Ethanol-Induced Male Infertility: Impairment of Spermatozoa

ROBERT A. ANDERSON, JR., BRIAN R. WILLIS, CHRISTINE OSWALD and LOURENS J. D. ZANEVELD

Departments of Physiology and Biophysics (R.A.A., C.O., L.J.D.Z.), Anatomy (B.R.W.) and Obstetrics and Gynecology (L.J.D.Z.), University of Illinois at the Medical Center, Chicago, Illinois

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ABSTRACT

Ethanol is generally regarded as a reproductive toxin. However, the mechanism(s) of ethanol-induced infertility remain poorly understood. As male fertility depends upon the ability of spermatozoa to fertilize ova, it was the purpose of the present study to examine the effects of chronic ethanol treatment on several parameters related to sperm fertility. Male C57B/6J mice of proven fertility were administered liquid diets as follows: 5% (v/v) ethanol for either 1) 5 weeks; 2) 10 weeks; 3) 20 weeks; or 4) 6% (v/v) ethanol for 5 weeks. After each treatment, epididymal spermatozoa were evaluated with respect to quantity, motility, morphology and the ability to fertilize. A biphasic effect on sperm content was noted: 5- and 10-week treatments with 5% ethanol increased content by 80 and 65%, respectively, whereas 20-week treatment with 5% ethanol and 5-week treatment with 6% ethanol decreased content by 52 and 71%, respectively. Although the proportion of motile spermatozoa was unaffected by ethanol, average forward progression velocity was reduced, the effect being dependent on ethanol dose and duration of exposure. Similarly, the frequency of abnormal spermatozoa was increased; 20-week treatment with 5% ethanol and 5-week treatment with 6% ethanol increased the frequency of sperm morphological anomalies by 50 and 40%, respectively. Fertility of spermatozoa was reduced as a function of ethanol dose and duration of exposure. The ability of sperm to fertilize mouse ova in vitro was reduced by 34% (P < .02) and 62% (P < .001) subsequent to 20-week treatment with 5% ethanol and 5-week treatment with 6% ethanol, respectively. An animal model has been developed which describes ethanol-induced male infertility. The degree of reproductive impairment varies with the amount of ethanol ingested, and the duration of ethanol exposure. The continuum of effects should make possible the evaluation of putative mechanisms of male sterility resulting from chronic ethanol consumption.

The association of chronic ethanol abuse with male reproductive failure is well recognized (Van Thiel and Lester, 1974; Weathersbee and Lodge, 1978; Gordon et al., 1979; Abel, 1980; Anderson and Willis, 1981; Heine, 1981). However, questions concerning the level and duration of ethanol exposure required to cause infertility, as well as the mechanism(s) by which ethanol exerts its toxic effect on the male reproductive tract, remain unresolved. To date, various pathological changes have been noted in male reproductive function subsequent to chronic ethanol administration to laboratory animals (Klassen and Persaud, 1978; Bhalla et al., 1979; Gordon et al., 1980; Van Thiel et al., 1979), but no indication of the severity of alcohol ingestion required to elicit such changes has been provided. Clinical studies describing varying degrees of male reproductive failure in alcoholics have utilized a rather heterogeneous population of subjects with regard to their drinking history (Van Thiel et al., 1974; Van Thiel and Loriaux, 1979; Majumdar, 1979). A criterion for patient selection has often been the presence of alcohol-induced hepatic damage (Van Thiel et al., 1974, 1978a,b), a pathological manifestation of alcoholism that has recently been shown not to correlate well with ethanol-induced reproductive dysfunction (Lindholm et al., 1978; Boersen et al., 1979; Van Thiel, 1981). Hence, little information is available concerning the extent to which a man must be exposed to ethanol before manifestations of reproductive failure become evident. Similarly, it remains unclear to what extent endocrinological changes, in the presence of alcoholic liver damage, relate to reproductive failure.

Experimentation with laboratory animals provides a more feasible approach to the identification of the level of ethanol exposure and other physiological correlates to chronic ethanol ingestion (e.g., tolerance, physical dependence) which may be associated with reproductive failure. Similarly, animal studies are well suited toward the elucidation of mechanisms by which ethanol perturbs the male reproductive tract. Ideally, results obtained with an appropriate animal model of ethanol-induced male infertility could be utilized not only to identify risk factors associated with alcoholism as it relates to reproductive dysfunction in man, but also toward eventual therapy of men of reproductive age currently suffering from alcohol-related reproductive failure.

Many studies have been performed clinically and with laboratory animals in which the acute and chronic effects of ethanol on hormonal homeostasis have been evaluated. Both acute and chronic ethanol administration alter the circulating levels of luteinizing hormone (Cicero and Badger, 1977; Cicero et al., 1978; Mendelson et al., 1977) and testosterone (Cicero and

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2 Present address: Biology Department, Indiana University, Bloomington, IN 47401.
Badger, 1977; Cicero et al., 1978; Gordon et al., 1976) and increasing circulating prolactin (Van Thiel et al., 1978a; Ylikahri et al., 1978). Additionally, reduced testicular weights (Van Thiel et al., 1975; Van Thiel et al., 1979; Klassen and Persaud, 1978) and altered testicular morphology (Van Thiel et al., 1975; Klassen and Persaud, 1978; Anderson et al., 1980) and steroidogenic activity (Gordon et al., 1980) have been observed in laboratory animals subsequent to chronic ethanol administration. On the other hand, the extent to which these changes contribute toward (or reflect) reproductive failure remains unknown, as fertility subsequent to chronic ethanol treatment has not usually been evaluated. Causative factors of ethanol-induced infertility cannot be empirically identified without first establishing conditions related to ethanol acquisition which result in sterility or subfertility. An animal model which describes ethanol-induced male reproductive dysfunction should therefore incorporate the assessment of fertility as an endpoint of the toxicity of ethanol to the reproductive tract.

As male fertility is largely dependent upon the quantity and quality of spermatozoa which successfully enter the female reproductive tract, the examination of various spermatozoal parameters subsequent to ethanol treatment represents an effective means of fertility evaluation. The objective of the present study was to determine the effects of various chronic ethanol treatment regimens upon spermatozoa within the male reproductive tract, as an assessment of male fertility.

Materials and Methods

Pregnant mare serum (Gestyl) and human chorionic gonadotropin were purchased from Organon, Inc. (West Orange, NJ), and Parke and Company (Detroit, MI), respectively. Lactoid dye was obtained from Matheson Coleman and Bell (Norwood, OH). Hema-tox- ylin was a product of Chroma-Grubler, distributed by Roboz Surgical Instrument Co., Inc. (Washington, DC). Ethanol (95% v/v) was obtained from the supply facility of the University of Illinois Medical Center. Carnation Slender was purchased from a local grocer. Bovine serum albumin (fraction V) was a product of Sigma Chemical Co. (St. Louis, MO). Mucolexx fixative (formaldehyde base) and Flo-Texx liquid coverslip were from Lerner Laboratories (Stanford, CA). All other reagents were of the highest quality commercially available.

Animals. Sexually mature male (C57Bl/6J mice (25 ± 2 g) were obtained from the Jackson Laboratory (Bar Harbor, ME). Animals were individually housed and acclimated to conditions of constant temperature (22 ± 1°C) and lighting (14/10 hr light/dark cycle) before any experimentation. Only proven breeder males (those males which mated at least one litter) were utilized. Fertility was established as described previously (Anderson et al., 1980).

Ethanol treatment. Alcohol was administered to the mice as part of a liquid nutrient diet (chocolate-flavored Carnation Slender) to which was added a vitamin supplement (3 g/l; ICN Pharmaceuticals, Inc., Cleveland, OH). Diet contained either 5% (v/v) or 6% (v/v) ethanol (ethanol-derived calories = 28 and 32% of total caloric content, respectively). The 5% ethanol diet was given to experimental animals for periods of either 5, 10 or 20 weeks. The 6% ethanol diet was administered for 5 weeks. Control animals were pair-fed liquid diets in which the ethanol was replaced by sucrose in amounts isocaloric to the ethanol-derived calories in each diet. Details concerning diet consumption, blood ethanol profiles and body weight gain subsequent to each of the treatment regimens have been previously reported (Willis et al., 1983).

At the end of each treatment period, animals were removed from their respective diets and given free access to laboratory chow and water. Forty-eight hours after removal of the liquid diets, the animals were sacrificed by cervical dislocation, and the epididymides were excised from the reproductive tract. Spermatozoa were obtained by making small cuts into the cauda epididymis and placing the caudae epididymides (two from each animal) into 1 ml of modified Krebs-Ringer-bicarbonate buffer (pH 7.4). After 10 minutes at 37°C, the epididymides were removed, and the resultant sperm suspension was evaluated for sperm content, percent motility, forward progression velocity, morphology and fertilizing capacity.

Assessment of sperm content and motility. The content of epididymal spermatozoa was determined with a Neubauer hemocytometer after dilution with 19 volumes of modified Krebs-Ringer-bicarbonate buffer. Percentage of motile spermatozoa was estimated by direct microscopic examination. Forward progression velocity was measured by determining the time required for 30 randomly selected spermatozoa to migrate from the center to the periphery of the microscopic field (diameter of field = 0.808 mm).

Estimation of fertilizing capacity of spermatozoa. The fertility of spermatozoa was estimated by measuring the extent to which they were capable of fertilizing mouse ova in vitro (Wolfe et al., 1976; Anderson et al., 1980). Spermatozoa from the cauda epididymides were capacitated by incubation in 1 ml of modified Krebs-Ringer-bicarbonate buffer containing 20 mg/ml of bovine serum albumin for 1 hr at 37°C under silicone oil. An aliquot (5 × 10^5 spermatozoa) of each sperm suspension was transferred to culture dishes containing ova from superovulated mice, and 0.1 ml of modified Krebs-Ringer-bicarbonate with 3 mg/ml of bovine serum albumin (culture medium). Sexually mature females (no prior treatment) were superovulated with 10 I.U. of pregnant mare serum (in 0.1 ml of saline i.p.), followed 48 hr later by i.p. injection of 10 I.U. of human chorionic gonadotropin. Superovulated ova were harvested from the ampullae of the ovaries 14 hr after the last injection. Twenty-four hours after insemination (37°C), ova were washed with culture medium, mounted onto microscopic slides, fixed with glutaraldehyde (2.5% w/v), flushed with 95% ethanol and stained with acetolacmoid (0.25% lacmoid dye in 45% acetic acid). Ova were examined for evidence of sperm penetration (i.e., presence of male pronucleus or the second polar body) and embryonic cleavage (two-celled embryo) under phase contrast optics. Fertilization rates were expressed as the percentage of nonfragmented ova which showed signs of fertilization.

Evaluation of sperm morphology. A portion of each epididymal sperm suspension was fixed with Mucolexx and smeared onto microscopic slides. The sperm were stained by a modification of the method of Zaneveld and Polakoski (1977). After air-drying, slides were advanced through Lillie-Mayer hematoxylina, sequentially dehydrated with 50, 70, 80 and 95% ethanol and cleared with xylene. Slides were coated with a liquid coverslip before microscopic examination.

Spermatozoa were examined by bright-field microscopy with an oil immersion lens (×97). Three hundred randomly selected spermatozoa from each sample were evaluated. Anomalies were grouped into five major categories: 1) Head anomalies—includes microcephaly, macrocephaly, denticles, amorphous, rounded (broken neck); 2) tail anomalies—coiled, kinked, duplicate; 3) occurrence of sperm precursors (i.e., spermatids, spermatocytes); 4) immature spermatozoa (i.e., those spermatozoa to which the cytoplasmic droplet remained attached); and 5) decapitated spermatozoa. Results were expressed as the frequency of spermatozoa displaying each of the above anomalies.

Statistical analysis of data. Significance of differences in sperm content between ethanol-treated and control groups was evaluated by Student's t test after first normalizing the data by logarithmic transformation (Sokal and Rohlf, 1981). Values are expressed as the antilogarithm of the average logarithmic transform, with the 90% confidence limits indicated in parentheses. Sperm motility and in vitro fertilization data were subjected to arc-sine transformation before parametric analysis. Values are expressed as the back-transform of the mean of the transformed data, with 90% confidence limits indicated in parentheses. Sperm morphology data were evaluated by χ^2 analysis of the number of abnormalities scored in three hundred spermatozoa which were examined from each of six males per treatment group. Differences in the average forward progression velocity between ethanol-treated and control groups were evaluated with Student’s t test.
without prior transformation of the data. Differences among treatment groups were evaluated by one-way analysis of variance. Differences were considered significant at the .05 level of confidence.

Results

Sperm content and motility. Sperm motility (i.e., forward progression velocity) was impaired subsequent to chronic ethanol treatment when data were compared with those obtained from the pair-fed groups (Table 1). The severity of impairment was a function of both duration of ethanol exposure and ethanol content in the diet. Thus, whereas the least severe treatment regimen (5 weeks of exposure to the 5% ethanol diet) produced no significant change in the average forward velocity of epididymal spermatozoa, 10 weeks of exposure to the same diet resulted in spermatozoa with significantly slower forward progression. A similar decrease in average sperm velocity was observed subsequent to 5 weeks of exposure to the 6% ethanol diet. On the other hand, the fraction of epididymal spermatozoa which was motile was not affected by ethanol treatment.

A biphasic effect was observed with respect to epididymal sperm content subsequent to chronic ethanol treatment (Table 1). The content of spermatozoa within the cauda epididymis was increased by 80% subsequent to 5 weeks of exposure to the 5% ethanol diet. A similar, though somewhat less pronounced, increase (65%) in sperm content was observed in animals maintained on the 5% ethanol diet for a period of 10 weeks. Both of these increases were significant (P < .01, two-tailed t test) when compared with data obtained from the appropriate pair-fed control animals. In contrast, 20 weeks of treatment with the 5% ethanol diet and 5 weeks of treatment with the 6% ethanol diet decreased the sperm content in the cauda epididymis by 52% (P < .01; two-tailed t test) and 71% (P < .001, two-tailed t test), respectively.

Fertilizing capacity of epididymal spermatozoa. Chronic ethanol treatment resulted in diminished capacity of epididymal spermatozoa to fertilize mouse ova in vitro (Table 2). The severity of impairment was directly proportional to both duration of ethanol treatment and ethanol concentration in the diet. Whereas the least severe ethanol treatment regimen had no significant effect upon the fertilizing capacity of epididymal spermatozoa, 10 weeks of exposure and 20 weeks of exposure to the same diet reduced the frequency of fertilization of mouse ova by 23% (P < .05, two-tailed t test) and 34% (P < .02, two-tailed t test), respectively. The fertilizing capacity of spermatozoa from animals treated for 5 weeks with the 6% ethanol diet was reduced by 62% (P < .001, two-tailed t test) when data were compared with those obtained from the appropriate pair-fed control group. No difference was noted among pair-fed control groups with respect to the fertilizing capacity of epididymal spermatozoa (F^2,19 = 1.307; P > .05), whereas a significant difference was observed among ethanol-treated groups (F^2,19 = 18.76; P < .001).

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>% Fertilization</th>
<th>No. of Ova Examined</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experimental: n = 7 (5 weeks, 5% ethanol)</td>
<td>69 (44-90)</td>
<td>190</td>
</tr>
<tr>
<td>Control: n = 8</td>
<td>66 (39-88)</td>
<td>184</td>
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<td>36* (9-68)</td>
<td>201</td>
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<td>192</td>
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<td>Experimental: n = 10 (5 weeks, 6% ethanol)</td>
<td>20* (6-59)</td>
<td>254</td>
</tr>
<tr>
<td>Control: n = 11</td>
<td>53 (25-81)</td>
<td>229</td>
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</table>

* Value differs from that of pair-fed control (P < .05, two-tailed t test).
* Value differs from that of pair-fed control (P < .02, two-tailed t test).
* Value differs from that of pair-fed control (P < .001, two-tailed t test).

Fertilizing capacity of epididymal spermatozoa obtained from ethanol-treated mice

TABLE 2

In vitro fertilization capacity of epididymal spermatozoa obtained from ethanol-treated mice

Forty-eight hours after withdrawing the animals from their respective diets, spermatozoa were obtained from the cauda epididymides. After capacitation, 5 x 10^6 spermatozoa from each animal were inseminated into culture dishes containing superovulated mouse ova. Twenty-four hours thereafter, oocytes were examined for fertilization. Details of the procedure are presented in the text. Data were normalized by arc-sine transformation before parametric statistical analyses. Values represent the backtransform of the mean arc-sine transformation, with the 90% confidence limits indicated in parentheses.

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* Value differs from that of pair-fed control (P < .05, two-tailed t test).
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Fertilizing capacity of epididymal spermatozoa obtained from ethanol-treated mice

TABLE 2

Ethanol-induced sperm abnormalities

TABLE 3

Ethanol-induced spermatozoal dysmorphology

Forty-eight hours after withdrawal from their respective diets, animals were sacrificed, and spermatozoa were obtained from the cauda epididymides. The sperm were fixed, stained and examined for morphological abnormalities, as described in the text. Values are expressed as the frequency (percentage) of 300 spermatozoa from each of six males per treatment group which displayed the indicated abnormality.

<table>
<thead>
<tr>
<th>Group (weeks and % ethanol)</th>
<th>Frequency</th>
</tr>
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<tbody>
<tr>
<td>5 weeks, 5% ethanol</td>
<td>6.0 9.0 15.0% 11.1% 23.0% 15.1% 20.5% 11.6%</td>
</tr>
<tr>
<td>Tail</td>
<td>1.2% 0.5% 0.6% 0.2% 23.7% 4.0% 5.6% 1.7%</td>
</tr>
<tr>
<td>Separated head/tail</td>
<td>4.4 5.5 3.3 3.5 1.9 2.0 7.4% 3.7%</td>
</tr>
<tr>
<td>Cytoplasmic droplet</td>
<td>1.8% 0.5% 0.4% 0.4% 0.6% 0.2% 1.0% 0.2%</td>
</tr>
<tr>
<td>Sperm precursors</td>
<td>2.0 2.1 4.8% 2.2 2.3% 1.4 7.2% 2.8%</td>
</tr>
<tr>
<td>Abnormal spermatozoa (one or more abnormalities)</td>
<td>15.0 17.4 22.0% 16.8 50.2% 20.5% 39.8% 18.2%</td>
</tr>
</tbody>
</table>

* Value differs from that of pair-fed control (P < .05, x^2 analysis).
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Epididymal sperm morphology. A micrograph of normal spermatozoa from a control (10 weeks, 5% ethanol control diet) epididymis is shown in figure 1. The frequency of anomalies of spermatozoa within the cauda epididymis increased with increasing period of ethanol exposure and with increasing diet ethanol content (table 3). Significantly greater (P < .05, \( \chi^2 \)) numbers of spermatozoa with tail anomalies (figs. 2 and 3) and with attached cytoplasmic droplets (P < .001, \( \chi^2 \)) were observed after the least severe treatment regimen (5% ethanol, 5 weeks). However, this treatment did not result in a difference in the overall frequency of spermatozoa displaying one or more anomalies. Ten weeks of exposure to the 5% ethanol diet increased the frequency of head anomalies (figs. 2 and 4) and the relative number of sperm precursors within the cauda epididymis by 35% (P < .05, \( \chi^2 \)) and 118% (P < .001, \( \chi^2 \)), respectively. Significant increases in the frequencies of these anomalies were also observed in epididymal spermatozoa subsequent to 20 weeks of exposure to the 5% ethanol diet and 5 weeks of exposure to the 6% ethanol diet. The number of categories having significantly increased anomalies were as follows: two categories—5 weeks/5% ethanol; three categories—10 weeks/5% ethanol; four categories—20 weeks/5% ethanol; and six categories—5 weeks/6% ethanol. Ten weeks and 20 weeks of exposure to the 5% ethanol diet increased the relative number of spermatozoa displaying one or more anomalies by 31 and 145%, respectively. Whereas 5 weeks of exposure to the 5% ethanol diet produced no significant change in the relative number of dysmorphic spermatozoa, 5 weeks of exposure to the 6% ethanol diet increased the proportion of abnormal spermatozoa by 119%.

Discussion

The data in the present report extend observations made by our laboratory indicating that the C57Bl/6J mouse can be used as an effective animal model for the study of ethanol-induced male reproductive failure (Anderson et al., 1978, 1980; Willis et al., 1983). Examination of various parameters associated with spermatozoa within the reproductive tract was warranted, as the integrity of the male gametes is a major determinant of male fertility. Several changes in epididymal spermatozoa were noted subsequent to various ethanol treatment regimens, and were dependent not only upon the dose of ethanol employed, but also upon the duration of ethanol exposure.

The forward progression velocity of spermatozoa from the cauda epididymis was impaired subsequent to chronic ethanol treatment, although the relative proportion of motile spermatozoa (i.e., those spermatozoa showing any movement) was unaffected (table 1). These data suggest that: 1) measurement of the frequency of spermatozoa showing signs of motility (whether the movement is forward progression or nondirectional) alone is not a sufficiently adequate index of their fertilizing capacity; and 2) the ethanol treatments used in the present study did not sufficiently alter spermatozoa such that they were incapable of movement. Thus, although a significant decrease in the proportion of motile spermatozoa may correlate with decreased fertilizing capacity, sperm motility cannot by itself serve as an accurate indicator of fertility (Mann, 1964).

Chronic ethanol treatment had an apparent biphasic effect on the content of spermatozoa within the cauda epididymis. Less severe treatments (i.e., 5 weeks and 10 weeks of exposure to 5% ethanol) resulted in significantly higher sperm content, whereas more severe treatments (i.e., 20 weeks of exposure to 5% ethanol, 5 weeks of exposure to 6% ethanol) resulted in significant reductions in sperm content. Increased sperm content within a particular locus of the male reproductive tract could be due to two mechanisms: 1) increased spermatogenesis by the testicular compartment; or 2) redistribution of existing spermatozoa within the reproductive tract. The testicular pathology observed subsequent to 5 weeks of exposure to 5% ethanol (Anderson et al., 1980; Willis et al., 1983) would argue against increased sperm production as a viable mechanism for the finding of increased sperm content within the cauda epididymis. On the other hand, Prins and Zaneveld (1979) have shown that the distribution of spermatozoa within the male reproductive tract is dependent upon the contractility of the

![Fig. 1. Normal spermatozoa. Note the typical sickle-shaped head, the orientation of the head with the sperm body and the elongated sperm tail (magnification, x605).](image)
Ethanol-Induced Sperm Abnormalities

Fig. 2. Ethanol-induced sperm dysmorphology. Epididymal spermatozoa were fixed, stained and examined for morphological anomalies, as described in the text. Micrographs show spermatozoa with typically occurring anomalies which resulted from ethanol treatment. This figure shows spermatozoon with head and tail anomalies. The sperm head is rotated (broken neck). The sperm tail is kinked at two locations (magnification, ×605).

Fig. 3. Spermatozoa with head and tail anomalies (magnification, ×472). Spermatozoon with coiled tail (a) and spermatozoon with amorphous heads (b). See text and legend to figure 2 for details.

vas deferens. Contractility of the vas deferens is thought to be under noradrenergic control; sympatholytic agents have been found nearly as effective as vasoligation in producing an increase in spermatozoa within the cauda epididymis (Zankl and Leidl, 1969). Depletion of noradrenergic stores within the vas deferens, either by ethanol or by its immediate oxidation product, acetaldehyde, may have a similar effect. In support of this possibility are the recent data of Degani et al. (1979), which demonstrated an ethanol-induced increase in the spontaneous release of norepinephrine from the vas deferens.

Decreased cauda epididymal sperm content subsequent to more severe treatment regimens, however, most probably reflects diminished spermatogenesis. This conclusion is supported by the findings of significant testicular atrophy, poor quality of spermatogenesis within the seminiferous tubules and an increased frequency of inactive tubules in testes taken from males subsequent to either 20 weeks of treatment with 5% ethanol or 5 weeks of treatment with 6% ethanol (Willis et al., 1983). The apparent biphasic effect of chronic ethanol treatment upon the sperm content of the cauda epididymis is therefore believed to be due to two distinct mechanisms: sperm redistribution within the male reproductive tract and decreased spermatogenesis. The former is the major determinant of sperm density within the cauda epididymis under less severe conditions of ethanol exposure, and the latter predominates under the conditions of ethanol-induced testicular failure. These proposed mechanisms are, of course, speculative in nature, and are subject to empirical verification.
The only direct measurement of male fertility is the assessment of the ability of spermatozoa to fertilize ova, either in vivo or in vitro. In vivo matings for assessment of male fertility are time-consuming, expensive and dependent upon the sexual behavior of both males and females. Additionally, in vivo matings are often unsuccessful, for reasons which may or may not be related to treatment. If, for example, chronic ethanol treatment impaired sexual behavior without affecting the fertilizing capacity of the spermatozoa, fertility could not be accurately assessed, due to diminished copulatory activity of the male. For these reasons, male fertility subsequent to chronic ethanol treatment was measured on the basis of the ability of sperm to fertilize mouse ova in vitro. The frequency at which spermatozoa fertilized mouse ova was significantly reduced by all but the least severe of the ethanol treatment regimens (table 2). The reduction in fertilizing capacity was dependent upon both ethanol dose and duration of exposure. The most severe impairment of fertilizing capacity was observed subsequent to 5 weeks of exposure to the 6% ethanol diet. A similar reduction in fertility in Swiss-Webster mice was noted subsequent to the same treatment regimen, as measured by a decrease in pregnancies resulting from successful in vivo matings (Anderson et al., 1981).

Of the several animal models which have been developed in recent years to study the effect of chronic ethanol on male reproductive function, only that described by Klassen and Persaud (1978) has included the assessment of fertility. In this study, fertility was assessed by in vivo matings. Of 13 females mated with ethanol-treated males, only 6 became pregnant as compared to a pregnancy rate of 100% in control matings. However, it was not clear whether the reduction in pregnancies was due to male infertility or decreased copulatory activity. Moreover, the matings were conducted for 21 of the 35 days during which the males were ingesting ethanol, making it difficult to assess the point at which ethanol exerted its effect on male reproductive performance. To our knowledge, the present study is the first report of an animal model in which chronic ethanol ingestion is associated with male reproductive tract pathology (also, see Willis et al., 1983), together with male infertility. Male infertility as measured by in vivo mating studies (Anderson et al., 1981) is probably a result of not only reduced sperm production subsequent to the more severe ethanol treatment regimens (table 1), but also a result of pathological alteration of the spermatozoa, which render them incapable of fertilizing ova. Chronic ethanol ingestion, therefore, affects both the quantity and quality of spermatozoa within the reproductive tract. The frequency of in vitro fertilization was significantly reduced subsequent to chronic ethanol
treatment, even when equal numbers of spermatozoa were inseminated with ova (table 2).

In spite of the well-known association between chronic ethanol consumption and male infertility, surprisingly few studies have examined the effect of alcohol on the quality of the male gametes. Doepfmer and Hinckers (1965) observed alterations in sperm motility and morphology in nonalcoholic volunteers after acute ingestion of relatively low levels of ethanol. Whether these anomalies were due to a direct effect of spermatozoa or due to an altered ejaculatory reflex was not assessed. A detailed semen analysis of chronic alcoholic patients remains lacking. Only one report by Van Thiel et al. (1974) has attempted to collect semen from chronic alcoholics. The patients selected in that study clearly manifested severe reproductive failure, as 82% of the subjects were unable to produce an ejaculate. No information beyond sperm concentration was given concerning the semen which was obtained. In view of the results in the present study, a detailed semen analysis (i.e., sperm motility, morphology, ejaculate volume, analysis of seminal fluid) of alcoholic patients having less severe signs of reproductive impairment would be highly warranted.

The present data lend further support to the use of the mouse as an animal model with which to study the adverse effects of ethanol on male fertility. The use of several treatment regimens has made it possible to produce a continuum of pathological changes of various parameters associated with the male reproductive tract, ranging from mild to severe. For example, whereas 5 weeks of exposure to a 5% ethanol diet resulted in minimal impairment of male fertility, longer exposure (i.e., 10 weeks) to higher levels (6% ethanol diet) or a shorter exposure (5 weeks) to lower levels (2% ethanol diet) of ethanol.

The mechanism(s) for ethanol-induced reduction in fertility remain unclear. Diminished fertilizing capacity of epididymal spermatozoa from ethanol-treated males could reflect, at least in part, the substantial increase in morphological sperm abnormalities which were observed in these animals. Decreased motility (as measured by forward progression velocity) may also be partially responsible. Other factors which could be involved in the effects of ethanol on fertility include incomplete sperm maturation within the epididymis, or the inability of spermatozoa to undergo physiologically important events (i.e., capacitation, acrosome reaction) which are prerequisites to their ability to fertilize ova (Zanевeld, 1978). Alternatively, abnormal spermogenesis, possibly secondary to ethanol-induced hormonal imbalance, could produce metabolically deficient spermatozoa, or spermatozoa deficient in the acrosomal lytic enzymes (i.e., acrosin, hyaluronidase) which are necessary for sperm penetration of the outer investments of the ovum (Zanевeld, 1975). Further experimentation is required to evaluate these possibilities. It is hoped that the continuum of adverse effects of ethanol on male reproductive tract function which are described in the present animal model will permit an evaluation of these and other putative mechanisms of ethanol-induced male infertility.

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References


Send reprint requests to: Robert A. Anderson, Jr., Department of Physiology and Biophysics, University of Illinois at the Medical Center, P.O. Box 6998, Chicago, IL 60680.