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REVIEW

Does alcohol have any effect on male reproductive function? A review of literature

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Although alcohol is widely used, its impact on the male reproductive function is still controversial. Over the years, many studies have investigated the effects of alcohol consumption on sperm parameters and male infertility. This article reviews the main preclinical and clinical evidences. Studies conducted on the experimental animal have shown that a diet enriched with ethanol causes sperm parameter abnormalities, a number of alterations involving the reproductive tract inhibition, and reduced mouse oocyte *in vitro* fertilization rate. These effects were partly reversible upon discontinuation of alcohol consumption. Most of the studies evaluating the effects of alcohol in men have shown a negative impact on the sperm parameters. This has been reported to be associated with hypotestosteronemia and low–normal or elevated gonadotropin levels suggesting a combined central and testicular detrimental effect of alcohol. Nevertheless, alcohol consumption does not seem to have much effect on fertility either in *in vitro* fertilization programs or population-based studies. Finally, the genetic background and other concomitant, alcohol consumption-related conditions influence the degree of the testicular damage. In conclusion, alcohol consumption is associated with a deterioration of sperm parameters which may be partially reversible upon alcohol consumption discontinuation.
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INTRODUCTION

Experimental and clinical studies suggest that alcohol consumption may alter both testosterone secretion and spermatogenesis. In fact, it is well known that alcohol consumption produces significant spermatozoon morphological changes which include breakage of the sperm head, distention of the midsection, and tail curling.[1] In addition, seminiferous tubules in alcohol users mostly contain degenerated spermatids with a consequent azoospermia.[1] These effects may be due to alteration of the endocrine system controlling the hypothalamic–pituitary–testicular (HPT) axis function and/or to a direct effect on testis and/or male accessory glands.[1–3] In particular, experimental evidence suggests that ethanol is a Leydig cell toxin,[3,4] although dose-dependent effects of alcohol on human spermatogenesis are not well known. A recent case report showed that an azoospermic patient recovered normal sperm parameters 3 months after alcohol consumption discontinuation,[5] which has raised the interest for this topic. The present article briefly reviews the main preclinical and clinical evidences on this topic.

PRECLINICAL EVIDENCE

C57B1 mice have been used to evaluate the effects of ethanol on the testicular function and its reversal following alcohol withdrawal.[2] In this study, the ingestion of 5% or 6% ethanol resulted in significantly decreased testicular (24% and 28%, respectively) and seminal vesicles/prostate (20%, 6% diet only) weights, increased frequencies of germ cell desquamation (480% and 400%, respectively), inactive seminiferous tubules (186% and 567%, respectively), spermatozoa with abnormal morphology (31% and 119%, respectively), and inhibition of mouse oocyte *in vitro* fertilization by epididymal spermatozoa (26% and 62%, respectively), as compared with their respective pair-fed control values. Significant decreases in epididymal sperm content (72%, 6% diet only), total motile spermatozoa (85%, 6% diet only) and seminal vesicles epithelial cell height (13% and 29%, respectively) were also observed. No abnormal sperm parameter was observed in mice fed with the 5% ethanol diet; whereas the 6% diet, significantly decreased sperm coagulum weight (50%), sperm count (85%), and acid phosphatase content (53%). An improvement of all parameters was observed in the contralateral half of the reproductive tract after 10 weeks of abstinence. Only germ cell desquamation remained significantly elevated (100% compared with control) in animals that ingested the 5% alcohol diet. In contrast, significant abnormalities persisting 10 weeks after treatment with the 6% ethanol diet included increased germ cell desquamation (200%), inactive seminiferous tubules (157%) and abnormal sperm morphology (39%), and decreased forward progressive motility (17%) of epididymal spermatozoa. This interesting and comprehensive study[2] showed that an ethanol-containing diet alters testicular function and that this effect is partially reversible upon discontinuation of alcohol consumption.

In another experimental study,[6] male mice were divided into four groups, one group was given *ad libitum* access to a liquid alcohol diet

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containing 35% ethanol-derived calories. A second group was pair fed with an isocaloric control diet containing 17.5% ethanol-derived calories. A third group was similarly treated with a 0% ethanol-derived calories diet for a minimum of 42 days. A fourth group served as *ad libitum* non-treated controls to assess the role of pair feeding. Male rats were then mated with non-treated females.[6] Rats consuming alcohol showed an increased percentage of morphological abnormal spermatozoa. There was a significant effect of paternal alcohol exposure on implantation rate, but no effects on pre- or postnatal mortality or fetal weight were observed.[6]

Dhawan and Sharma[7] reported that ethanol (3 g kg^{-1} orally for 30 days) resulted in a decreased *libido* (evaluated by mating behavior) and decreased sperm number. These detrimental effects on sexual/reproductive function were counteracted by the administration of a tri-substituted benzoflavone moiety isolated from *Passiflora incarnata linneaus*. Another experimental study was carried out to evaluate the effects of the intraperitoneal administration of alcohol (15 ml kg^{-1}, 25%, v/v) and/or acetylsalicylic acid (ASA, 15 mg kg^{-1}) for 10 weeks on sperm parameters and fertility in male Sprague–Dawley rats. ASA was given 1 h before alcohol to determine whether the effects of ethanol can be prevented by pre-treatment with this drug which has been reported to antagonize the rate-depressant effects of ethanol. The results of this study confirmed that alcohol significantly reduced sperm concentration and motility compared with control rats and that pre-treatment with ASA was not able to revert these effects.[8] Talebi and colleagues[9] evaluated the effect of ethanol consumption on sperm parameters and chromatin integrity of spermatozoa aspirated from the epididymal cauda of rats allowed to drink *ad libitum* ethanol compared to control rats. The results showed that progressive and non-progressive motility were significantly lower in ethanol-consuming rats compared with control animals, whereas the percentage of aniline-blue-reacted spermatozoa were similar in both groups. However, the percentages of spermatozoa positive to chromomycin A3, toluidine blue, or acridine orange were significantly higher in ethanol-drinking rats compared with controls.

Although it has been well established that spermatogenic cells undergo apoptosis when treated with ethanol, the mechanisms are not clear. In this regard, adult male mice were given ethanol intraperitoneally at a dose of 3 g (15%, v/v) per kg body weight per day for 14 days and testicular steroidogenesis and germ cell apoptosis were evaluated.[10] Western blotting analysis revealed that repeated ethanol treatment decreased the expression of steroidogenic acute regulatory protein, 3β-hydroxysteroid dehydrogenase (HSD) and 17β-HSD, increased the expression of active caspase-3, p53, Fas and Fas-L and led to upregulation of Bax/Bcl-2 ratio and translocation of cytochrome c from mitochondria to cytosol in testis. Moreover, repeated ethanol treatment led to upregulation of caspase-3, p53, Fas, Fas-L transcripts, increase in caspase-3 and caspase-8 activities, diminution of 3β-HSD, 17β-HSD and GPx activities, decrease in the mitochondrial membrane potential along with ROS generation and depletion of glutathione pool in the testicular tissue.

Finally, in a recent study, Stouder and colleagues[11] evaluated the possible transgenerational effects of alcohol administration in pregnant mice on the methylation pattern of five imprinted genes (*H19*, *Gtl2*, *Peg1*, *Snrpn* and *Peg3*) in somatic and sperm cell DNA of the male offspring. A 3% decrease in the number of methylated CpGs of *H19* in the F1 offspring spermatozoa, a 4% decrease in the number of methylated CpGs of *H19* in the F2 offspring brain, and a 26% decrease in the mean sperm concentration were observed.

CLINICAL EVIDENCE

Alcohol has been shown to have a deleterious effect at all levels of male reproductive system. It interferes with the HPT axis regulation resulting in an impairment of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) secretion.[12–14] Moreover, a progressive testicular damage and the consequent decrease of sex hormones leads to a loss of secondary sexual characteristics and the onset of erectile dysfunction and infertility.[7,15]

Alcohol consumption and sperm parameters

In a historical study performed on 685 men who drank alcohol (beer and wine) systematically in a 30- to 60-min period,[16] a delayed seminal fluid liquefaction was found associated with low sperm motility. The authors speculated that the asthenozoospermia of these patients was secondary to inflammatory or irritative prostatitis due to the excessive alcohol intake. In a study conducted on 3000 men, the proportion of cases with a positive history for urethritis was found to be very higher among those men whose daily alcohol consumption was equivalent to more than a liter of wine.[17] Similarly, a pro-inflammatory state was reported by Close and colleagues[18] in heavy alcohol users who had significantly higher leukocyte concentrations in the seminal fluid compared with non-users. However, after controlling for past sexually transmitted diseases and multiple substance exposures in a multivariate model, alcohol users had only a trend toward increased leukocyte concentrations in the seminal fluid.

A significant seminal fluid volume and sperm concentration decrease has been reported in 20 men with alcohol dependence syndrome.[19] Hormonal serum levels, measured in only five of them, showed low testosterone levels, and normal LH, FSH and prolactin values. Thus, hypotestosteronemia may explain the observed reduction of the seminal plasma volume. In addition, a higher percentage of morphologically abnormal spermatozoa was observed in these men compared with controls, but no correlation has been found with the amount or the duration of alcohol consumption. The lack of a compensatory increase of LH and FSH concentrations suggests that alcohol has an inhibitory effect on the central component of the HPT axis.[20,21] Indeed, alcohol may alter gonadotropin-releasing hormone receptor function at the pituitary levels or the interaction of these receptors with gonadotropin-releasing hormone, resulting in a diminished LH release. In addition, alcohol seems to interfere negatively with the LH biological activity.[22,23] Furthermore, the increased β-endorphin levels observed after acute or chronic alcohol consumption may contribute to testicular damage. Indeed, β-endorphin produced in the hypothalamus results in a suppression of gonadotropin-releasing hormone neuronal function. In addition, β-endorphin produced within the testis suppresses testicular testosterone production and release.[24] Furthermore, opioids may increase programmed cell death (i.e. apoptosis).[25]

On the other hand, sperm parameter abnormalities have been reported to be significantly associated with elevated serum LH, FSH, and 17β-estradiol levels and significantly decreased serum testosterone levels, thus suggesting the presence of a primary testiculopathy in men drinking ethanol.[13] Goverde and colleagues[26] did not find any statistically significant difference for seminal fluid volume (4.1±1.9 ml *vs.* 3.3±1.3 ml), sperm concentration (10.6±7.8 million spermatozoa per ml *vs.* 8.9±5.8 million spermatozoa per ml), and percentage of motile spermatozoa (27.0%±15.1% *vs.* 25.5%±16.1%) in daily drinkers and subfertile patients. Similarly, alcohol users among 34 healthy Argentine medical students had a non-significant reduction in sperm concentration, motility, viability and normal morphology.[27] On the

other hand, a significantly lower percentage of morphologically normal spermatozoa in daily-drinkers (17.6%±7.2%) compared with subfertile patients (23%±6.5%) was reported.[26] Semen volume, sperm count, motility and the percentage of morphologically normal spermatozoa were reported to be significantly decreased in 66 nonsmoking and drug-free alcoholics who consumed a minimum of 180 ml alcohol per day (brandy and whisky, both 40%–50% alcohol content) for a minimum of 5 days per week for ⩾1 year. The morphological abnormality was mainly relative to the sperm head.[28] Gaur and colleagues[29] reported that only 12 out of 100 alcoholics (12%) had normozoospermia compared with 37 out of 100 nonalcoholic control men (37%).

A significant negative association was observed between daily alcohol consumption and polycyclic aromatic hydrocarbon-DNA adducts (early marker of sperm genotoxicity) in spermatozoa.[30] Horak and colleagues[31] analyzed the levels of bulky DNA adducts in spermatozoa of 179 healthy donors or infertile patients by ^{32}P-postlabeling method and did not find any correlation between alcohol and sperm DNA adducts. Finally, Loft and colleagues[32] evaluated the level of oxidative DNA damage in terms of 7-hydro-8-oxo-2′-deoxyguanosine (8-oxodG) in spermatozoon DNA among 225 first-pregnancy planners; 8-oxodG level were not significantly associated with alcohol consumption.

Alcohol consumption and testicular pathology

Some studies have explored the effects of alcohol consumption on human testicular pathology. Kuller and colleagues[2] evaluated testicular and liver pathology and related the findings with the estimated alcohol consumption among men who had died suddenly from a variety of causes. Out of the 137 men studied, 20 (14%) had a moderate-to-severe decrease in spermatogenesis, but only nine of these men had also severe or very severe fatty infiltration of the liver. These findings suggest that testicular spermatogenesis seem to be more sensitive to alcohol than liver tissue. A subsequent prospective autopsy study further explored the relationship between alcohol consumption, spermatogenesis and morphometric analysis of the human testis.[14] The autopsy series comprised non alcohol users (daily intake <10 g) (controls) and heavy-drinkers (daily intake >80 g). Among controls, 81.3% of subjects had normal spermatogenesis, whereas the remaining 18.7% had partial spermatogenic arrest. A significant lower percentage of heavy drinkers (36.4%) had normal spermatogenesis, while 52.3% showed partial or complete spermatogenic arrest. The mean testicular weight of heavy-drinkers was slightly, but significantly lower compared with that of controls. Compared to men with normal spermatogenesis, testicular weight was slightly lower in heavy-drinkers with spermatogenic arrest, and significantly lower in heavy-drinking men with Sertoli-only cell syndrome. Spermatogenic arrest was not correlated with fatty liver or cirrhosis of the liver, whereas four of the five men with Sertoli-only cell syndrome exhibited a fatty liver.

Alcohol consumption and couple's fertility

Several clinical studies have not shown any effect of alcohol consumption on the male reproductive performance. Dunphy and colleagues[33] evaluated the relationship between male alcohol intake and fertility in 258 couples attending an infertility clinic. In this cohort, 21% consumed <1 unit of alcohol per week, 10% consumed 1–5 units per week, 23% consumed 6–10 units per week, 27% consumed 11–20 units per week, and 19% consumed >20 units per week. No association between the amount of alcohol and sperm parameters was observed in this study. In addition, there was no significant difference in the alcohol intake between normal and abnormal female groups. Couples were followed-up for up to 32 months. Sixteen women had a treatment independent conception within the normal female group. Finally, there was no significant association between the amount of alcohol consumed per week and the fertility outcome.

An European multicenter study was conducted on: (1) a population-based (census register and electoral rolls) randomly selected women between 25 and 44 years in the different European countries, and (2) a pregnancy-based study of consecutive pregnant women (at least 20 weeks of pregnancy) recruited during prenatal care encounters.[34] More than 4000 couples were included in each arm of the study, and 10 different regions in Europe took part in the data collection. Data were collected through personal interviews in all population-based samples and in all but four regions of the pregnancy study. Results of this evaluation showed no strong nor coherent association between alcohol intake and subfecundity.

Data from the Ontario Farm Family Health Study were analyzed to determine whether alcohol use among men and women impact upon fecundability.[35] In this retrospective cohort study, farm operators, husbands and wives completed questionnaires during 1991–1992, yielding information on 2607 planned pregnancies that had occurred over the previous 30 years. Fecundability ratios were calculated using an analog of the Cox proportional hazards model. Results of this analysis showed that alcohol use among women and men was not associated with fecundability.

We reported the achievement of a pregnancy by the female partner of an infertile couple 3 months after alcohol consumption withdrawal by the male partner who had azoospermia secondary to heavy alcoholic intake (mean daily alcohol consumption: 90 g).[36] In this study, alcoholism was the putative cause of the infertility of this patient because, during alcohol consumption, he first had teratozoospermia characterized by a high percentage of spermatozoa with large heads, associated with a non-megaloblastic macrocytic anemia in the blood smear, and subsequently azoospermia. A multicenter prospective study evaluated whether the amount and the timing of female and male alcohol use during *in vitro* fertilization program affected the reproductive outcome. The risk of not achieving a live birth increased by 2.28 (1.08–4.80) to 8.32 (1.82–37.97) times, depending on the time period, in men who drank one additional drink per day; drinking beer also affected live births (odds ratio=5.49–45.64).[37]

Alcohol consumption during pregnancy: effects on male offspring sperm parameters

The effects, if any, of maternal alcohol consumption during pregnancy and sperm parameters and reproductive hormone levels were evaluated in the sons of these women. From a cohort of Danish pregnant women established in 1984–1987, 347 young adult sons were selected for a follow-up study conducted in 2005–2006.[38] The results of this study showed that the sperm concentration decreased with increasing prenatal alcohol exposure. The adjusted mean sperm concentration among sons of mothers drinking ⩾4.5 drinks per week during pregnancy was 40 (95% confidence interval (CI): 25–60) million per ml. This concentration was approximately 32% lower compared with the sons of mother exposed to <1 drink per week (sperm concentration of 59 (95% CI: 44–77) million per ml). The semen volume and the total sperm count were also associated with mothers' prenatal alcohol exposure; sons prenatally exposed to 1.0–1.5 drinks per week had the highest values. No associations were found for sperm motility, sperm morphology, or any of the reproductive hormones, including testosterone.

INDIVIDUAL VARIABILITY TO ALCOHOL CONSUMPTION: ROLE OF GENETIC BACKGROUND AND OTHER FACTORS

Although the post-alcoholic liver disease in general is considered as the most common expression of organ-end disease from alcohol, and it is well compared with the total amount of alcohol ingested over the years, we are not quite able to explain why only 30% heavy drinkers of alcoholic hepatitis can present and 10% cirrhosis[39] or to predict which patients will have these lesions in the future. Individual susceptibility factors are likely to have a relevant influence. The same is true for the onset of sperm abnormalities and fertility in male alcohol users.

The glutathione S-transferase (*GST*) *M1* genotype may be associated with a greater susceptibility to develop, via direct mechanism at testicular level, alcohol-induced spermatogenesis disorders.[40] Association between the occurrence of *GST M1* 'null' genotype and alcoholic liver disease has been described.[41,42] The 'null' genotype indicates the absent activity of class μ-glutathione transferase. In particular, the homozygous deletion of the *GST M1* gene may indicate increased susceptibility to develop irreversible liver damage in response to the toxic effects of ethanol. Moreover, there is a significant association between the occurrence of the 'null' genotype and the occurrence of alcoholic liver cirrhosis.[41,42] The association between alcohol-induced alteration of human spermatogenesis and *GST M1* genotype was investigated in an autopsy study comprising 271 subjects.[43] The results of this study showed that among 50 moderate drinking men (reported mean daily alcohol consumption $<$40 g), 42% of the subjects had normal spermatogenesis, whereas 48% had partial, and 10% completed spermatogenic arrest. Among the 21 men with normal spermatogenesis, 42.9% had the *GST M1* genotype with a frequency similar to that found in men with partial or complete spermatogenic arrest (44.8%). Among the 212 heavy-drinking men (reported mean daily alcohol consumption $>$80 g), 21.2% of the subjects had normal spermatogenesis, 36.3% had partial spermatogenic arrest, 38.2% showed complete arrest spermatogenic arrest, and 4.2% showed Sertoli-only cell syndrome. Interestingly, 27 out of the 45 heavy drinkers with normal spermatogenesis (60%) men had the *GST M1* genotype (OR 2.7 with 95% CI: 1.0–4.0, when compared with those with disorders of spermatogenesis). The frequency of *GST M1* genotype in heavy drinkers with normal spermatogenesis also differed from that of corresponding moderate drinkers, whereas the frequency of *GST M1* genotype in heavy drinkers with disorders of spermatogenesis was similar to moderate drinkers with or without disorders of spermatogenesis. The finding that $>$20% of heavy drinkers had normal spermatogenesis suggests that the *GST M1* genotype exerts a protective effects on alcohol-induced spermatogenesis disorders. The same authors were unable to demonstrate a significant association between polymorphisms of the *CYP2E1* gene and spermatogenesis abnormalities.[43]

Among factors that may potentiate the toxic action of alcohol protein malnutrition, other nutritional deficiencies or imbalances and the associated liver disease are frequently encountered. Due to a low dietary intake or excessive loss of micronutrients, caused by vomiting or diarrhea, the lack of certain minerals is often present in alcohol users. These include Zn (which plays an important role for the activation of alkaline phosphatase, carbonic anhydrase and alcohol dehydrogenase), Mg (important in some metabolic processes and for stabilizing DNA, RNA and ribosomes) and possible states of folate deficiency and hypovitaminosis (A, D, E) in many organs (liver, muscle, heart, testis, and male accessory glands). In this regard, recently, Sobral-Oliveira and colleagues,[44] evaluating the nutritional profile of 48 men, found a significant reduction of Mg in alcohol consumers, and a correlation between vitamin D and vitamin B12 levels with alcohol abuse, as well as between C-reactive protein and serum amyloid A with duration of excessive drinking and this condition was associated with a reduced lean body mass.

In a study performed on a group of alcohol abusers and control group, the patients had significantly low plasma testosterone with low LH and FSH concentrations, associated with oligo-asthenozoospermia and increased oxidative stress. The latter was due to high thiobarbituric acid reactive substances, superoxide dismutase, glutathione S-transferase, low glutathione, ascorbic acid, catalase, glutathione reductase and glutathione peroxidase.[45]

CONCLUSION

Overall the studies presented in this review suggest that alcohol consumption seems to alter sperm parameters and testicular pathology. As far as sperm parameters, the more frequently abnormality reported is the higher percentage of morphologically abnormal spermatozoa. In addition, a decrease in the seminal fluid volume and an increased seminal fluid leukocyte concentration have also been reported. Interestingly, alcohol intake has been associated with a greater frequency of urethritis. Sperm parameter abnormalities are often associated with hypotestosteronemia which results from ethanol effects on both the central component of the HPT axis and at the testicular levels (primary testiculopathy). Finally, the genetic background as well as nutritional deficiencies may modulate the impact of alcohol on spermatogenesis.

AUTHOR CONTRIBUTIONS

SLV, RAC, GB, EV and AEC made a substantive intellectual contribution to this study. Equally they searched the databases, critically reviewed the articles, and wrote the manuscript.

COMPETING FINANCIAL INTERESTS

The authors declare no conflict of interest.

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