Pharmacology Biochemistry & Behavior, Vol. 19, pp. 583–585, 1983. © Ankho International Inc. Printed in the U.S.A.

Estrous Cyclicity in Rats Fed an Ethanol Diet for Four Months

WAYNE A. KRUEGER

Discipline of Anatomy, University of Osteopathic Medicine and Health Sciences
3200 Grand Ave., Des Moines, IA

WALTER J. BO

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

AND

P. KEVIN RUDEEN

Department of Anatomy, School of Medicine
University of Missouri, Columbia, MO

Received 25 January 1983

KRUEGER, W. A., W. J. BO AND P. K. RUDEEN. *Estrous cyclicity in rats fed an ethanol diet for four months.* PHARMACOL BIOCHEM BEHAV **19**(4) 583–585, 1983.—This study is designed for better understanding the effect of chronic ethanol treatment on ovarian function in sexually mature rats by assessing the estrous cycle. Rats were fed the following diets for 17 weeks: a liquid diet containing 5% (w/v) ethanol, a liquid diet without ethanol (pair-fed) or laboratory chow and water ad lib. Estrous cycles were determined throughout the 17 weeks and the rats were necropsied at proestrus and metestrus. Ethanol-fed rats had significantly more irregular estrous cycles than did controls, and the duration of an estrous cycle in the ethanol-treated rats was statistically longer than that of controls. However, ovarian and uterine weights and ovarian and vaginal morphology of ethanol-fed rats were similar to those of controls.

Estrous cycle Ovarian function Ethanol

THE data concerning the effect of ethanol on ovarian morphology and function in the rat are conflicting. Van Thiel *et al.* [11] studied the toxic effect of ethanol on ovarian morphology and function in immature rats that consumed 5% ethanol in a liquid diet from 28 to 77 days of age; they observed atrophy of the ovaries and the histological appearance of the uteri and vaginae resembled that seen in ovariectomized rats. However, our studies [2] showed that in immature rats given 5% ethanol from 20 to 75 days of age, ovarian function was suppressed as evidenced by a delay in vaginal patency, an absence of estrous cycles and only a single generation of corpora lutea. There was no indication of ovarian atrophy in our previous studies [2,9].

Using the sexually mature rat, Gavaler *et al.* [6] found that administration of a liquid diet containing 5% ethanol for four months resulted in ovarian atrophy as manifested by loss of ovarian mass, decreased progesterone levels and atrophy of steroid-responsive tissues. On the other hand, Henderson and Schenker [7] found "no decreased incidence of conception" in mature rats exposed to alcohol for up to 21 weeks prior to mating; while their report was not concerned with ovarian morphology or function per se, the data indicated indirectly that ethanol treatment did not result in ovarian atrophy since the rats were capable of ovulating, conceiving and delivering.

Because our previous reports [2,9] showed that ethanol resulted in a delay in ovarian maturation rather than in ovarian atrophy or failure in immature rats, and because Henderson and Schenker [7] observed that rats were capable of mating after 21 weeks of alcohol treatment, the present study was undertaken to better understand the effect of chronic ethanol treatment on ovarian morphology and function in sexually mature rats by assessing the effect of ethanol on the estrous cycle.

METHOD

Young adult female Holtzman rats weighing 150–165 grams were housed in a room maintained at 20–22°C and exposed to 14 hours of artificial light per day (lights on at 0600 hours, EST). Each animal was housed individually in a hanging wire-meshed cage measuring 18×9×9 inches. Male rats, nor any other animals, were present in the same facilities during the duration of the experiment. At 60 days of age, the rats were placed into three groups of 18 rats each according to diet: The animals in Group I received a liquid diet (Bio-Serv., Inc., Frenchtown, NJ) containing 5% ethanol (w/v; 36% of the daily caloric intake); the rats in Group II were weight-matched and pair-fed isovolumetrically and isocalorically (maltose-dextrin was substituted for ethanol); and the rats in Group III received laboratory chow

TABLE 1

THE EFFECT OF ETHANOL ON BODY, OVARIAN AND UTERINE* WEIGHTS (±SEM)

Group	Treatment	Body Weight (grams)	Paired Ovarian Weight (mg%)	Paired Uterine Weight (mg%) Proestrus	Metestrus
I	5% ethanol	286 ± 6	33.7 ± 3.1	217 ± 14†	169 ± 13‡
II	Pair-fed	288 ± 9	26.8 ± 2.6	218 ± 16†	166 ± 10‡
III	Ad lib	281 ± 5	28.0 ± 2.1	228 ± 19†	148 ± 5‡

*For uterine values, n=9; for body and ovarian weights, n=18.

Significant differences: † vs. ‡, $p<0.01$, ANOVA and Duncan's multiple range t-test.

(Purina) and tap water ad lib. The rats were fed daily at 0900–1000 hours, except on the morning of autopsy.

Estrous cycles were determined throughout the entire 17 weeks of treatment; daily vaginal lavages were read blindly by the same investigator to ascertain the stage of the cycle. The rats were killed by decapitation at 0900–1100 hours on the days of proestrus (n=9/group) and metestrus (n=9/group) and the trunk blood was collected for serum ethanol determination. The ovaries were dissected from the oviducts and surrounding adipose tissue, and the uterine horns were cut at the junction with the cervix and separated from the adjacent mesentery. The paired ovaries and paired uterine horns were each weighed to the nearest 0.1 mg. A portion of the vagina and the ovaries were fixed in cold picric acid-alcohol-formalin for 24 hours and processed for routine paraffin sectioning. Four of the nine rats killed at proestrus and four of the nine rats taken at metestrus were selected randomly from each of the three groups; at least 50 sections of ovary and 15 sections of vagina from each of these rats were stained with hematoxylin and eosin and microscopically examined. Serum ethanol levels were determined spectrophotometrically using the procedure of Bonnichsen and Theorell [3]. The data were analyzed statistically by ANOVA, Duncan's multiple range t-test and the corrected chi square test.

RESULTS

The average duration (±SEM) of an estrous cycle for the ethanol-fed rats was 5.8±0.2 days, which was significantly longer than that of the pair-fed controls (5.1±0.2, $p<0.05$) and that of the ad lib rats (4.6±0.2, $p<0.01$). A "regular" estrous cycle for the Holtzman rats in our laboratories is one which is four to six days in duration; an "irregular" cycle is shorter than four or longer than six days. By this criterion, 99% of the estrous cycles exhibited by the ad lib-fed rats and 95% of the cycles of the pair-fed animals were regular. These percentages were statistically similar to each other, and both were significantly greater ($p<0.05$) than that of the ethanol-treated rats (88%). Regardless of diet, all of the irregular cycles were greater than six days and were characterized by a prolonged diestrous phase. Diestrus was prolonged by one to five days; none of the cycles were as long as that of pseudopregnancy (12 days) as observed for the Holtzman rat in our laboratory. The percentage of regular estrous cycles did not change during the 17 weeks of treatment.

The amount of ethanol consumed by the alcohol-fed rats ranged from 13 to 20 grams of alcohol per kilogram of body weight per day. The ethanol-fed rats displayed signs of intoxication and physical dependence on alcohol during the course of the experiment: lacked ability to groom themselves, strob-tail sign, and hyperactivity and hypersensitivity to touch. Trunk blood was collected at autopsy (0900–1100 hours) for serum ethanol determination; the rats were fed 24 hours prior to autopsy. The average serum alcohol level for the rats on the ethanol diet was 149±31 mg% (±SEM).

For each group of rats autopsied at proestrus, the body weights were statistically similar ($p>0.05$) to those autopsied at metestrus, as were the ovarian weights (Table 1), so these values were pooled. For each group, the average uterine weight of rats killed at proestrus was significantly greater ($p<0.01$) than that of rats taken at metestrus (Table 1). When comparing the body weights or the weights of the ovaries or uteri of the ethanol-fed rats to those of either the pair-fed or ad lib controls, no differences were observed ($p>0.05$).

Microscopic examination of the ovaries of rats killed at proestrus, regardless of treatment, showed several large antral follicles and numerous small follicles at varying stages of development as well as several generations of corpora lutea. The ovaries of rats killed at metestrus, irrespective of previous treatment, contained new corpora lutea, corpora lutea of previous cycles and numerous developing follicles. The vaginae of ethanol-fed rats were histologically similar to those of pair-fed or ad lib control animals. At proestrus the epithelium was stratified cuboidal, with the cornified cells lying deep to the surface cells. The epithelium was stratified squamous at metestrus and numerous white blood cells were present in the lumen of the vagina.

DISCUSSION

The data from our study show that rats placed on a 5% ethanol diet for 17 weeks starting at 60 days of age had significantly more irregular estrous cycles than the controls, and the length of the cycles was significantly greater than that of the controls. In both mice [4] and rats [5], ethanol has been reported to alter the estrous cycle. Irregularities of the menstrual cycle are often seen in alcoholic women [8,10].

Although irregularities in the estrous cycles were observed in ethanol-treated rats in the present report, the weights of the ovaries and uteri and the histology of the ovaries and vaginae were the same as those in the ad lib and pair-fed controls. Therefore, based on organ weight and morphology, which are ovarian dependent [12], our study shows that ethanol did not significantly alter ovarian func-

tion. It is difficult to explain why our data differ from those of Gavaler *et al.* [6] who reported that similar treatment resulted in ovarian atrophy. There are several similarities in protocol between our study and that of Gavaler *et al.* [6]: the age of the rats at the start of treatment (60 days), the duration of treatment (4 months), the diet (Bio-Serv., Inc.), the concentration of ethanol (5%, w/v) and several of the parameters analyzed (ovarian weight, ovarian morphology, and uterine weight).

The only apparent differences between the two reports is the strain of rats; we used Holtzman rats while Gavaler *et al.* [6] used rats of the Wistar strain. In their study, the rats weighed only 160 and 166 grams (alcohol and pair-fed, respectively) at the time of autopsy (six months of age) despite the fact that the rats "gained weight steadily" throughout the experiment. Our Holtzman rats weighed 150–165 grams at the start of the experiment (two months of age) and 286 ± 6 and 288±9 grams at autopsy (alcohol and pair-fed, Table 1). It is not clear if the variability in body weights of the rats could account for the difference in results obtained by the two laboratories.

Although with the morphological parameters used in our study ovarian function appeared normal, it is interesting to speculate that ethanol may have an effect on follicular development, resulting in a suppression of the number of ova which ovulate. Data from a previous study in our laboratory [1] suggest that this may occur. Following treatment with pregnant mares serum gonadotropin (PMSG), the number of rats that superovulated in the ethanol-fed group was statistically similar to that of the pair-fed controls, but the number of ova shed was significantly lower in the ethanol-treated rats than in the controls [1]. However, whether follicular development is suppressed by ethanol still remains to be determined.

REFERENCES

1. Bo, W. J., W. A. Krueger and P. K. Rudeen. Ethanol suppression of PMS-induced superovulation in the immature rat. *Anat Rec* **202:** 17–18A, 1982.
2. Bo, W. J., W. A. Krueger, P. K. Rudeen and S. K. Symmes. Ethanol-induced alteration in the morphology and function of the rat ovary. *Anat Rec* **202:** 255–260, 1982.
3. Bonnichsen, R. K. and H. Theorell. An enzymatic method for micro-determination of ethanol. *Scand J Clin Lab Invest* **3:** 58–62, 1951.
4. Cranston, E. M. Effect of tranquilizers and other agents on sexual cycles of mice. *Proc Soc Exp Biol Med* **98:** 320–322, 1958.
5. Eskay, R. L., R. S. Ryback, M. Goldman and E. Majchrowicz. Effect of chronic ethanol administration on plasma levels of LH and the estrous cycle in the female rat. *Alcoholism: Clin Exp Res* **5:** 204–206, 1981.
6. Gavaler, J. S., D. H. Van Thiel and R. Lester. Ethanol: a gonadal toxin in the mature rat of both sexes. *Alcoholism: Clin Exp Res* **4:** 271–276, 1980.
7. Henderson, G. I. and S. Schenker. The effect of maternal alcohol consumption on the viability and visceral development of the newborn rat. *Res Commun Chem Pathol Pharmacol* **16:** 15–32, 1977.
8. Jones-Saumty, D. J., M. S. Fabian and O. A. Parsons. Medical status and cognitive functioning in alcoholic women. *Alcoholism: Clin Exp Res* **5:** 372–377, 1981.
9. Krueger, W. A., W. J. Bo and P. K. Rudeen. Female reproduction during chronic ethanol consumption in rats. *Pharmacol Biochem Behav* **17:** 629–631, 1982.
10. Ryback, R. S. Chronic alcohol consumption and menstruation. *J Am Med Assoc* **238:** 2143, 1977.
11. Van Thiel, D. H., J. S. Gavaler, R. Lester and R. J. Sherins. Alcohol-induced ovarian failure in the rat. *J Clin Invest* **61:** 624–632, 1978.
12. Velardo, J. T. The anatomy and endocrine physiology of the female reproductive system. In: *The Endocrinology of Reproduction*, edited by J. T. Velardo. New York: Oxford University Press, 1958.