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Effects of Prepregnancy Ethanol on Neuromotor Development, Activity, and Learning

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TORRES, F. K. AND B. ZIMMERBERG. *Effects of prepregnancy ethanol on neuromotor development, activity, and learning.* PHARMACOL BIOCHEM BEHAV 41(3) 587–597, 1992.—This study examines the behavioral consequences of both long-term chronic and acute high-dose administration of ethanol before pregnancy. Female rats were assigned to one of five conditions: a self-administered liquid diet with 35% ethanol-derived calories, a pair-fed liquid diet with 0% ethanol-derived calories, a gavage intubation with 4 g/kg ethanol, a gavage intubation control, or a standard lab chow control. Offspring were weighed at various ages and tested for neuromotor development, activity, and learning a Morris maze water task. Offspring of mothers in the high-dose condition were hyperactive as juveniles but not as adults. Hyperactivity was not seen in the low-dose chronic ethanol condition, contrary to previous reports. Neither ethanol administration paradigm was associated with learning deficits. Prepregnancy stress effects were apparent from both the restricted liquid diet feeding and the gavage treatment on neuromotor development. A possible mechanism for the effects of prepregnancy ethanol treatment is linked to ethanol's potential to alter hormone levels. Since hormones are directly responsible for the maturation of the egg, hormonal imbalances as a result of ethanol exposure may result in developmental deficiencies in the offspring.

Fetal Alcohol Syndrome Prepregnancy Alcohol Ethanol Rat Neuromotor development
Activity Learning Alcohol-related birth defects Stress

THE teratogenic effects of alcohol consumed during pregnancy range from spontaneous abortion to a range of specific birth defects that characterize what is now known as Fetal Alcohol Syndrome (FAS) to a number of less severe problems in the offspring that characterize Fetal Alcohol Effects (FAE) (28). Despite the abundance of research on FAS since 1973, the degree and specificity of alcohol's teratological effects are difficult to predict. The effects are dependent on the amount of alcohol ingested, the time during gestation when alcohol is ingested, and the duration of exposure (12). The CNS develops throughout gestation. The extended critical period available for alcohol consumed during pregnancy on a regular basis may not induce any dramatic effects such as facial malformations but may nevertheless lead to behavior dysfunctions. Learning disabilities, hyperactivity, sleep disorders, and language dysfunctions, as well as motor problems and difficulties in conceptualizing money and time, are examples of alcohol's teratogenic effects on the brain (26).

Recent studies demonstrating a paternal effect of ethanol exposure added a new dimension to the study of alcohol's teratogenicity. These findings suggested that, in addition to a direct effect on the developing fetus, ethanol can also act as teratogen via an indirect pathway. The number of dead implants observed in females mated to male mice gavaged with ethanol 4–13 days prior to mating was greater than the number observed in controls (6). Other studies reported growth deficiencies in the offspring of paternally exposed offspring (17). No differences in litter size nor in body or organ weights, typical of FAS, were detected in the offspring of male mice administered varying doses of ethanol prior to mating with untreated females (3,25). However, paternal alcohol-exposed juvenile offspring were hypoactive, required fewer trials to reach criterion in a passive avoidance task, and took longer to reach a choice point in a T-maze than control mice. There was also a dose-related decrease in serum testosterone levels at postnatal day (PN) 55 (3). Paternal alcohol-exposed juvenile rats were also less active than controls, but were no different in passive avoidance learning or spontaneous alternation (4). In contrast, Abel and Bilitzke (2) reported hypoactivity in mice offspring but hyperactivity in rat offspring during a swimming test. Grooming activity was also affected by paternal alcohol exposure; offspring sired by these males groomed

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significantly less than control rats when placed in situations that elicited grooming (1).

Given a paternal alcohol effect, it should follow that maternal drinking prior to pregnancy might also cause behavioral deficits in the offspring. There is only one report of a child who appeared affected by alcohol despite the fact that the mother stopped drinking before conception. The effects, however, did not meet the criteria for diagnosis as FAS (26). Nevertheless, the few reports from animal studies that have just begun to look at this issue make it clear that, similar to paternal alcohol exposure, maternal alcohol exposure prior to conception has teratogenic potential on the offspring of some individuals. Teratogenic effects prior to pregnancy is not a new concept. Friedler and Cochin (13) treated female rats with morphine and then mated them after a 5-day withdrawal period. The retarded growth in their offspring at 3–4 wk of age revealed that teratogens are potentially dangerous before conception.

Recently, Ledig and coworkers (18) reported behavioral and biochemical effects in the offspring of dams given a 20% (v/v) alcohol solution as the sole liquid source for a period of 1 month prior to mating. Two control groups were included in the study. The first received 15% (w/v) sucrose and the second received water. All groups were fed with standard laboratory chow ad lib. Significant increases in open-field activity and novelty-seeking behavior were observed in the adult prepregnancy alcohol-exposed offspring. In addition, the number of rearings was significantly greater for the offspring of alcohol-exposed dams; however, the number of defecations was significantly reduced compared to controls. Finally, when given 20% alcohol solutions, the offspring of alcohol-treated dams significantly reduced intake as compared to controls.

In the liver, at 8 wk of age, the levels of cytosolic superoxide dismutase (SOD) activity and the levels of mitochondrial SOD were reduced in the prepregnancy group compared to control rats. In the brain, at 2 wk of age, the cytosolic SOD activity was also lower than controls and at 4 wk of age the mitochondrial SOD activity was again lower than controls. These altered enzyme activities in the brain were no longer observed at 24 wk of age. The level of nonneuronal enolase in the brains of offspring of alcohol-exposed dams was significantly lower than controls at 4 and 8 wk of age. There were also significant decreases in alcohol-metabolizing enzymes at various ages. These results were similar to those reported in a previous study (20). More recently, Ledig and Tholey reported that offspring of dams drinking alcohol prior to conception had altered turnover and levels of GABA in hypothalamus, olfactory tubercle, and frontal cortex (19).

Ledig and coworkers (18) acknowledged that controlling for caloric intake with an ad lib sucrose condition was problematic and only compared their alcohol offspring to the standard lab control offspring. In fact, sucrose mothers were 10% heavier after treatment, while no weight gain was observed in the groups receiving alcohol or water. In the subsequent study, Ledig pooled the results from his two control groups (19). A previous unpublished pilot study in this laboratory found behavioral effects in a sucrose control group similar to Ledig's, making it difficult to interpret the effects seen in offspring of dams who drank a 12% (v/v) alcohol solution with sucrose.

Since the results reported by Ledig's group would have important implications, this research further investigated the question of prepregnancy alcohol exposure using more appropriate control groups and varying the duration and dose of alcohol exposure. Two ethanol administration paradigms were used. The first was a high-dose binge condition that would limit ethanol exposure to the time period during which the egg matured. The second was a low-dose, long-term condition similar to the one used by Ledig and coworkers. A variety of dependent measures were examined to determine the effects of prepregnancy alcohol exposure on growth, neuromotor development (11), activity, and learning. Learning was assessed using a common test of spatial memory and learning, the Morris water maze test (24). Rats exposed to alcohol pre- and postnatally have exhibited learning deficits in the Morris Maze (8,14,15).

METHOD

Subjects

Subjects were the offspring of female Long-Evans rats (Charles River Farms, Wilmington, MA). There were five prepregnancy treatment groups. Group A (n = 12) was given ad lib access to a liquid diet containing 35% ethanol-derived calories (EDC) (Bio-Serv, Frenchtown, NJ). Group B (n = 12) was a pair-fed control group given a similar liquid diet except that the ethanol was replaced isocaloricaly with maltose-dextrin (0% EDC). Each female was yoked to a female in Group A and fed the same amount consumed by the Group A female on a ml/kg body weight basis. Group C (n = 12) received standard lab chow and water ad lib. Group D (n = 11) was intubated by gavage with 4 g ethanol per kg body weight using a 30% (v/v) ethanol solution (USP ethyl alcohol, 95%, US Industrial Chemicals Co.). Group E (n = 11) was intubated (gavaged) with an equivalent volume of distilled water as a control for Group D.

Water and lab chow were removed from Groups A and B and replaced with the control liquid diet (0% EDC) for an adjustment period of 24 h. Treatment diets (Group A, 35% EDC; Group B, 0% EDC) were then administered for 20 days, allowing each female to complete a minimum of four estrus cycles. Rectal temperatures were taken at 2 and 3 wk after starting the ethanol administration. Diets were replaced with lab chow and water, and Groups A, B, and C were mated; the presence of a vaginal plug marked day 1 of gestation. Rectal temperatures were also measured for 4 days following cessation of the chronic diets for the two liquid diet groups to determine if there were any withdrawal effects.

For Groups D and E, the duration and specific stage of the estrus cycle was assessed by vaginal smears. Gavage intubation began the day estrus was reached and continued daily throughout the duration of the estrus cycle. Upon reaching proestrus, treatment stopped and that evening rats were mated. This procedure attempted to limit ethanol exposure to the time during which the egg matured.

Rats were housed in standard plastic breeding cages during administration of liquid diets and intubation and during gestation. The nursery was maintained at a constant temperature (20–22°C) and humidity (55–60%) with a 12 L:12 D cycle; lights were on at 0600. Dams were checked three times daily for births starting on gestational day 21. The day a birth was noted was deemed PN 0; litters were not disturbed at this time. The next day (PN 1), litters were culled to 10 pups (5 males and 5 females when possible) and each was marked by toe clip so naive subjects could be used for each of the behavioral tests. Only one male and one female from any one litter was used for either the neuromotor development tests (righting, negative geotaxis, and ramp climbing), juvenile activity, Morris maze test, or adult activity to avoid litter confounds.

All pups were weighed on PN 1, 5, 11, and 16. Subjects were also weighed at 9 wk when tested for activity. At 11 wk of age, remaining naive subjects were weighed and decapitated and their brains removed and weighed.

Apparatus

Materials for behavioral testing consisted of two ramp structures for use in negative geotaxis and in ascending a wire-mesh surface: a wooden structure at a 30° angle with an overlapping layer of fine sandpaper and a plastic structure at a 60° angle with overlapping wire-mesh. The apparatus for activity testing on PN 16 consisted of plastic boxes (44 × 44 × 14 cm) marked into 25 equally sized squares. For activity testing (9 weeks), a plastic box (77 × 90 × 47 cm) marked into 42 equally sized squares was used. Apparatus for the Morris maze consisted of a children's swimming pool (122 cm base diameter), a stainless steel insert (114 cm diameter), and a circular plastic platform (8 cm diameter, 20 cm height) filled with marble chips. The circle formed by the steel insert was subdivided into four quadrants. The quadrants were visible from above but did not interfere with testing. They were formed by placing two wire strips on the steel insert and across the center point of the pool. Water level was maintained at a depth of 21 cm and a temperature of 23°C. Water was colored white with nontoxic Crayola powder paint. Behavior for activity testing and for Morris water maze was recorded with an overhead videocamera.

Behavioral Testing

Latency to right. Latency to right was measured on PN 1-PN 5 and PN 7. The pup was placed on its back and the latency to turn onto its stomach was recorded. If a subject failed to right by 90 s it was assigned a ceiling value of 90 s.

Negative geotaxis. Testing for negative geotaxis began on PN 3 and continued every other day through PN 13. The pup was placed face downward at a 30° angle and the latency to turn 180° was recorded. Criteria for completing the 180° turn required that the pup place both front paws in a position parallel to the sides of the ramp and directing upward. Three trials were given daily. A 90-s ceiling limit was again used. If a pup was unable to complete a task before the time expired because it lost its balance and rolled down the ramp, the trial was assigned the ceiling score.

Latency to ascent a wire-mesh surface. Latencies were measured on PN 5, 9, and 13. The 60° ramp was placed in a clear plastic container with a half inch of cold water. Pups were placed at the bottom of the ramp with their hind paws and tail touching the water. The latency to ascend the ramp was recorded. A 90-s ceiling limit was again used. If a pup was unable to complete a task before the time expired because it lost its balance and rolled down the ramp, the trial was assigned the ceiling score. Three trials were given daily.

Activity testing. Testing was performed on PN 16 and at 9 wk of age. Rats were first weighed, then placed in the center square of the appropriate box and timed for 5 min at PN 16 and for 9 min at 9 wk of age. For a square to be counted, all four paws had to have entered that square. After each rat was tested, the box was cleaned.

Morris water maze. Testing was performed on PN 23, 24, and 25. These days were chosen because they represent a critical period where substantial behavioral differences, with respect to the Morris maze, were observed in normal rats and rats exposed to alcohol prenatally (8). Each rat performed six trials daily. On the first day, trials were preceded by a habituation run and on the last day they were followed by a probe test. Each rat was randomly assigned a quadrant for all three testing days. The platform was placed in this quadrant. The other three quadrants were then randomly assigned to each of the six daily trials with the stipulation that each quadrant would appear twice per day and that the same quadrant would not be repeated consecutively.

Testing procedure for the daily trials consisted of placing the rat in the assigned quadrant facing the steel insert and timing the latency to start swimming, as well as the latency to find the platform and climb onto it. The trial was terminated after a 2-min ceiling time. The time between trials was also 2 min. For the habituation and probe trials, the platform was removed. Rats were then placed in the center of the pool and allowed to swim for 2 min. For the probe test, the percentage of time spent in the quadrant assigned to the platform was recorded.

Data Analysis

Data were analyzed by analysis of variance (ANOVA) with group and sex as between-subject factors and day or trial (as appropriate) as repeated measures within subjects. Significant effects were analyzed by posthoc comparison of means using Fisher's test and a significance level of 0.05. Since sex did not interact with any other factor, data are presented in figures and graphs with the results from males and females combined.

RESULTS

Maternal and Litter Characteristics

The mean average daily ethanol intake for the liquid diet ethanol group was 11.35 g ethanol per kg body weight per day. There were no significant differences between conditions in rectal temperature observed on the first temperature recording day. The second recording day revealed a significant effect of condition on rectal temperature, $F(2,30) = 3.74$, $p = 0.05$. The liquid diet ethanol animals had significantly lower temperatures (36.80°C) compared to temperatures of the liquid diet control animals (37.33°C). No significant differences, however, were observed when compared to the lab chow control group (37.16°C). The lab chow control and liquid diet control groups did not differ.

No hyperthermic withdrawal effects were observed for the liquid diet ethanol group as measured by rectal temperature. There was, however, a significant interaction between condition and day of measurement (four daily repeated measurements), $F(3,66) = 7.46$, $p = 0.05$. The first day after the termination of ethanol treatment revealed a significant difference in temperature between the liquid diet ethanol group (36.82°C) and the liquid diet control group (38.02°C). The temperatures for the ethanol group rose to a normal level on subsequent days (37.32, 37.04, 37.33°C).

All dams in Groups A, B, and C gave birth, while only six and seven dams in Groups D and E, respectively, gave birth. There was a significant effect of condition on maternal weight gain, $F(4,44) = 3.43$, $p = 0.01$ (see Table 1). The mean percent weight gain was significantly lower for both gavage groups when compared to the liquid ethanol and lab chow control groups. The gavage groups did not differ from each other. The number of male pups born in each litter also differed significantly by condition, $F(4,44) = 4.79$, $p = 0.003$, with the number of males smaller in the two gavage groups compared to the other groups. The number of female pups was not affected by condition.

TABLE 1

PERCENT GESTATIONAL WEIGHT GAIN AND LITTER CHARACTERISTICS (LITTER SIZE AND BIRTH WEIGHTS) FOR PRE-PREGNANCY TREATMENT GROUPS

Group	% Gestational Weight Gain (±SEM)	Number of Pups Born (±SEM)		Birth Weights (g) (±SEM)	
		Male	Female	Male	Female
LQ EtOH	38.7% ± 1.77	7.7 ± 0.620	7.2 ± 0.405	6.37 ± 0.158	5.98 ± 0.157
LQ Control	33.9% ± 2.32	7.8 ± 0.410	7.8 ± 0.683	6.35 ± 0.171	5.92 ± 0.145
LC	39.2% ± 2.43	7.6 ± 0.434	7.0 ± 0.492	6.28 ± 0.105	6.13 ± 0.119
GV EtOH	26.2% ± 2.94	4.8 ± 0.703	6.7 ± 0.760	6.67 ± 0.434	6.17 ± 0.396
GV Control	28.5% ± 5.99	5.7 ± 0.606	6.3 ± 0.644	7.11 ± 0.361	6.98 ± 0.401

There was a main effect of condition on female pups' birth weights, $F(4,44) = 3.28$, $p = 0.02$; female pups born to mothers in the gavage control group were significantly heavier than female pups in all other groups (see Table 1). Pup weights on PN 5, 11, and 16 did not reveal any additional weight differences between any of the five treatment conditions.

Behavioral Testing

Righting reflex. There was a significant interaction between condition and day of testing for the latency to right onto the stomach, $F(20,405) = 2.28$, $p = 0.001$. Posthoc means comparisons, shown in Fig. 1, revealed that on PN 1 the gavage ethanol (EtOH) group had significantly longer latencies than either the lab chow control group or the gavage control group, which did not differ from each other. In addition, on PN 1 both the liquid diet ethanol group and the liquid diet control group had longer latencies to right than did the lab chow control group or the gavage control group. However, the latencies for both liquid diet groups did not differ from each other, indicating a stress effect of liquid diet treatment. On PN 3 and 4, the gavage control group had longer latencies than all other groups. There was also a significant effect of day, $F(5,405) = 54.90$, $p < 0.001$; latencies to right decreased over days of testing.

Negative geotaxis. Figure 2 shows a significant interaction

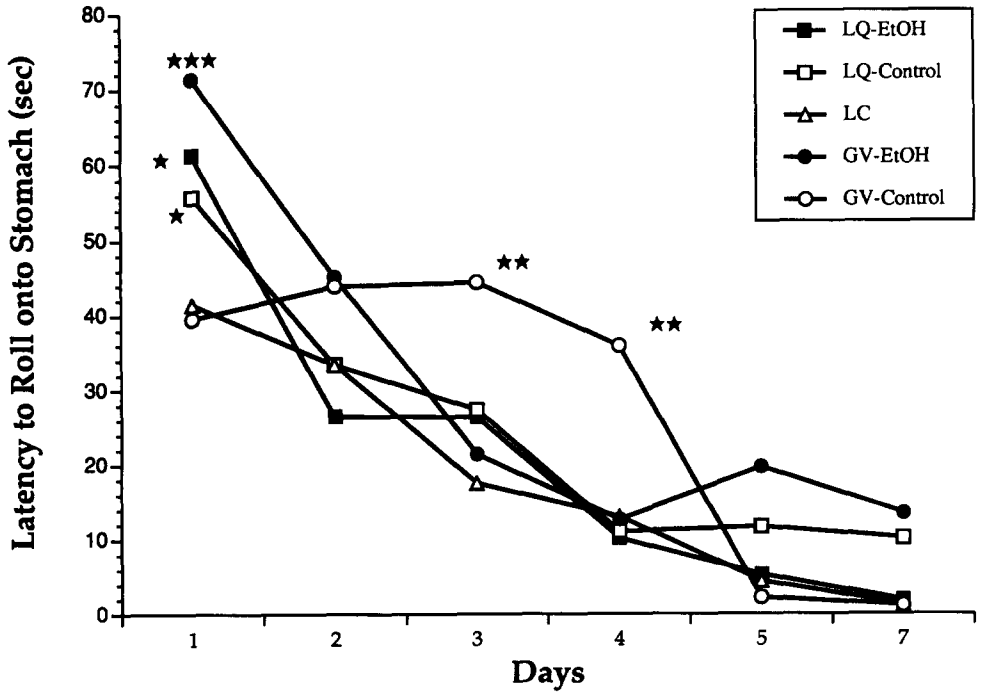

FIG. 1. Righting latency (time in s to roll onto the stomach from the back) in offspring of dams administered either a chronic ethanol liquid diet (LQ-EtOH), a pair-fed liquid diet (LQ-Control), standard laboratory chow (LC), an acute ethanol gavage intubation of 4 g/kg (GV-EtOH), or a control vehicle gavage intubation (GV-Control). All treatments were administered prior to mating. Pups were tested on PNI-5 and PN 7. *Significantly different from LC and GV-Control; **significantly different from all other groups; ***significantly different from all three control groups.

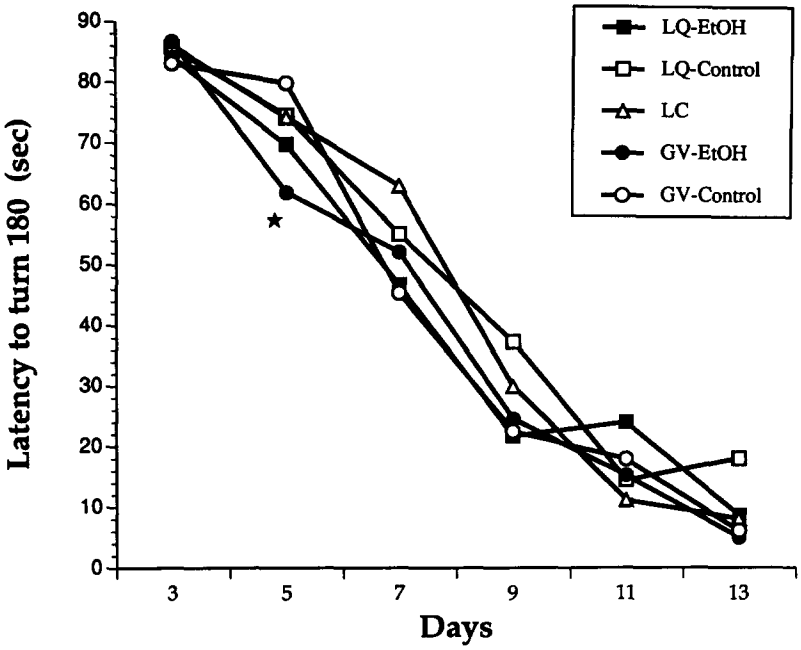

FIG. 2. Latency in seconds to make a 180° turn on a negative geotaxis test in offspring of dams administered either a chronic ethanol liquid diet (LQ-EtOH), a pair-fed liquid diet (LQ-ControlO), standard laboratory chow (LC), an acute ethanol gavage intubation of 4 g/kg (GV-EtOH), or a control vehicle gavage intubation (GV-Control). All treatments were administered prior to mating. Pups were tested every other day from PN 3-13. *Significantly different from LC and GV-Control.

between condition and day for the latency to turn 180° on a ramp, $F(20,405) = 1.86$, $p = 0.01$. Posthoc means comparisons revealed the only significant difference was found on PN 5. The gavage ethanol (EtOH) group had shorter latencies when compared to the gavage control group and the lab chow control group. Control groups did not differ from each other. There was also a significant effect of day, $F(5,405) = 276.76$, $p < 0.001$; latencies to turn decreased over days of testing.

Latency to ascend a wire-mesh surface. There was a significant interaction of day of testing, trial, and condition, $F(32,648) = 2.15$, $p = 0.0003$. Results for PN 5, 9, and 13 are shown in Figs. 3, 4, and 5, respectively. Posthoc analysis of the three-way interaction of day, trial, and condition revealed a stress effect of the liquid diets on PN 5, shown in Fig. 3, and on PN 13, shown in Fig. 5. Both the liquid diet ethanol and the liquid diet control group had significantly longer latencies than did the lab chow and gavage control groups for the first daily trial on PN 5 and longer latencies than the lab chow control group for the last daily trial on PN 13. These two liquid diet groups did not differ from each other.

Figure 4 illustrates a stress effect of gavage treatment on PN 9. Both gavage groups had significantly longer latencies than all other groups for the first trial. Additional gavage stress effects were observed; however, results were complicated by an interaction with a gavage EtOH effect. Figure 3 demonstrates an effect of ethanol administered by gavage. Significantly longer latencies were observed for the gavage ethanol group when compared to the gavage control group and the lab chow control group for Trial 1 on PN 5. The same effect is present for Trial 3 on PN 13, shown in Fig. 5. On both days, the latencies for the gavage control are significantly lower than the lab chow control while the latencies for the gavage ethanol are higher.

Activity testing PN 16. There was a main effect of condition on the number of squares crossed in a 5-min period, $F(4,81) = 2.73$, $p = 0.03$ (see Fig. 6). Posthoc analysis demonstrated that the gavage ethanol group crossed significantly more squares than all other treatment groups, which did not differ from each other.

Activity testing 9 weeks. Figure 7 shows the number of squares crossed in an open field for the five conditions. There was a significant effect of time, $F(2,162) = 9.51$, $p = 0.0001$. By the last 3-min time period, all animals had reduced the number of squares crossed. No condition group effects were observed.

Morris water maze. No significant effects of sex or condition were observed for latency to find the platform or for the probe test, although significant effects of day, $F(2,126) = 106.80$, $p < 0.001$, and trial, $F(5,315) = 22.63$, $p < 0.001$, indicated that all groups learned to find the platform. However, a significant interaction was observed between the day of testing and condition for the latency to start swimming, $F(8,136) = 2.29$, $p = 0.02$, as shown in Fig. 8. Posthoc means comparisons indicated that on days 2 and 3 of the test, PN 24 and 25, respectively, both gavage groups had longer latencies than all other groups, which did not differ from each other.

Adult brain and body weights. As shown in Table 2, at 11 wk the liquid ethanol group weighed significantly less than both the liquid control group and the lab chow control group, which did not differ from each other, $F(4,46) = 3.04$, $p =$

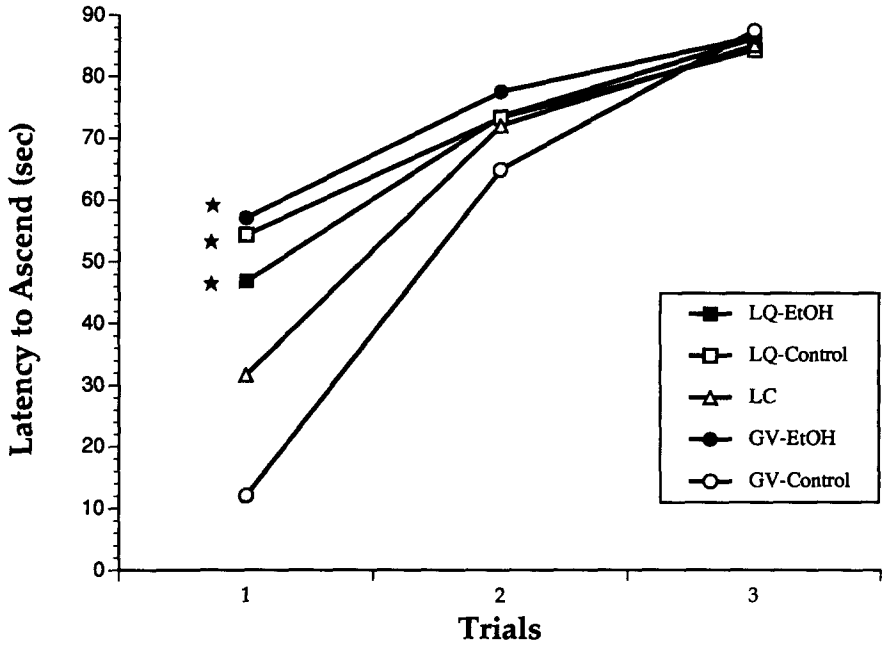

FIG. 3. Latency in seconds to ascend a wire-mesh ramp in 5-day-old offspring of dams administered either a chronic ethanol liquid diet (LQ-EtOH), a pair-fed liquid diet (LQ-Control), standard laboratory chow (LC), an acute ethanol gavage intubation of 4 g/kg (GV-EtOH), or a control vehicle gavage intubation (GV-Control). All treatments were administered prior to mating. Pups were tested for three trials. *Significantly different from LC and GV-Control.

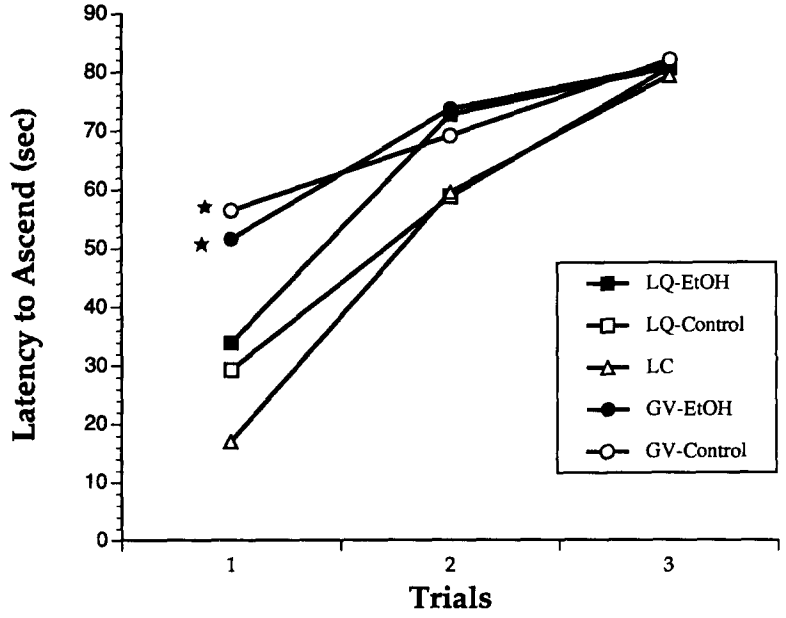

FIG. 4. Latency in seconds to ascend a wire-mesh ramp in 9-day-old offspring of dams administered either a chronic ethanol liquid diet (LQ-EtOH), a pair-fed liquid diet (LQ-Control), standard laboratory chow (LC), an acute ethanol gavage intubation of 4 g/kg (GV-EtOH), or a control vehicle gavage intubation (GV-Control). All treatments were administered prior to mating. Pups were tested for three trials. *Significantly different from all other groups.

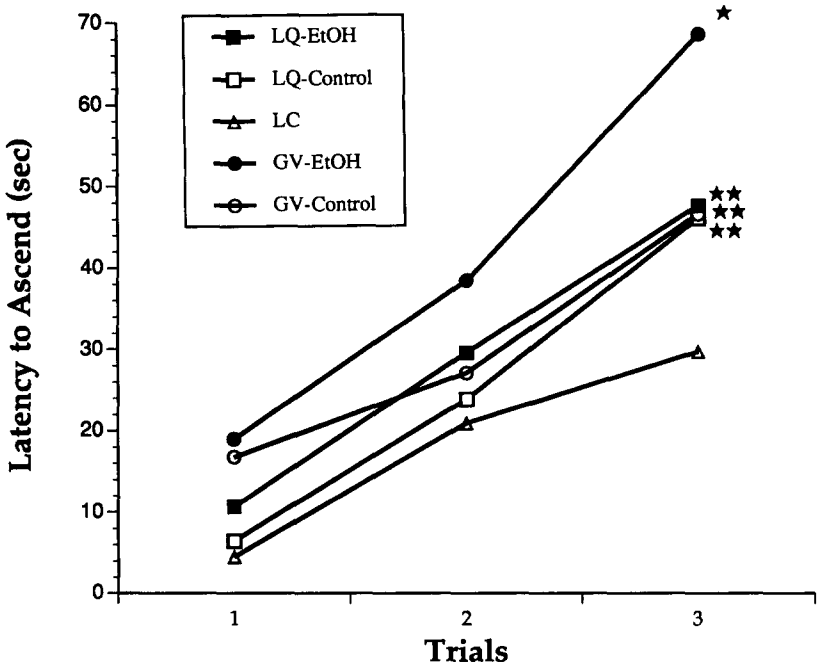

FIG. 5. Latency in seconds to ascend a wire-mesh ramp in 13-day-old offspring of dams administered either a chronic ethanol liquid diet (LQ-EtOH), a pair-fed liquid diet (LQ-Control), standard laboratory chow (LC), an acute ethanol gavage intuation of 4 g/kg (GV-EtOH), or a control vehicle gavage intubation (GV-Control). All treatments were administered prior to mating. Pups were tested for three trials. *Significantly different from all other groups; **Significantly different from LC.

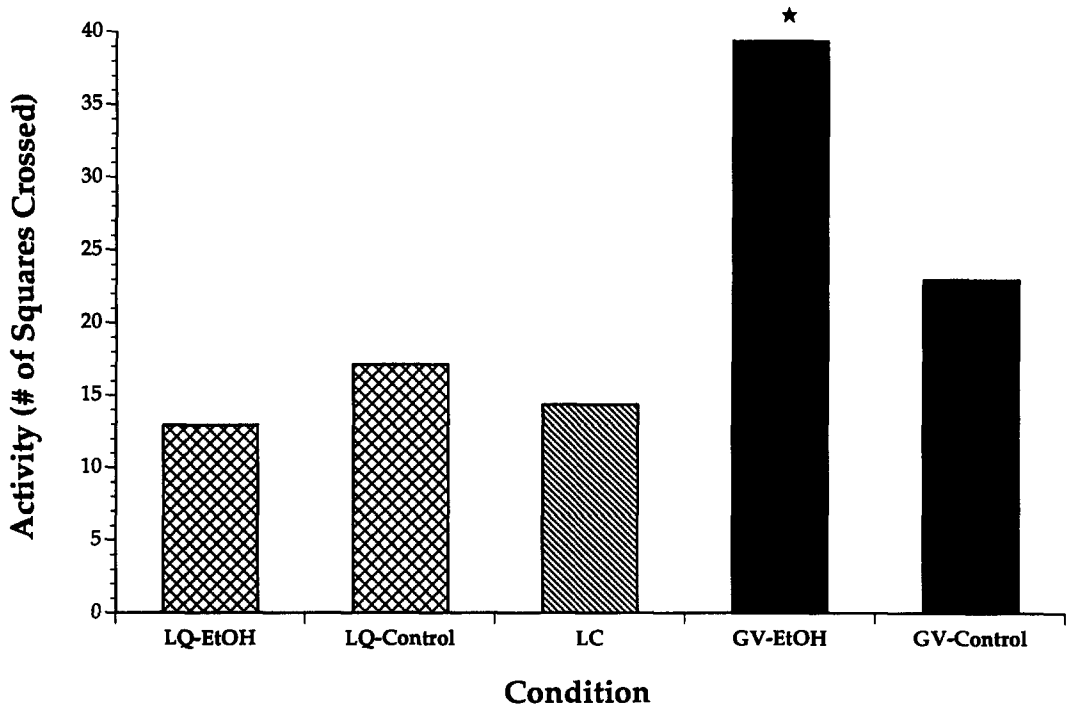

FIG. 6. Activity in an open field (number of squares crossed) in 16-day-old offspring of dams administered either a chronic ethanol liquid diet (LQ-EtOH), a pair-fed liquid diet (LQ-Control), standard laboratory chow (LC), an acute ethanol gavage intubation of 4 g/kg (GV-EtOH), or a control vehicle gavage intubation (GV-Control). All treatments were administered prior to mating. *Significantly different from all other groups.

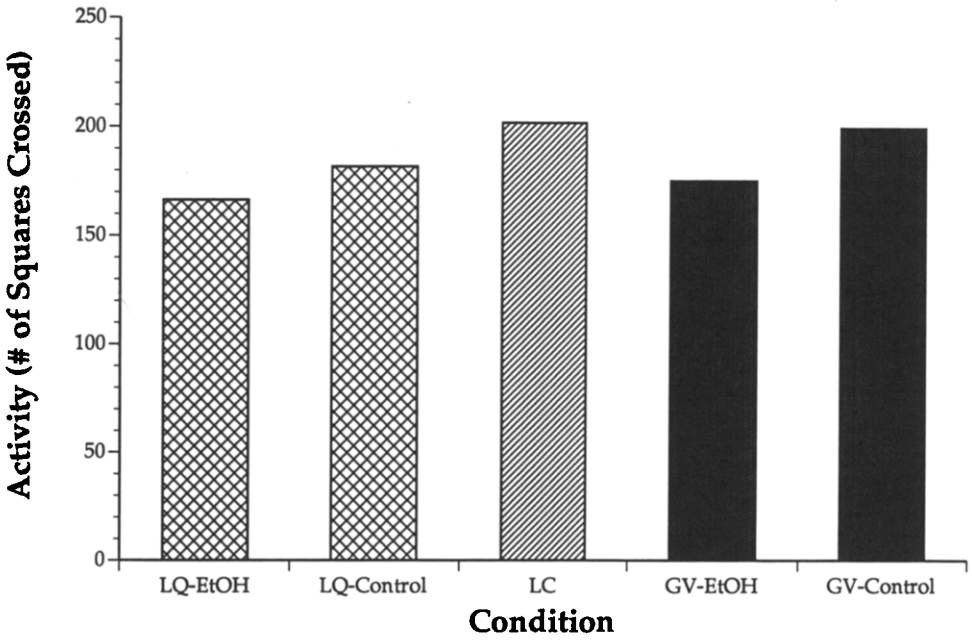

FIG. 7. Activity in an open field (number of squares crossed) in 9-wk-old offspring of dams administered either a chronic ethanol liquid diet (LQ-EtOH), a pair-fed liquid diet (LQ-Control), standard laboratory chow (LC), an acute ethanol gavage intubation of 4 g/kg (GV-EtOH), or a control vehicle gavage intubation (GV-Control). All treatments were administered prior to mating. There were no significant differences between groups.

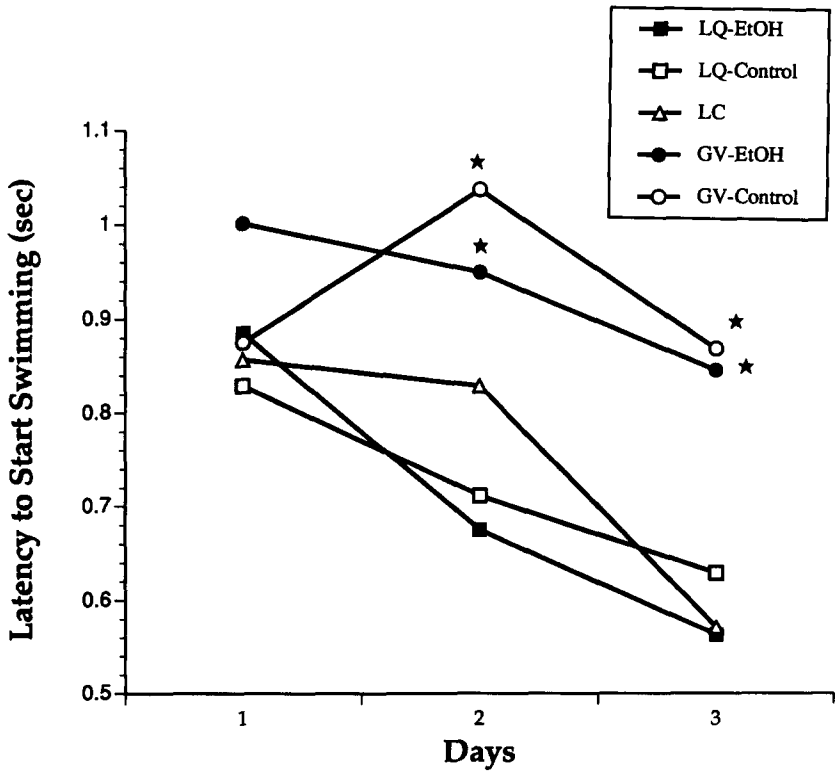

FIG. 8. Latency in seconds to start swimming in the Morris water maze test in 3-week-old offspring of dams administered either a chronic ethanol liquid diet (LQ-EtOH), a pair-fed liquid diet (LQ-Control), standard laboratory chow (LC), an acute ethanol gavage intubation of 4 g/kg (GV-EtOH), or a control vehicle gavage intubation (GV-Control). All treatments were administered prior to mating. Rats were tested for 3 days. *Significantly different from all other groups but not from each other.

0.03. Brain weights did not significantly differ among groups at 11 wk (see Table 2).

DISCUSSION

Maternal and Litter Characteristics

Maternal percent weight gain is an important dependent variable because of the teratogenic potential inherent in nutritional deficits. Nevertheless, in this study no differences that could be attributed to ethanol were observed. The significantly lower maternal percent weight gains for both gavage groups as compared to the lab chow control group and the liquid diet control group are probably attributable only to a stress effect of gavage treatment.

Given the stress-related deficiency in maternal percent weight gain in the gavage groups, it was not surprising to find significantly smaller numbers of male pups in both gavage groups as compared to all other groups. Ethanol did not affect the number of pups, as there was no significant difference between the means in the gavage control group and the gavage ethanol group. However, ethanol did affect the birth weights of female pups from the ethanol gavage group in that they weighed significantly less than those of the gavage control group.

During the third week of ethanol administration, rectal temperatures for the liquid diet ethanol group were lower than temperatures for the liquid diet control group on the second day measurements were taken. However, temperatures did not differ between the liquid diet ethanol group and the lab chow control group. Typically, ethanol withdrawal is accompanied by hyperthermia. However, this characteristic rebound increase in temperature was not observed for the liquid diet ethanol animals. The lack of such a withdrawal effect is important to rule out because hyperthermia itself is teratogenic (27).

At 11 wk of age, offspring from the liquid diet ethanol condition weighed significantly less than offspring from either the liquid diet control or the lab chow control condition, which did not differ from each other. The fact that adults weighed less suggests that they ate less as adults or had an abnormal metabolic state, as suggested by Ledig's group (20). Additional studies are needed to confirm this delayed effect of low-dose, chronic prepregnancy ethanol exposure on the growth of adult offspring and to study its physiological processes.

Behavioral Testing

In light of the seemingly random results obtained from the neuromotor tasks as a whole, it is necessary to examine the results of each task separately and consider the conditions inherent to that particular task. It is proposed that these results are not the function of one deficit but two: abnormal activity levels and motor coordination developmental delays. Neuromotor tasks such as righting reflex, negative geotaxis, and ascending a wire-mesh surface are designed to test motor coordination but not activity per se. Nevertheless, in light of the fact that the gavage ethanol offspring were hyperactive at PN 16, activity levels may also have been a factor in their performance of the neuromotor tasks. This hyperactivity could be masked in some of the tasks because the tasks were too challenging in terms of motor skills. In such a case, offspring would demonstrate longer latencies than normal to complete the task. For tasks that were not as challenging, hyperactivity could be expressed, resulting in shorter latencies than normal to complete the task.

Righting reflex. The gavage ethanol group had longer latencies on PN 1, suggesting delayed motor development due to ethanol. By PN 2, a developmental effect of ethanol was not apparent.

Stress effects of both treatment procedures are seen in these results. On PN 1, both liquid diet groups have significantly longer latencies than the lab chow control group. This effect is temporary. On PN 3 and 4, the gavage control group had longer latencies to turn from their stomach onto their back compared to all other groups. This group had essentially the same latencies on the first 4 days of testing, unlike the other groups, whose latencies dropped markedly. These significant differences on the third and fourth days indicate that the stress of gavage may be associated with delayed motor coordination. An ethanol-associated hyperactivity in the gavage ethanol group could have reversed the gavage effect in the gavage ethanol group.

Negative geotaxis. Significant differences for negative geotaxis were observed only during one day of experimentation. On PN 5, the gavage ethanol group had significantly shorter latencies to turn 180° on a ramp when compared to the gavage control and lab chow control groups. These shorter latencies may be indicative of hyperactivity. However, given the number of test days and trials this small effect is probably not meaningful.

Wire-mesh surface. An effect of ethanol in the gavage group was noted on all three days of testing, suggesting a developmental delay in motor coordination. The effect on PN 9, however, cannot be reliably attributed to ethanol since the gavage control group was also slower than the three other groups and did not differ from the ethanol gavage group. Gavage alone effects on PN 9 and 13 parallel the righting reflex results, indicating that the stress of gavage may be associated with a deficit in motor coordination.

There was no apparent effect of ethanol in the chronic, low-dose condition, but there did seem to be an effect of liquid diet administration per se on PN 5 and 13. On both occasions, the liquid diet control group and the liquid diet ethanol group had significantly longer latencies than the lab chow control but did not differ from each other.

Activity testing. An effect of prepregnancy acute, high-dose ethanol administration was observed for 16-day-old pups but not for adult offspring, in contrast to Ledig's groups' results (18). For activity testing on PN 16, the number of squares crossed by the gavage ethanol group was significantly higher than all other treatment groups. This effect is indicative of hyperactivity.

Morris maze. There was no prepregnancy effect of ethanol on learning in the Morris water maze. Another stress effect of gavage treatment was observed for the latency to start swimming on PN 24 and 25. Both gavage groups demonstrated longer latencies to start swimming. This effect could be reflective of motor incoordination deficits due to the stress of prepregnancy gavage intubation.

CONCLUSIONS

The administration of high-dose, binge ethanol treatment prior to pregnancy did result in behavioral deficits in the offspring. Offspring of the gavage ethanol condition were hyperactive when tested in an open field at 16 days of age. Hyperactivity may have also been responsible for significant differences in righting reflex and negative geotaxis tasks. Evidence for delayed motor coordination was also observed in these tasks in the high-dose, gavaged ethanol offspring.

TABLE 2

ADULT BODY AND BRAIN WEIGHTS AT 11 WK OF AGE FOR PREPREGNANCY TREATMENT GROUPS

Group	Adult Body Weights (g) (±SEM)		Brain Weights (g) (±SEM)	
	Male	Female	Male	Female
LQ EtOH	426.4 ± 19.6	266.5 ± 6.69	1.86 ± 0.04	1.70 ± 0.03
LQ Control	507.2 ± 30.7	280.6 ± 13.9	1.83 ± 0.04	1.66 ± 0.04
LC Control	502.0 ± 17.8	310.3 ± 9.32	1.92 ± 0.09	1.63 ± 0.03
GV EtOH	462.8 ± 21.0	282.0 ± 8.04	1.84 ± 0.04	1.73 ± 0.06
GV Control	480.0 ± 40.0	287.0 ± 18.0	1.78 ± 0.08	1.71 ± 0.06

The effects of low-dose, long-term exposure to ethanol prior to pregnancy was minimal; only weight deficits were observed in adult offspring. These results thus do not directly support those of Ledig's group (18), but do suggest that further research in this animal model is necessary since in both studies hyperactivity was associated with prepregnancy ethanol.

Interestingly, the administration of solutions by gavage just prior to mating was stressful enough to produce behavioral effects in the offspring. Gavage animals experienced lower percent maternal weight gain and gave birth to fewer male pups. In addition, offspring were slower for the righting reflex, in ascending the wire-mesh surface, and to start swimming in the Morris water maze. Gavage treatment prior to conception appears to retard the motor performance of offspring. The chronic administration of a restricted liquid diet to dams prior to mating was also associated with subsequent behavioral deficits in offspring. Offspring were slower for the righting reflex and in ascending a wire-mesh surface.

The gavage stress effects may overlap with gavage ethanol effects because of similar effects upon corticosterone levels. Baron and Brush (7) observed that acute restraint stress during estrus resulted in higher corticosterone levels than controls. Similarly, Cicero (9) reported that acute ethanol administration at high doses can result in elevated corticosterone levels in animals and humans. Whether these elevated levels of corticosterone have a subsequent effect on the maturation of the oocyte or on behavioral characteristics of the dams is unclear. There is also no data on whether such effects on maturation or behavior will result in deficiencies in the offspring such as hyperactivity and poor motor performance. Nevertheless, corticosterone levels are a factor to keep in consideration.

Weight differences found in adult offspring of the liquid diet ethanol condition are reflective of the metabolic effects found by Ledig and coworkers (18) after administration of a similar low-dose liquid diet ethanol solution prior to pregnancy. Weight differences were not observed by Ledig's group during early offspring development, in agreement with the findings of this study. Ledig's group found lower levels of nonneuronal enolase, SOD, and alcohol dehydrogenase (ADH) in the brains and livers of offspring treated with ethanol prior to pregnancy. Although all these enzymes are associated with the breakdown and removal of ethanol from the body, they are also associated with regular metabolic processes. For example, nonneuronal enolase is also used in the glycolytic pathway. It is very likely that the weight differences at 11 wk are connected to the deficiencies of these metabolic and enzymatic chemicals.

In addition, adult weight differences at 3 to 4 wk of age were observed by Friedler and Cochin (13) after prepregnacy treatment with morphine. Obviously, the effects of morphine and ethanol as teratogens will vary considerably. However, it is important to note that both prepregnancy treatments resulted in weight differences not at birth or early in development, as is typical in FAS, but much later at 3–4 wk and at 11 wk, respectively.

It is possible that the mechanism of prepregnancy ethanol teratogenesis is associated with ethanol's effect on hormone levels. Progesterone is directly involved in the maturation of the egg, while estrogen regulates the production of structural and maintenance materials for the maturing egg, regulates the liver, which provides yolk proteins, carrier proteins for cholesterol, and vitamins, and regulates the oviduct, which provides the surface membrane for the maturing egg (29). Moor and coworkers (23) and Moor and Osborn (22) reported that steroid alterations, specifically the reduction of estrogen levels and the enhancement of progesterone activity in the first 8 h of maturation, resulted in developmental abnormalities at fertilization. These hormonal alterations were achieved through the in vitro use of SU 10603, which selectively inhibits 17 ∂-hydroxylase. Developmental abnormalities present at fertilization will vary depending on the specific steroid environment. For the reduction of estrogen and the enhancement of progesterone, the abnormalities consisted of irregular patterns of protein synthesis in the maturing oocyte. In addition, only 13% of oocytes treated with SU 10603 underwent normal fertilization. This was raised to 50% if the administration of SU 10603 was delayed until the eighth hour of maturation or exogenous steroids were added to the follicle cells.

Hormonal imbalances as a result of ethanol exposure have the potential to cause abnormalities in the ococyte at fertilization. This in turn may result in developmental deficiencies or other problems in the offspring. Imbalances in both estrogen and progesterone have been reported as a result of ethanol exposure both during pregnancy and prior to pregnancy. Depending on the dose and duration of ethanol administered, estrogen and progesterone levels were significantly reduced or increased (5,16,21,30). Dees and coworkers (10) recently reported that prepubertal female rats administered a liquid ethanol diet had significantly lower body and reproductive organ weights as compared to control animals. A significant decrease in the serum concentration of luteinizing hormone (LH) was also observed for the ethanol-treated females. A decrease in LH can be linked to abnormalities in the maturation of the oocyte via its regulation of the follicle cells, where estrogen and progesterone are released.

This proposed mechanism is supported by the fact that behavioral effects of ethanol in offspring were limited to the gavage group and gavage treatment limited ethanol exposure to the time when the oocyte was developing. Whether the effect is the result of hormonal imbalances as a result of etha-

nol teratogenesis remains to be determined; future studies should include assays to determine estrogen and progesterone levels before and during gavage treatment. From the data gathered by this study, in addition to previous research by Ledig's group and associated research by Abel's laboratory, it is apparent that ethanol intake prior to pregnancy is something that must be considered by potential mothers. Although the effects are significantly less extensive than FAS effects and will vary from individual to individual, it is nonetheless a significant aspect of FAS and is potentially dangerous to offspring.

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