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Female Reproduction During Chronic Ethanol Consumption in Rats

WAYNE A. KRUEGER,[1] WALTER J. BO AND P. KEVIN RUDEEN[2]

Department of Anatomy, Bowman Gray School of Medicine, Wake Forest University, Winston-Salem, NC 27103

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KRUEGER, W. A., W. J. BO AND P. K. RUDEEN. *Female reproduction during chronic ethanol consumption in rats.* PHARMAC. BIOCHEM. BEHAV. 17(4) 629–631, 1982.—This study was undertaken to ascertain ovarian function under the conditions of ethanol withdrawal and continued ethanol treatment to distinguish between a temporary delay in ovarian activity and a permanent suppression of ovarian function. Immature rats were fed the following diets for 16 weeks: a liquid diet containing 5% ethanol, a liquid diet without ethanol (pair-fed controls), a liquid diet with 5% ethanol for eight weeks followed by laboratory chow and water for eight weeks, or chow and water ad lib. Vaginal patency was significantly delayed in both groups of ethanol-treated rats compared to controls. The duration of an estrous cycle for the rats in the ad lib group was 5.0±0.3 days, while a "regular" estrous cycle was four to six days in duration. The rats which received ethanol for 16 weeks exhibited more irregular estrous cycles (both <4 and >6 days) than the rats with other treatments and the cycles were significantly longer. After 16 weeks of treatment, the rats were mated; ethanol was not given during pregnancy. The average number of pups per litter and body weight of the offspring were similar for all groups. These data show that although ethanol alters normal cyclic activity, it does not totally suppress ovarian function since alcohol-treated rats were capable of mating and delivering viable offspring.

Estrous cycles Vaginal patency Ethanol Pregnancy Ovarian function

ETHANOL has been shown to have an adverse influence on ovarian function of prepuberal rats. Van Thiel *et al.* [10] observed that ovarian atrophy, as evidenced by a lack of developing follicles and corpora lutea (in the majority of ovaries), occurred in rats that consumed 5% ethanol in a liquid diet from 28 to 77 days of age. On the other hand, Bo *et al.* [2] demonstrated that 5% ethanol in a liquid diet given to rats from 20 to 75 days of age (eight-week duration) resulted in a delay of ovarian maturation indicated by retardation of vaginal patency and a single generation of corpora lutea. It was not determined in our previous study [2] if normal ovarian function would occur following removal of the ethanol after eight weeks of treatment or if normal ovarian activity would occur spontaneously with continued (i.e., beyond eight weeks) ethanol treatment. Therefore, the present study was undertaken to ascertain ovarian function under the conditions of ethanol withdrawal and continued ethanol treatment. To assess ovarian activity, the regularity of estrous cycles was determined and rats were mated following 16 weeks of alcohol consumption; the number and body weight of the offspring were determined.

METHOD

Female Holtzman rats, 20 days of age and weighing 45–55 g, were housed in a room maintained at 21–22°C, exposed to 14 hours of artificial light per day (lights on at 0600 hr) and divided into groups according to diet. The animals in Group I (n=10) received a liquid diet (Bio-Serv, Inc.) containing 5% ethanol (w/v); the rats in Group II (n=6) were pair-fed isocalorically (maltose-dextrin was substituted for ethanol) and isovolumetrically with six of the rats from Group I; the 10 rats in Group III received the diet containing 5% ethanol for eight weeks followed by lab chow (Purina) and tap water ad lib for eight weeks; and the animals in Group IV (n=7) received Purina chow and tap water ad lib for sixteen weeks. Each rat was examined daily for vaginal patency, after which a daily lavage was made (0900–1100 hr) to establish regularity of estrous cycles; the vaginal smears were read blindly by the same investigator. Body weights were determined weekly throughout the experiment. Sixteen weeks after the start of treatment, three rats from Groups II–IV were autopsied at vaginal estrus, and the ovaries and uterine horns were prepared for histological examination as described previously [2]. All of the other rats were presented to males over a two-week period; the males were placed with the females at 1700–1800 hours on the day of proestrus (prior to anticipated mating) and removed the following morning (0700–0800 hours). Therefore, some of the male rats had access to ethanol diet overnight. On the first day of pregnancy (sperm positive=day 1), the rats that received liquid diets (ethanol and their pair-fed controls) for 16 weeks were

[1]Current address (address for reprint requests): Discipline of Anatomy, University of Osteopathic Medicine and Health Sciences, 3200 Grand Avenue, Des Moines, IA 50312.

[2]Current address: Department of Anatomy, School of Medicine, University of Missouri, Columbia, MO 65212.

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TABLE 1

EFFECT OF ETHANOL ON ESTROUS CYCLES

Group	Treatment (n)	Average duration of estrous cycle ± SEM	Percentage of estrous cycles 4–6 days in duration	<4	>6
I	Ethanol for 16 weeks (10)	6.2 ± 0.2[a]	68%[c]	10%	22%
II	Pair-fed with Group I (6)	5.4 ± 0.3[b]	86%[d]	4%	10%
III	Ethanol for 8 weeks, water-chow for 8 weeks (10)	5.2 ± 0.2[b]	90%[d]	3%	7%
IV	Ad lib (7)	5.0 ± 0.3[b]	89%[d]	5%	6%

Statistical differences: a vs b, $p<0.05$; c vs d, $p<0.01$.

placed on a chow-water diet ad lib. Therefore, rats were not given alcohol during gestation. The rats were examined periodically between 0700 and 1700 hours for evidence of delivery. Following delivery, the number and body weight of each pup were determined. The data were analyzed by one-way ANOVA.

RESULTS

The average age (±SEM) of vaginal patency for the rats given ethanol for 16 weeks was 72±4 days, while it was 77±5 days for the rats fed ethanol for eight weeks followed by chow and water for eight weeks. These values were similar ($p>0.05$) to each other, and both were significantly greater ($p<0.01$) than the ages of patency for the pair-fed (58±3) and ad lib (41±2) controls. Vaginal opening in the pair-fed rats was significantly delayed compared to the ad lib animals ($p<0.01$).

The average duration of an estrous cycle (i.e., the number of days between the first day of complete vaginal cornification of a cycle and the first day of cornification of the subsequent cycles) for the ad lib control rats was 5.0 days (0.7=SD, 0.3=SEM). Therefore, a "regular" estrous cycle was considered as one of 4 to 6 days in duration, while an "irregular" cycle lasted less than four or more than six days.

The average length of an estrous cycle for the rats on the ethanol diet for 16 weeks was significantly greater ($p<0.05$) than that of all other groups of rats (Table 1). Furthermore, the rats which received ethanol for 16 weeks exhibited significantly fewer ($p<0.01$) regular estrous cycles (Table 1).

Because of both the increased duration of estrous cycles and increased frequency of irregular estrous cycles for the rats on ethanol for 16 weeks, we chose to mate all of these rats to have a maximum number of rats in this experimental group. Three rats from each of the other groups (i.e., II–IV) were autopsied at estrus. Microscopic examination of the ovaries showed numerous follicles at varying stages of development and several generation of corpora lutea. The ovaries of the rats from the different groups were histologically indistinguishable from each other. Likewise, the uteri of rats from the different groups were similar in appearance: the luminal epithelium was tall columnar and had numerous infoldings, typical of the estrous animal.

Except for two of the rats given ethanol for 16 weeks and one rat from the ad lib control group that did not mate, all of the other rats mated and delivered live pups; none of the offspring were stillborn. After delivery, the sex, number and body weight of offspring were determined (Table 2). The data showed that there were no statistical differences among the four groups with regards to the number or weight of the offspring.

Serum ethanol levels were not determined in this study. However, the amount of ethanol consumed ranged from 14 to 21 grams of alcohol per kilogram of body weight per day. In another report, the same ethanol diet from Bio-Serv, Inc., was used and the average serum ethanol level was 249 mg% (±57 mg%, SEM) in rats that consumed 16–20 grams of ethanol per kg per day for eight weeks [2].

DISCUSSION

The data that ethanol suppresses reproductive maturation in the rat, as evidenced by a delay in vaginal patency, confirmed our earlier report [2]. Furthermore, rats treated with alcohol for 16 weeks had a significantly greater percentage of irregular estrous cycles than did control animals. This observation of an increased frequency of irregular estrous cycles in ethanol-treated rats is in agreement with a recent report by Eskay *et al.* [5]. Menstrual dysfunction or irregularities of the cycle are often seen in alcoholic human females [7,9].

Although our data show that ethanol given to rats results in alteration of normal ovarian function, they do not support the hypothesis of Van Thiel *et al.* [10] that ethanol in rats is a gonadal toxin resulting in ovarian atrophy. Van Thiel *et al.* [10] administered ethanol in a liquid diet to rats from 28 to 77 days of age. Based on estradiol and progesterone levels and ovarian and uterine weight and morphology, these investigators concluded that ethanol produces ovarian atrophy. In our previous report [2] alcohol was given to rats in a liquid diet from 20 to 75 days of age and, like Van Thiel *et al.* [10], we found that ovarian and uterine weights were suppressed

TABLE 2

EFFECT OF ETHANOL, GIVEN PRIOR TO MATING, ON THE NUMBER AND BODY WEIGHT OF THE OFFSPRING

Treatment	Number of rats which were exposed to males	Number of rats which became pregnant and delivered live pups	Average number of pups per litter ± SEM*	Average body weight (grams) of pups ± SEM*	
				Males	Females
Ethanol for 16 weeks	10	8	8.3 ± 1.9	7.22 ± 0.10	6.44 ± 0.11
Pair-fed with rats given ethanol for 16 weeks	3	3	9.7 ± 2.8	7.12 ± 0.15	6.39 ± 0.12
Ethanol for 8 weeks, water-chow for 8 weeks	7	7	10.0 ± 1.3	7.20 ± 0.08	6.56 ± 0.07
Ad lib	4	3	10.5 ± 1.5	7.08 ± 0.20	6.50 ± 0.13

*No significant differences ($p > 0.05$) among groups were observed; all pups were live.

in the ethanol-treated rats compared to controls and that the uterine and vaginal morphology of the alcohol-fed rats appeared infantile. Since vaginal patency occurred in 75% of the alcohol-treated rats and because all of the ovaries of these rats contained corpora lutea (although only a single generation), we postulated that the ovaries showed signs of becoming functional [2]. The present report supports this hypothesis. While ethanol alters normal cyclic activity, it does not result in ovarian failure, since the animals receiving alcohol for 16 weeks mated, carried the young to term and had offspring of similar number and comparable weight to control rats. Henderson and Schenker [6] also reported that rats given a liquid diet containing ethanol for periods of time up to 21 weeks were capable of mating. Therefore, our studies and that of Henderson and Schenker [6] show that ethanol does not produce ovarian atrophy in rats.

Although the data show that ethanol affects normal ovarian cyclic activity, the manner(s) by which this occurs are not understood. Several possibilities may exist. Ethanol may be acting directly at the hypothalamic level by affecting the production and/or release of GnRH; Cicero *et al.* [3,4] have reported that ethanol inhibits LH-RH in males. Alternatively, ethanol may interfere with the secretion of FSH and/or LH; ethanol has been shown to inhibit the LH surge at proestrus [1,8].

Nevertheless, the data in this report indicate that alcohol consumption delays ovarian maturation in sexually immature female rats, but the effects of continued alcohol consumption fail to completely suppress ovarian function. The effects of alcohol on the female reproductive system appear to be more complex than previously indicated [7,10] and alcohol may have only subtle effects on the mature ovary.

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