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Male Reproductive Tract Sensitivity to Ethanol: A Critical Overview

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ANDERSON, R. A., JR., B. R. WILLIS, C. OSWALD AND L. J. D. ZANEVELD. *Male reproductive tract sensitivity to ethanol: A critical overview.* PHARMACOL BIOCHEM BEHAV **18:** Suppl. 1, 305–310, 1983.—While endocrinological effects of acute and chronic ethanol ingestion have been extensively reviewed, a survey of ethanol induced functional and physical perturbations of the male reproductive tract remains lacking. A brief overview of recent literature concerned with ethanol sensitivity of various components of the reproductive tract is presented. Clinical findings are reviewed as they relate to the possible pathogenesis of alcohol-related testicular atrophy. Currently available animal models for the study of ethanol-induced male reproductive failure are discussed. Attempts have been made to separate the contribution of ethanol per se from secondary factors, such as hepatic dysfunction and nutritional deficiency, to manifestations of male infertility. Studies directed toward elucidating the mechanism(s) by which ethanol exerts its inhibitory effect on testicular steroidogenesis are discussed. Finally, evidence suggesting an effect of ethanol on the functional integrity of other components of the reproductive tract is reviewed. It is concluded that ethanol is a male reproductive tract toxin. Future clinical studies of alcoholics afflicted with testicular atrophy, but having normal liver histology, will be of great value in efforts to identify the mechanisms by which chronic ethanol ingestion results in reproductive inpairment. Similar benefits will be realized in laboratory experimentation, in which models are employed that describe ethanol-induced infertility, while minimizing nutritional factors and hepatic involvement, and control for the reproductive maturity of the organism.

Ethanol Male fertility Testicular function Reproductive tract Testosterone Animal models

THE association of chronic alcoholism with male reproductive failure has been recognized for many years (for reviews, see [1, 4, 21, 40, 44]). Loss of libido, testicular atrophy and impotence occur in as many as 70–80% of chronic alcoholics [35]. However, the factors underlying the etiology of these alcohol-related afflictions remain, for the most part, undefined. For many years, it was believed that impotence, sterility and feminization were secondary manifestations of alcoholic liver disease, as a result of altered hepatic steroid metabolism. Additionally, studies conducted over the last several years have documented the profound effects of both acute and chronic ethanol ingestion on pituitary and gonadal hormone homeostasis, providing evidence of the possible endocrinological basis of ethanol-induced reproductive dysfunction.

On the other hand, more recent evidence indicates that male reproductive failure observed in chronic alcoholics seems to be independent of the extent of hepatic histopathology [8, 19, 29, 30]. Similarly, the role of certain ethanol-induced hormonal alterations, particularly that of depressed testosterone, in the pathogensis of alcohol-related infertility and hypogonadism, has recently been questioned [1,19]. While it would not be surprising that ethanol-induced hormonal imbalances would exacerbate pathological changes in the reproductive tract of chronic alcoholics, it is not known whether such imbalances play a causative role. The mechanism(s) by which ethanol exerts its effects as a reproductive toxin might be better understood if the actions of ethanol on various components of the male reproductive tract were delineated. The following overview summarizes some of the more recent experimental data regarding male reproductive tract sensitivity to both acute and chronic ethanol exposure.

EFFECT OF ALCOHOLISM ON REPRODUCTIVE FUNCTION: CLINICAL STUDIES

Several clinical studies have recently been conducted with chronic male alcoholics having varying degrees of reproductive failure. Basal hormone levels, as well as hor-

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monal responses to various stimuli, have been measured in an attempt to elucidate underlying mechanisms by which ethanol exerts its adverse effects on reproductive function. These studies have made a valuable contribution to our understanding of the endocrinological changes associated with chronic alcoholism. However, the heterogeneity of the patient population in such studies has impaired identification of hormonal perturbations responsible for alcohol-related reproductive deficiencies.

A high incidence of reproductive failure and endocrine imbalance in patients suffering from various degrees of alcohol-related liver disease has been documented [38,39]. Although there is no clear relation between testicular atrophy and duration of alcoholism (ranging from 5–44 years), this affliction is observed more frequently in patients with more severe liver damage. However, alcohol-related reproductive dysfunction also occurs in patients having no or minimal liver damage [29]. These findings indicate that both hepatic dysfunction, as well as the hypothalamic-pituitary-gonadal effects of ethanol, contribute to reproductive failure (thus, the so-called "triple effect" of ethanol [21]). The contribution of ethanol per se to changes in reproductive function is therefore difficult, if not impossible, to evaluate in a patient population afflicted with alcoholic liver disease.

Most hypogonadal alcoholics have circulating testosterone and estradiol levels within the normal range. Luteinizing hormone and follicle stimulating hormone (gonadotropin) levels are frequently higher than normal. However, no relation exists between elevated gonadotropin levels and testicular atrophy. Below-normal testosterone responses to human chorionic gonadotropin (hCG) stimulation are often observed, but these are not well-correlated with the incidence of testicular atrophy.

Alcoholics with gynecomastia have significantly higher circulating levels of estrone and prolactin than alcoholics without gynecomastia [38]. These findings may have significance with respect to the etiology of gynecomastia and other signs of feminization seen in chronic male alcoholics.

Additional clinical evidence of hypothalamic-pituitary-gonadal hormonal imbalance in alcoholics was provided by the observation of an exaggerated prolactin response to administered thyrotropin releasing hormone (TRH) [43]. This exaggerated response is indicative of primary gonadal failure [33]. On the other hand, alcoholics with both cirrhosis and gynecomastia had reduced prolactin responses to TRH stimulation, suggesting pituitary impairment [33]. Increased basal levels of prolactin in this group of patients supported earlier studies [38] which suggested a relation between elevated prolactin and gynecomastia.

Similar clinical findings have been reported by Lindholm *et al.* [29,30], insofar as no relation was apparent between testicular impairment and plasma testosterone levels in alcoholics. Additionally, patients showing signs of impaired spermatogenesis tended to be cirrhotic, although there were several exceptions. As expected, most of the alcoholics with severe testicular pathology exhibited an exaggerated prolactin response to TRH stimulation. In contrast to the studies of Van Thiel *et al.* [42], however, elevated gonadotropin levels were generally associated with impaired spermatogenesis. Hormonal imbalance was not associated with the severity of liver pathology.

Clinical studies have provided convincing evidence that male alcoholics often experience various manifestations of reproductive failure and hormonal imbalance. However, it is very difficult to evaluate possible cause-effect relations among these variables, due to heterogeneity of the patient populations with regard to drinking history, age and alcoholic liver disease. These shortcomings can be at least partially overcome through experimentation with laboratory animals under more controlled conditions.

ANIMAL MODELS FOR ETHANOL-INDUCED REPRODUCTIVE DYSFUNCTION

Relatively few investigations have been conducted concerning the development of an animal model in which reproductive tract dysfunction occurs subsequent to chronic ethanol ingestion. This is somewhat surprising, in view of the numerous studies concerned with endocrinological effects of chronic ethanol ingestion. However, all the four animal models utilized most frequently describe various pathological alterations of the reproductive tract subsequent to chronic ethanol administration, including testicular atrophy, reduced seminiferous tubule diameter, impaired spermatogenesis and histopathological alterations of the seminal vesicles/prostate.

Important differences exist concerning the exact protocol of ethanol administration. For example, a relatively early model [37] utilized liquid diets in which ethanol comprised 36% of the total calories. Diets were administered to sexually immature (age 20 days) male rats for a period of 41 days. Although control animals were given diets containing an isocaloric amount of sucrose, the alcohol-fed group gained significantly less weight than the controls. Livers from alcohol-treated males showed fatty metamorphosis, and significantly higher liver/body weight ratios were observed in ethanol-treated, as compared to control animals. Additionally, a trend toward abnormal liver function, as measured biochemically, was observed. While this model did clearly demonstrate reproductive tract pathology subsequent to ethanol treatment, it is not clear whether reproductive impairment was due to ethanol per se, or to ethanol plus other confounding variables related to nutritional status or hepatic function. It is also difficult to conclude to what extent the observed pathological findings were due to inhibition of sexual maturation, impairment of sexually mature reproductive tract function, or both, since alcohol was administered during the period of pubertal development.

An improved model was subsequently developed [36] in which the same liquid diet was administered to sexually mature rats for a period of 164 days. Similar pathological alterations of the reproductive tract were observed as in the previous model. However, again, body weights of ethanol-treated animals were significantly less than those of controls. For this reason, a group of chow-restricted animals were included, whose body weights were kept equal to those of the ethanol-fed animals. Some degree of hepatic impairment was noted in ethanol-treated animals, as measured by morphological and biochemical analyses.

This model successfully described reproductive impairment subsequent to chronic ethanol ingestion by adult males. Hepatic dysfunction was minimal, and the problem of decreased weight gain in the ethanol-fed animals was considered by inclusion of a weight-reduced control group. Unfortunately, subsequent studies concerned with the effects of ethanol on reproductive function have utilized the earlier model with sexually immature males [10, 17, 23].

An animal model has also been described by Klassen and Persaud [26,27]. Some improvements were included, such as the assessment of male fertility prior to initiation of a four-

week ethanol treatment of sexually mature rats. However, this model lacked proper nutritional controls, and the ethanol (58% of the total calories) in the diets was too high to maintain the good health of the animals. Although reproductive impairment was evident, ethanol-fed animals lost 42% of their initial body weight. Other signs of nutritional deficiency included dull ruffled hair, small pale eyes and hypoglycemia. Liver and kidney pathology was also apparent. It was difficult to determine to what extent reproductive impairment was secondary to nutritional deficiency.

An experimental design utilizing sexually mature mice of proven fertility has recently been described [6]. Various histopathological alterations of the testes, in addition to reduced plasma testosterone, in the absence of significant hepatic damage, were noted following 35 days of exposure to a liquid diet in which ethanol comprised 28% of the caloric content. This treatment resulted in no loss in body weight. However, the model failed to describe ethanol-induced infertility, as measured by the ability of epididymal spermatozoa to fertilize ova, *in vitro*. Increasing the duration of ethanol exposure to either 70 days [3] or 140 days [5], or increasing the ethanol content of the diet (5 week exposure) to 32% of the total calories [45], resulted in a continuum of reproductive tract pathology ranging from mild to severe, the extent being dependent upon dose and duration of ethanol exposure. Male fertility was also impaired, as measured by the decreased ability of sperm to fertilize *in vitro*, and by decreased fertile matings *in vivo* [2]. The mouse may therefore serve as an effective animal model with which the effects of ethanol exposure on reproductive function may be examined, with minimal effects due to nutritional deficiency or hepatic damage.

ACUTE AND CHRONIC EFFECTS OF ETHANOL ON TESTICULAR FUNCTION

Perhaps the most direct evidence supporting the action of ethanol as a gonadal toxin has been the demonstration of an inhibitory effect of ethanol on steroidogenesis by testicular preparations *in vitro*. Inhibition of testicular testosterone production by ethanol has been observed in the perfused testis [14], Leydig cell preparations [11,18], broken cell homogenates [18] and microsomal preparations [25]. Since acetaldehyde is several times more effective than ethanol as an inhibitor of testosterone synthesis [11, 12, 18], it has been suggested that the inhibitory effect of ethanol is mediated by its oxidation to acetaldehyde by testicular alcohol dehydrogenase. However, this putative mechanism is not consistent with the observed dose-dependent inhibition of testosterone production at ethanol concentrations ranging from 50–700 mg% [12, 14, 18]. Assuming that the kinetic properties of testicular ADH are similar to those of the hepatic enzyme [31], these ethanol concentrations would result in maximal ethanol oxidation. Therefore, acetaldehyde formation would be constant over this range of ethanol concentrations, and no dose-response relationship would be expected. On the other hand, evidence is available which suggests that a testicular ADH with a much higher K_m for ethanol than the prominent hepatic enzyme may exist [12]. Whether this enzyme is similar to the high-K_m hepatic π-ADH [28], remains to be determined.

In an attempt to identify the mechanism by which ethanol inhibits testosterone production, the effects of ethanol upon various steroidogenic reactions have been measured. Johnston *et al.* [25] determined that ethanol inhibited 17α-hydroxyprogesterone aldolase ($C_{17,20}$-lyase) activity in testicular microsomal preparations. No effect of ethanol was observed upon either 3β-hydroxysteroid dehydrogenase/$\Delta^{5,4}$ isomerase or 17β-hydroxysteroid dehydrogenase activities. No consistent effect of acetaldehyde was observed on any of the enzymic activities. This finding suggests that ethanol and acetaldehyde may inhibit steroidogenesis by independent mechanisms.

Cicero and Bell [11] determined that both ethanol and acetaldehyde inhibit 17β-hydroxysteroid dehydrogenase activity in Leydig cells preparations, based upon an accumulation of labeled androstenedione from labeled pregnenolone. Recovery of labeled products suggested that 17β-hydroxysteroid dehydrogenase was the rate-limiting step in testosterone biosynthesis via the Δ^4 pathway. This could, at least in part, explain their failure to detect ethanol-induced inhibition of a non-rate-limiting step ($C_{17,20}$-lyase) [25].

Since Johnston *et al.* [25] measured enzyme activities in the presence of saturating concentrations of substrates and cofactors, enzyme inhibition other than noncompetitive or irreversible inhibition, would go undetected. This may explain their failure to detect inhibition of 17β-hydroxysteroid dehydrogenase activity. Alternatively, enzyme activities in Leydig cells and microsomal preparations could display differential sensitivities to ethanol.

Ellingboe and Varanelli [18] noted that NAD^+ added to broken cell preparations exerted a protective effect on ethanol-induced inhibition of testosterone production. These data suggest that steroidogenic enzymes which utilize oxidized pyridine cofactor (i.e., 3β-hydroxysteroid dehydrogenase) are inhibited by ethanol when cofactor concentration is less than saturating. Inhibition of 3β-hydroxysteroid dehydrogenase would go undetected in assays employing saturating concentrations of NAD^+, or in studies which only examine the products of the Δ^4 pathway [11], which are formed subsequent to the action of 3β-hydroxysteroid dehydrogenase.

It seems that ethanol and/or acetaldehyde exert an inhibitory effect upon at least three enzyme activities involved in testicular steroidogenesis: 3β-hydroxysteroid dehydrogenase, $C_{17,20}$-lyase and 17β-hydroxysteroid dehydrogenase. Interestingly, Cicero *et al.* [12] noted that ethanol- and acetaldehyde-induced inhibition of testosterone production was dependent upon addition of gonadotropin (i.e., basal testosterone production was not affected). The extent of inhibition seems to be dependent upon the extent to which steroidogenesis is stimulated by gonadotropins. This observation may at least partially explain the rather poor correlation between blood ethanol levels and plasma testosterone concentrations observed *in vivo* [6]. It would be of interest to measure the effect of ethanol on the conversion of cholesterol to pregnenolone, since this transformation is extremely sensitive to stimulation by luteinizing hormone.

Testicular steroidogenesis is also altered subsequent to chronic ethanol ingestion. Chronic ethanol exposure results in decreased activity of 3β-hydroxysteroid dehydrogenase and increased 17α-hydroxylase activity [10,23]. However, it is not clear whether the animals used in these studies had undergone pubertal changes prior to ethanol administration. Rats weighing approximately 150 grams were used; this body weight corresponds to approximately 40 days of age. Puberty occurs in the male rat at 40–60 days. Meaningful interpretation of such data would be dependent upon the use of sexually mature animals, since testicular steroidogenic enzyme levels differ between prepubertal and sexually mature rats

[32]. If these effects are also seen in sexually mature males, one would expect an accumulation of testicular dehydroepiandrosterone.

The physiological significance of the observed changes in enzyme activities after chronic ethanol treatment is not entirely clear. If testosterone synthesis occurs primarily via 17α-hydroxyprogesterone, as suggested by Cicero and Bell [11], 3β-hydroxysteroid dehydrogenase activity is most probably not rate-limiting. Moderate decreases in this activity may not result in decreased testosterone synthesis. Increased 17α-hydroxylase activity could possibly provide more substrate to the rate-limiting step via the Δ^4 pathway (conversion of androstenedione to testosterone) [11] and actually increase the rate of testosterone synthesis. On the other hand, if 3β-hydroxysteroid dehydrogenase is rate-limiting [10], testosterone production would be decreased, and the Δ^5 pathway would become more prominent. Interaction between these two pathways may offer at least a partial explanation of the frequent observation of normal testosterone levels in alcoholics.

OTHER TESTICULAR EFFECTS OF ETHANOL

Alcohol-induced inhibition of testicular steroidogenesis strongly implicates ethanol as a Leydig-cell toxin. On the other hand, most examinations of Leydig cells from either chronic alcoholics, or animals chronically exposed to ethanol, reveal no histological abnormalities, at least at the light microscopic level [37,41]. Both increased [8,36] and decreased [27] numbers of Leydig cells have been reported subsequent to chronic ethanol ingestion. Van Thiel, *et al.* [36] suggested that the apparent increase may be due to decreased testicular volume. The decreased number of Leydig cells [27] may have been secondary to general malnutrition; ethanol-treated animals in this study lost greater than 40% of their initial body weights. Recent electron microscopic observations have indicated a decrease in the smooth endoplasmic reticulum (ER) in Leydig cells from rats chronically treated with ethanol [20]. Reduction of the ER would be expected to decrease Leydig cell steroidogenic activity; a strong positive correlation exists between smooth ER volume and LH-stimulated testosterone release in several species, including the rat [47].

Chronic ethanol treatment also decreases the number of testicular gonadotropin receptors. This may be due to decreased receptor biosynthesis [7]. Alternatively, the observed decrease in receptor content could be due to increased degradation of previously unexposed receptor sites. For example, exposure of luteal membrane preparations to ethanol *in vitro* increases the apparent number of gonadotropin receptors [9]. Evaluation of these possibilities is dependent upon further experimentation.

Since the Sertoli cells are thought to have an important supportive role in maintenance of the germinal epithelium, ethanol-induced impairment of spermatogenesis suggests that Sertoli cells may be sensitive to the toxic effects of alcohol. Indirect evidence for functional impairment of Sertoli cells is provided by observations of elevated FSH levels in chronic alcoholics [42]; pituitary secretion of FSH is under negative feedback control by Sertoli cell secretions (i.e., inhibin). Little, if any, attention has been given to the effects of ethanol on Sertoli cell function. Klassen and Persaud [27] noted no histological change, at the light microscopic level, in rat Sertoli cells after chronic ethanol treatment. It remains to be determined whether ultrastructural changes occur (as apparently is the case for Leydig cells), or whether functional activity of the Sertoli cell changes subsequent to chronic ethanol exposure.

In addition to the direct effect of ethanol on testosterone synthesis, ethanol also exerts a direct inhibitory effect on testicular nucleic acid and nucleoside biosynthesis [24]. These inhibitory effects may be significant as they relate to the effect of ethanol on spermatogenesis.

EFFECT OF ETHANOL ON OTHER COMPONENTS OF THE MALE REPRODUCTIVE TRACT

Relatively few studies have been conducted regarding the effect of ethanol on extra-testicular reproductive tract function. Detailed semen analyses can provide information concerned with the quality and quantity of spermatogenesis, the patency of the reproductive tract and the functional integrity of the accessory sex glands. Surprisingly, however, only one report of semen analysis of chornic alcoholics has appeared [41]. The only parameter reported was sperm count, and ejaculates were obtained from only four patients. A more complete analysis of a larger patient population would provide valuable information concerning the integrity of the male reproductive tract of chronic alcoholics.

Histological observation of the reproductive tracts of laboratory animals chronically treated with ethanol has revealed pathologic changes in the prostate [36], seminal vesicles [27,38], epididymis [27] and epididymal spermatozoa [27,45]. Analysis of semen from mice chronically treated with ethanol suggested impaired seminal vesicle and prostatic function, as measured by decreased fructose and acid phosphatase content [3]. Indirect evidence of epididymal dysfunction has been provided by the observation of increased frequency of caudal epididymal spermatozoa which have retained their cytoplasmic droplets. This indicates impairment of sperm maturation, a process normally occurring in the epididymis [45]. Normal epididymal function is essential for sperm fertility. Inhibitors of epididymal 5α-reductase have been shown to reduce the fertilizing capacity of spermatozoa [13]. Whether chronic ethanol treatment reduces epididymal 5α-reductase activity as it does the activity of the hepatic enzyme [22], remains to be determined.

Ethanol also exerts direct effects on the vas deferens, *in vitro*, by increasing the spontaneous release of norepinephrine [15]. Depletion of noradrenergic stores within the vas deferens may result in accumulation of spermatozoa within the epididymis [46]. This contention is supported by the observation of a significant increase in the epididymal sperm content subsequent to chronic ingestion of relatively low levels of ethanol [6]. The influence of ethanol on the contractility of the seminiferous tubules [34] may also contribute to abnormal distribution of spermatozoa within the male reproductive tract.

Contractility of the vas deferens is thought to be under noradrenergic control. Increased release of norepinephrine in the vas deferens may offer an explanation for the increased frequency of damaged spermatozoa found in ejaculates from non-alcoholic volunteers subsequent to acute ethanol intoxication [16]. This may be caused by increased shear forces produced by the vas deferens during the ejaculatory response. These speculations are, of course, subject to experimental verification.

In summary, studies conducted over the last several years clearly indicate that ethanol is a reproductive toxin. In addition to the adverse effects of ethanol on the hypothalamus

and pituitary, ethanol also exerts direct toxic effects on the male reproductive tract. The etiology of ethanol-induced male infertility, however, remains poorly understood. Although alcohol exerts profound effects on hormonal homeostasis, the poor correlations between reproductive failure and altered levels of steroid hormones and gonadotropins pose serious questions concerning possible cause-effect relationships. The use of animal models to study the effect of chronic ethanol ingestion on male fertility will undoubtedly increase our understanding of underlying factors responsible for reproductive failure. On the other hand, it is extremely important that secondary contributing factors (e.g., liver impairment, nutritional deficiency) be minimized. Studies employing animal models must also demonstrate infertility before mechanisms can be adequately identified. Additionally, concern for the age of the animals must be exercised. Many investigations have been conducted with animals whose sexual maturity was uncertain. Continued studies of the effects of ethanol upon the male reproductive tract under controlled laboratory conditions will be valuable with respect to the eventual treatment of patients afflicted with alcohol-related reproductive impairment.

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