hr of ejaculation. In the clinical setting, semen samples can often be collected at the health care facility. The various fractions of semen can be collected by a technique known as split ejaculate (38). This procedure exploits the fact that distinct, sequential muscular contractions are responsible for ejaculation of the various semen fractions. If sperm fractions are to be studied, clinicians ask that two fractions be collected. The first fraction contains the majority of the sperm and the sperm with highest motility, while the second consists of secretions from the seminal vesicles.

The initial evaluation of the specimen is conducted when the sample arrives at the laboratory and consists of recording the temperature, turbidity, color, liquefaction time, volume, osmolality, and pH of the semen. Video recordings are useful for motility assessments and sperm counts.

Sperm concentration and motility characteristics should be measured in a chamber at least 10 μm deep for the sperm to move freely in all planes.

Measurements of sperm motility and velocity are 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 videotapes 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. If a computer-assisted sperm analysis (CASA) system is employed for motility and velocity estimates, the number of sperm per field must be reduced with iso-osmotic buffer to minimize cell collisions.

Sperm morphology should be estimated on air-dried, stained semen smears. Variations in sperm size and shape are not distinct, but rather represent a continuum. This presents a challenge within, and especially among, laboratories to establish a repeatable system for morphological classification. With recent advances in computerized image analyses, several methods of sperm morphometry have been introduced (39–42). 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 (43).

Sperm viability can be determined by two methods: eosin Y stain exclusion (44) and hypo-osmotic swelling (HOS assay)(45). These techniques test for the structural and functional integrity of the cell membrane, respectively (46).

Biochemical analysis of seminal plasma provides insights into the function of the accessory sex glands. Specific chemicals serve as markers for each gland. For example, the epididymis is represented by GPC, the seminal vesicles by fructose, and the prostate gland by zinc. This type of analysis provides only gross information on glandular function and little or no information on other secretory constituents. Measuring semen pH and osmolality provide additional general information on the nature of seminal plasma.

Seminal plasma can also be analyzed for the presence of a toxicant or its metabolites. Heavy metals have been detected in seminal plasma using atomic absorption spectrophotometry (47), while halogenated hydrocarbons have been measured in seminal fluid by gas chromatography after extraction (48) or protein-limiting filtration.

Interpretation of Semen Analysis

The proper interpretation of a semen analysis is critical. It is important not to overemphasize the normal/abnormal ranges, overinterpret a single semen analysis, or unduly weigh the importance of one variable over another. Fertility assessment is further complicated by the many variables that determine the reproductive status of the prospective mate.

The historical data in the literature can be confusing. Studies may report sperm counts in terms of "normal" means and ranges, "fertile" means and ranges, or control population mean and range. Generally, reports from proven fertile men indicate an average sperm count of approximately 100 million, while studies of populations not selected for fertility status report lower means of approximately 50–80 million. Each laboratory must establish its own "normal" or "fertile" range for comparison. Some basic guidelines have been published by the World Health Organization (49).

It is generally believed that there is a fecundity curve for each semen characteristic, rather than a magical threshold below which conception does not occur (Fig. 1.5). For example, while oligospermia is commonly defined as a sperm count below 20 million/ml, successful conception can occur at lower counts. At the same time, at values below 20 million/ml, the probability of conception decreases steadily with reductions in sperm count (50). For some men, even a modest toxicant-induced reduction in sperm count could bring them into the oligospermic range, thereby decreasing the likelihood of successful impregnation.

Another issue in conducting semen analysis is the reliability of a single semen sample for representing an individual's true semen quality. Data from a recent study of unexposed workers lend insight into this issue (51). In this study, sperm count had a high within-subject coefficient of variation, i.e., a single sample sperm count was not very representative of the true mean concentration based on multiple samples over time. However, intraclass correlation was high, indicating that men with high counts generally tended to stay high and vice versa. The percentage of motile sperm also had poor precision, and its repeatability was poorer than that for sperm count. The percentage of sperm with normal morphology was both precise and quite stable over time.

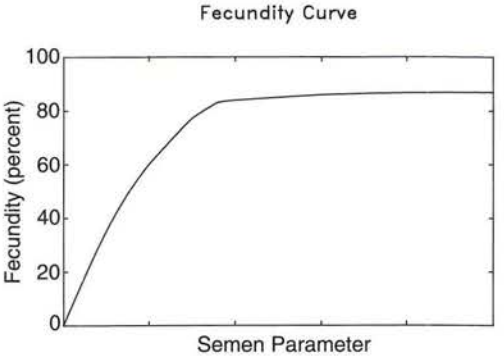

Figure 1.5. This fecundity curve illustrates the relationship between changes in a sperm parameter such as sperm count and male fecundity (a measure of the ability of the male to fertilize a female). This model suggests that decreased male fecundity may be a nonthreshold phenomenon, i.e., as the sperm parameter decreases, the chance of successful conception decreases.

Various semen parameters measure different aspects of the reproductive potential. No single variable is a measure of fecundity. Thus, a semen sample with 100 million sperm with 10% motility is probably no better than a semen sample with 10 million sperm with 100% motility.

PROMISING NEW METHODS

Promising new methods are being evaluated for assessing the effects of toxicants on the male reproductive system. The sperm penetration assay (SPA), also known as the zona free hamster egg penetration assay, provides information on sperm function. To perform this assay, sperm are capacitated and then incubated with hamster eggs with the zona pellucida removed. The frequency of sperm penetration is then calculated. This procedure has been used in the clinical setting and is quite routine in several laboratories (52).

The DNA stability assay may provide information regarding genetic damage to sperm. To perform the assay, the sperm DNA is stressed thermally or chemically and then stained with acridine orange. Double-stranded DNA stains green, while single-stranded DNA stains red. Animals exposed to known mutagens have an increase in single-stranded DNA (53, 54), indicating an increase in genetic damage. The fertility rate of bulls is correlated with the percentage of double-stranded DNA (55). A recent report indicates that the DNA stability assay is highly repeatable between ejaculates from the same man (56). At present, this procedure has been developed in only a few laboratories. Methods to evaluate DNA adducts and DNA probes are on the horizon. DNA adducts, the complex formed between a toxicant or its metabolites and the sperm nucleotide, may be carried in the sperm chromatin to the site of fertilization. New methods for labeling nucleotides may permit the detection of these adducts in ejaculated sperm. DNA probes are monoclonal antibodies to distinct modifications of the DNA molecule (57) and may allow for the detection of subtle changes in the DNA code.

Toxicant Effects

Toxicants can disrupt spermatogenesis at several points. The stage(s) of spermatogenesis targeted by a toxicant determines both the time required for clinical expression of the injury and the prospects for recovery. Due to irreversibility, the most damaging toxicants are those that kill or genetically alter (beyond repair mechanisms) Type A spermatogonia or Sertoli cells.

Animal studies have been useful to determine the stage at which a toxicant attacks the spermatogenic process. Sampling occurs after short-term exposure to a toxicant. By knowing the duration required for each spermatogenic stage, one can extrapolate to estimate the affected stage. For example, exposure of male rats to 2-methoxyethanol resulted in reduced fertility after 4 weeks (58). This evidence, corroborated by histological examination, indicates that the target of toxicity is the spermatocyte (59). Semen analyses of serial ejaculates of men inadvertently exposed to potential toxicants for a short time may provide similar useful information.

Exposure to DBCP reduced sperm concentration in human ejaculates from a median of 79 million cells/ml in unexposed men to 46 million cells/ml in exposed workers (60). Long after removal from exposure, workers with reduced sperm counts experienced partial recovery, while men who had been azoospermic remained sterile (61). Testicular biopsy revealed that the target of DBCP was the spermatogonia, substantiating the severity of the effect seen with stem cell injury.

Genetic damage is difficult to detect in human sperm. Laboratory animal studies using the dominant lethal assay (62) indicate that paternal exposure can produce early pregnancy loss. In humans, an association between paternal occupational exposures and spontaneous abortion has been suggested for agents such as anesthetic gases, organic solvents, and metallurgic factory exposures (63, 64). While human data are still limited, such findings indicate a need for methods to detect genetic damage in human sperm. The issue of paternally mediated developmental effects is further discussed in Chapter 5.

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 (65) and affect the sperm, be absorbed through the vaginal mucosa after intercourse, or be carried to the site of fertilization on the sperm membrane and affect the ova or conceptus.

Ethylene dibromide (EDB) is one example of a toxicant that exerts post-testicular effects. Short-term exposure to the toxicant reduced sperm velocity and semen volume (66). Chronic exposure decreased sperm motility and viability, decreased seminal fructose levels, and increased semen pH (66). An EDB metabolite was present within the semen of some exposed workers (48). Other potential toxicants that have been detected in semen include lead, cadmium, hexachlorobenzene, hexachlorocyclohexane, dieldrin, and PCBs (47).

SEXUAL FUNCTION

Human sexual function refers to the integrated activities of the testes and secondary sex glands,

the endocrine control systems, and the CNS-based behavioral and psychological components of reproduction. A brief overview follows. If more detail is desired, several reviews are available (67–69).

Erection, ejaculation, and orgasm are three distinct, independent physiological and psychodynamic events that normally occur concurrently in men.

Erection

Erection results from the engorgement of blood within the corpora cavernosa of the penis. Parasympathetic stimulation induces dilation of the penile arterioles and closure of the venous valves to cause accumulation of blood within the cavernosa. Two independent mechanisms may induce erection. A sacral reflex arc triggers erections induced by tactile stimulation of the genitalia. Nontactile, erotic stimuli stimulate several regions of the brain including the thalamic nuclei, the rhinencephalon, and the limbic structures. These neural signals are mediated by the autonomic nervous system (69).

Impotence, the failure to achieve and/or maintain an erection, may have either a psychogenic or organic etiology. Organic impotence is characterized by reduced ability to achieve an erection at any time, whether awake or asleep. In most cases, psychologically impotent men can experience spontaneous erections during rapid eye movement (REM) sleep (69). To differentiate between the two etiologies, devices can be worn on the penis to detect changes associated with nocturnal erections including penile circumference, pulsatile blood flow, volume, or axial rigidity (the resistance of the penis to buckling when a known weight is applied to the glans penis).

Priapism is a condition in which an erection occurs without sexual arousal and without resolution after ejaculation. This condition appears to reflect an autonomic nervous system imbalance resulting in inappropriate arteriole dilation without subsequent venous drainage.

Ejaculation and Orgasm

Ejaculation involves emission of seminal components into the urethra and the pulsatile ejection of the semen from the urethra. The sensation that typically accompanies ejaculation is orgasm. Emission of seminal components from the seminal vesicles, prostate gland, and vas deferens into the prostatic urethra is controlled by reflex activity from the thoracolumbar sympathetic neural network. Parasympathetic innervation controls the pulsatile contractions of bulbocavernosus and ischiocavernosus muscles that orchestrate to eject the semen rhythmically from the urethra. Contraction of muscles generates afferent signals that are transmitted to the cerebral cortex, generally eliciting the sensory experience of orgasm (67). Thus, emission of seminal components to the urethra can be blocked with α-adrenoceptive antagonists without blocking the sensations of ejection and orgasm, even though there will be little or no semen ejaculated.

Libido

Loss of interest in sexual activity may also be of either psychogenic or organic origin. Most organic causes are associated with endocrine imbalances of testosterone and/or prolactin.

Assessment Methods

Assessment of occupationally induced sexual dysfunction is difficult, inasmuch as it usually relies on the testimony of the worker. This testimony may be confounded by the bias of the individual to guard his masculine image or to attribute a pre-existing libido problem to exposures at work.

Burris et al. (70) recently reported application of a monitor for assessing erection at home. This method may provide a convenient means by which to evaluate erectile function of exposed workers.

Assessment of ejaculate volume can provide information on the integrity of the emission phase of ejaculation. This measurement also reflects the secretory capacities of the accessory sex glands. Thus, a semen sample of reduced volume, but with a normal ratio of marker chemicals, supports a diagnosis of an emission phase defect.

Toxicant Effects

Few reliable data are available on toxicant-induced effects on sexual function. Drugs have

been shown to affect each of the three stages of male sexual function (71), indicating the potential for occupational exposures to exert similar effects. Antidepressants, testosterone antagonists, and stimulants of prolactin reduce libido in men. Antihypertensive drugs that act on the sympathetic nervous system induce impotence in some men, but paradoxically, priapism in others. Phenoxybenzamine, an α-adrenoceptive antagonist, has been used clinically to block seminal emission, but not orgasm (72). Anticholinergic effects of some antidepressant drugs permit seminal emission, while blocking seminal ejection and orgasm.

Recreational drugs also affect sexual function (71). Ethanol can reduce impotence while enhancing libido. Cocaine, heroin, and high doses of cannabinoids reduce libido. Opiates also delay or impair ejaculation.

SUMMARY

Toxicants can attack the male reproductive system at several sites. As summarized in Table 1.2, an arsenal of laboratory assessment procedures is available for detecting the effects of toxicants at many of these sites. While most of the methodologies were originally developed and validated for the clinical assessment of infertility, they have also proven useful in the evaluation of cohorts of exposed men in population-based studies.

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Table 1.2.
Assessment of Reproductive Function[a]

Assessment Method	Endocrine System	Testes	Post-testicular	Sexual Function
FSH	X			
LH	X			
Testosterone	X			
Prolactin	X			
Sperm count		X		
Sperm morphology and morphometry		X		
Sperm viability: Vital stain and HOS			X	
Sperm motility: % motile and velocity		?	X	
Semen biochemistry			X	
Semen pH			X	
Sperm penetration assay		X	X	
Nocturnal penile measurements				X
Personal history				X

a FSH = follicle-stimulating hormone; LH = luteinizing hormone; HOS = hypo-osmotic swelling assay.

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Occupational and Environmental Reproductive Hazards:

A GUIDE FOR CLINICIANS

Edited by

MAUREEN PAUL, M.D., M.P.H.

Assistant Professor

Departments of Obstetrics and Gynecology and Family and Community Medicine

(Occupational Health Program)

University of Massachusetts Medical Center

Worcester, Massachusetts

Illustrated by

PETER ROSENBLATT, M.D.

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