Deteriorating trends in male reproduction: idiopathic or environmental?

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Abstract

Recent reports portend deterioration in male reproductive health in several human populations. Similar trends might exist in domestic animals, but data are not available because of the inherent nature of animal husbandry practices — culling of the reproductively inefficient food- and fiber-producing animals at an early age. Although the causes for this deterioration are unknown, a variety of endocrine-mimicking environmental pollutants have been implicated. Data for relevant laboratory animal models exposed to several classes of suspect chemicals indicate that a variety of chemicals ubiquitously present in the environment can disrupt normal reproductive phenomena in the male at exposure rates encountered in nature. Data are presented for occurrence of cryptorchidism, carcinoma in situ of the testis, acrosomal malformations, and impaired sexual function following in utero and/or postnatal exposures to pesticides (e.g., DDT and vinclozolin), high-volume industrial chemicals (e.g., alkylphenols and phthalates), and commonly occurring organic and inorganic chemical contaminants in drinking water (e.g., chemical mixtures and water disinfection byproducts). These observations are discussed in the context of similar, so-called idiopathic conditions encountered in stallions. © 2000 Elsevier Science B.V. All rights reserved.

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1. Introduction

Recent studies of human populations in several countries (Carlsen et al., 1992, Auger et al., 1995) showed that the incidence of abnormalities of male genitalia is on the rise...
and that the quality of semen has declined over the past 50 years. Although other reports (Fisch et al., 1996; Paulsen et al., 1996) revealed no downward trend in semen quality, the subjects involved in these studies represented a limited geographic area. Another meta-analysis of data involving bulls, boars, and rams also did not find any decline in sperm counts over the last 60 years (Setchell, 1997). Whether test populations of these farm animal species already had been selected for their reproductive efficiency, or if there was any decline in the quality of sperm is not known. Although data for many animal species might be compiled, this would likely have limited value because of non-standard conditions of evaluation, particularly collection of semen in a manner precluding estimation of daily sperm production.

The incidence of testicular cancer, the most frequent malignancy in young men, also has increased three- to four-fold during the past 50 years in several human populations, particularly in the Western world (Adami et al., 1994; Bosl and Motzer, 1997). Likewise, epidemiologic data (Laumann et al., 1999), together with increased demand for clinical services to correct erectile dysfunction, indicate that sexual dysfunction in human populations is more prevalent than previously thought. Similar trends might also exist in domestic animals, but data are not available largely because of the inherent nature of animal husbandry practices — culling of the reproductively inefficient food- and fiber-producing animals at an early age. The implications of these data for human populations have caused great concern. The relatively short time period during which these changes have occurred in human populations has been inferred as evidence that the cause is environmental rather than genetic.

Many chemicals in the environment, including some pharmaceutical compounds, are capable of mimicking the inherent actions of reproductive hormones and, hence, have the ability to disrupt the neuroendocrine system or function of the gonads directly. Estrogens or estrogen-like chemicals have been implicated as the causative agents for cryptorchidism, testicular cancer, or other testicular abnormalities and declining sperm counts (Sharpe and Skakkebaek, 1993; Toppari et al., 1996). There is convincing evidence that a variety of compounds with endocrine-mimicking characteristics — pesticides, alkylphenols, phthalates, disinfection byproducts of water purification, common chemical contaminants, phytoestrogens and estrogenic mycotoxins — are found in packaged foods, drinking water, lakes, and oceans (Colborn et al., 1993; Jobling et al., 1995). Surprisingly, in spite of an ominous potential for catastrophic health hazards, there is little information on long-term consequences (in adult life) of exposure to endocrine-disrupting chemicals in utero or during infancy. In most cases of reproductive dysfunction, because the cause and effect are separated by a long interval, it is impossible to ascribe a current clinical condition to an etiologic agent or event that impacted many years earlier and thus the existing condition commonly would be labeled as idiopathic.

Our laboratory is studying whether some common perturbations of the male reproductive system or sperm are truly idiopathic or sequelae to developmental exposure to common environmental pollutants. Some of these experimental paradigms in laboratory animals vis-à-vis clinical cases of stallions manifesting perturbed reproductive phenomena — occurrence of testicular carcinoma in situ, unique defects in morphogenesis of sperm such as acrosomal dysgenesis, and sexual dysfunction — are discussed herein.
2. Carcinoma in situ of the testis

Skakkebaek (1972) first coined the term “carcinoma in situ” (CIS) of the testis to describe atypical intratubular germ cells in humans that were shown to later result in testicular germ cell tumors. Since germ cell tumors are not true carcinomas, a variety of names including testicular intraepithelial neoplasia and testicular intratubular germ cell neoplasia also have been suggested. It is widely accepted that CIS cells are present in a testis many years before clinical manifestation of a tumor. Although it is not definitively known which differentiating germ cell type undergoes this neoplastic transformation, there is evidence that the CIS cell is a gonocyte with stem cell potential (Skakkebaek, 1998). Whether all gonocytes are fully differentiated into spermatogonia neonatally, or if a few remain undifferentiated in the adult is uncertain. While the cause and pathogenesis of such transformation into CIS are not known, occurrence of CIS cells (Bettocchi et al., 1994; Moller and Skakkebaek, 1999) in infertile men indicates that male subfertility and testicular cancer share common etiological factors. Estrogenic xenobiotics that are present as natural constituents of the environment (phytoestrogens and fungal products) or as synthetic byproducts (chlorinated hydrocarbons and alkylphenols) are considered to be the most likely factors involved in these phenomena. However, a major factor limiting verification of this hypothesis or study of pathogenesis of testicular germ cell tumors has been lack of an animal model (Rajpert-De Meyts and Skakkebaek, 1993; Looijenga and Oosterhuis, 1999).

We were the first to document atypical germ cells resembling CIS cells of human testis in an 8-year-old, subfertile, unilaterally cryptorchid stallion (Veeramachaneni and Sawyer, 1998) and a 3-year-old, infertile, rabbit (Veeramachaneni and VandeWoude, 1999). In both cases, these cells were found in association with a developing intratubular seminoma directly linking them to CIS — a first observation for any animal species. The etiological factors in these two spontaneous cases are unknown.

We induced similar lesions in rabbits under controlled conditions by exposing them to octylphenol in utero (Veeramachaneni and Palmer, unpublished) or to \( p,p' \)-DDT/DDE in utero and during infancy (Veeramachaneni et al., 1999) or to zeranol during infancy (Veeramachaneni and Amann, 1994). Octylphenol, an estrogenic chemical, is commonly used in a variety of industrial applications including cosmetics, detergents, lubricants, etc.; \( p,p' \)-DDT, an androgen receptor antagonist with a half-life of \( \sim 50 \) years, still is being used as an insecticide in several countries, although it is banned in Western countries; zearalenones, found in moldy cereal grains, can be food-borne, and their synthetic metabolites, such as zeranol, are commercially used as growth promotants in meat-producing animals. Although the incidence varied, developmental exposures to these chemicals resulted in undescended testes, which manifested CIS cell-like lesions (Fig. 1). While CIS-like atypical cells were also found occasionally in scrotal testes (Fig. 2) of animals exposed to these agents, they were predominant in undescended testes. To verify if abdominal location of the testis was a major factor in the development of CIS, we surgically induced cryptorchidism in rabbit pups (not exposed to chemicals) before the testes transcended the inguinal canal (VandeWoude et al., 1999) and evaluated histopathological features of testes at 6 months of age (post-puberty). The germ cells that were present in surgically induced cryptorchid
animals were either unaffected gonocytes (Fig. 3) or spermatogonia (Fig. 4) similar to those normally present in neonatal or adolescent testes. Germ cell differentiation did not progress beyond the spermatogonial stage, as is common for the cryptorchid condition. No cellular atypia was evident in surgically induced cryptorchidism. This indicates that transformation of stem germ cells (gonocytes or pre-spermatogonia) into CIS cells in chemically induced cryptorchid animals was not due to the intra-abdominal location of the testes per se but only due to actions of the chemicals administered.
In another experiment using rabbits (Higuchi et al., 1999), we found that dibutylphthalate, following in utero exposure during the later half of gestation, caused undescended testes, ambiguous genitalia, hypospadias, regressed prostate, and missing bulbourethral glands. Similar effects were observed in male rats following gestational and/or lactational exposures to dibutyl- and diethylhexyl phthalates (Mylchreest et al., 1998; Gray et al., 1999). Phthalates are utilized as plasticizers to produce polymeric materials. Environmental contamination and exposure occur when phthalates leach out of plastic products. Due to high-volume production and usage, phthalate esters are distributed globally and are potentially ubiquitous pollutants.

Cryptorchidism occurs in humans and many species of animals including dogs, pigs, and horses. CIS has been associated with the coexistence or a history of testicular maldescent in 20% to 30% of human cases (Berthelsen et al., 1982; Loy and Dieckmann, 1993). We have examined, by light and transmission electron microscopy, undescended testes of 41 stallions aged 1 to 8 years and found atypical germ cells and CIS-like lesions in eight; a 20% incidence (Veeramachaneni, Hendrickson and Sawyer, unpublished). In three of these eight stallions, CIS cells were found coincident with beginning seminomatous lesions!

Migration and descent of the testes, differentiation of germ cells and specialized somatic cells, and initiation and maintenance of spermatogenesis require trophic and regulatory hormones. Any imbalance in the hormonal milieu, caused by endogenous or exogenous factors, will alter these processes. While some exogenous agents cause abnormal transformation of differentiating germ cells in addition to interfering with normal testicular descent, others might only cause cryptorchidism without affecting stem cell differentiation. Could these lesions in stallions be sequelae of exposure to pollutants early in life? Perhaps these animals were unknowingly exposed at critical periods of development to endocrine-mimicking chemicals similar to the ones we tested in rabbits.

Fig. 1. A CIS cell, transformed gonocyte, from the undescended testis of a 6-month-old Dutch-Belted rabbit exposed in utero to octylphenol (150 mg/kg body weight; administered orally on alternate days between gestation days 15 and 30). Note the size, irregular nuclear contours, chromatin clumps, and cytoplasmic inclusions including glycogen granules and lipid droplets characteristic of germ cell neoplasia. Scale bar represents 1 μm (for ease of comparison, all micrographs are photographed and printed at the same magnification).

Fig. 2. An atypical germ cell, probably a spermatogonium, from the scrotal testis of a 6-month-old Dutch-Belted rabbit exposed to octylphenol. Scale bar represents 1 μm (for ease of comparison, all micrographs are photographed and printed at the same magnification).

Fig. 3. A normal gonocyte from the abdominal testis of a 6-month-old Dutch-Belted rabbit in which testicular descent was prevented surgically at 3 weeks of age. Note that gonocytes are not normally found in postnatal scrotal testes, particularly in neopubertal animals. Compare this cell with that in Fig. 1. Scale bar represents 1 μm (for ease of comparison, all micrographs are photographed and printed at the same magnification).

Fig. 4. An unaffected spermatogonium from the abdominal testis of a surgically induced cryptorchid 6-month-old Dutch-Belted rabbit. Compare this with Fig. 2. Scale bar represents 1 μm (for ease of comparison, all micrographs are photographed and printed at the same magnification).
3. Acrosomal dysgenesis

It is well established that the acrosome of a mammalian spermatozoon plays a pivotal role in unassisted fertilization of an oocyte and that any defect in formation or function of the acrosome results in subfertility/sterility. Using light and transmission electron microscopic procedures to evaluate equine spermatozoa as a part of breeding soundness examination (Veeramachaneni and Sawyer, 1996), we consistently have observed unique
acrosomal malformations in subfertile or sterile stallions. During the last 3 years, we 
evaluated semen collected from 55 subfertile stallions: Of these, 52 (95%) had acroso-
mal vesiculation, acrosomal dysplasia resulting in nuclear invasion, acrosomes shared 
together with two or more sperm, and occasionally aplasia or complete lack of an acroso-
me (Veeramachaneni, Moeller, and Sawyer, unpublished). Thirteen of the 52 stallions were 
evaluated multiple times over a period of 6 to 30 months during which time they 
consistently manifested these lesions. Although a few of these stallions were from 
Europe, most originated from various regions of the USA. Because it is impossible to 
make a definitive association with a probable cause based on recent clinical history, the 
etiology behind acrosomal dysgenesis in these otherwise valuable stallions remains 
unknown.

Interestingly, we have also observed acrosomal malformations identical to those 
found in the 52 subfertile stallions in: (1) rabbits exposed in utero to octylphenol (Figs. 
5–8); (2) rabbits exposed in utero and during infancy to a mixture of common chemical 
contaminants in drinking water [viz., arsenic, lead, chromium, benzene, chloroform, 
phenol, and trichloroethylene, which are most frequent contaminants in ground water 
near industrial sites in the USA] (Veeramachaneni et al., 1995); (3) rats exposed 
postnatally to trichloroethylene (Veeramachaneni, unpublished); (4) rats exposed to 
dibromoacetic acid, a water disinfection byproduct (Linder et al., 1997); and (5) rabbits 
exposed in utero and throughout postnatal life to dibromoacetic acid (Veeramachaneni, 
unpublished). Also, exposure to cadmium has been implicated in abnormal acrosomal 
function, i.e., reduced mannose receptor expression and acrosome exocytosis (Benoff et 
al., 1997). While other causes such as genetic factors cannot be ruled out, it is very 
likely that the 52 stallions in question had been exposed to one or more of these 
contaminants present in feed and/or drinking water. Therefore, it appears that exposure

Fig. 5. Two round spermatids in golgi phase sharing a common golgi apparatus. Transmission electron 
micrograph of a section from scrotal testis of a 6-month-old, octylphenol-exposed rabbit depicting acrosomal 
dysgenesis. Scale bar represents 1 μm.

Fig. 6. Two spermatids sharing a developing acrosome. Note the irregular orientation/spread of the acrosomal 
cap (arrows). This results in two disoriented sperm heads conjoined by a common acrosome as seen in Fig. 8. 
Large arrow points to acrosomal granule and arrowheads point to ends of the acrosomal cap. Transmission 
electron micrograph of a section from scrotal testis of a 6-month-old, octylphenol-exposed rabbit depicting 
acrosomal dysgenesis. Scale bar represents 1 μm.

Fig. 7. Condensing or elongating spermatids. As the spermatids sharing a common acrosome (arrow) 
condense, the acrosomal matrix surrounds both nuclei resulting in the development of two spermatozoa as a 
single unit. In this process, the acrosome sometimes invades nucleus resulting in nuclear as well as acrosomal 
malformations as seen in Fig. 8. Transmission electron micrograph of a section from scrotal testis of a 
6-month-old, octylphenol-exposed rabbit depicting acrosomal dysgenesis. Scale bar represents 1 μm.

Fig. 8. Acrosomal malformations: acrosomal vesiculation (arrow head), acrosomal dysplasia resulting in 
invasion of the nucleus (arrow), and shared, dysplastic acrosome between two elongated spermatids (open 
arrows). Transmission electron micrograph of a section from scrotal testis of a 6-month-old, octylphenol-ex-
posed rabbit depicting acrosomal dysgenesis. Scale bar represents 1 μm.
to one or more of these insidious pollutants during critical periods of development can cause lasting and irreversible effects on spermatogenesis.

4. Sexual dysfunction

Inability to copulate is a major cause of functional sterility and a major reason for culling domestic animals; it had been documented by the 1930s (Lagerlof, 1936). It is very difficult to pinpoint the causes for impaired sexual function/capacity since they can vary widely from physical to psychogenic factors. However, a few examples of how environmental pollutants affect sexual interest and capacity will be presented to illustrate how common pollutants, even at low, environmentally relevant concentrations, can alter these processes. These observations were recorded in an as-observed manner rather than during ethological studies.

Male rabbits exposed in utero and/or during infancy to a mixture of common chemical contaminants in drinking water (same mixture as described above), or to disinfection byproducts of water purification (dibromoacetic acid) or to vinclozolin (a fungicide widely used in European agriculture and horticulture) were studied (Veeramachaneni et al., 1995; Veeramachaneni, unpublished). Several treated animals, but no control animal, failed to show any sexual interest in the female or failed to ejaculate. These observations might indicate that developmental exposure to certain chemicals can permanently alter sexual behavior/capacity in adult life.

5. Conclusion

Experimental evidence strongly indicates that many chemical pollutants in the environment can permanently alter reproductive function in male laboratory animals at doses likely encountered by domestic animals. Use of a relevant laboratory animal model, such as the rabbit, that facilitates longitudinal seminal evaluation and monitoring of the sexual capacity is imperative to delineate cause and effect relationships in many so-called idiopathic clinical conditions in males. The causative factors for the conditions such as cryptorchidism, testicular carcinoma in situ, and poor seminal quality described here for stallions are unknown and the evidence to date is limited to a few unrelated studies with rabbits and rats. Nevertheless, based on the morphological findings presented herein, the effects of some environmental pollutants appear to have a common thread across several animal species including humans. Given the impact of this global phenomenon, more studies focusing on the areas of mechanisms of action of endocrine-mimicking chemicals, pathogenesis of reproductive anomalies resulting from exposure to these chemicals, and environmental remediation are obligatory.

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References


