Ovarian stimulation by exogenous gonadotrophins in fetal ethanol-exposed immature rats

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Summary. Adult pregnant rats were given either an ad libitum liquid diet containing 5% ethanol, a pair fed liquid diet or an ad libitum diet of rat chow and water administered throughout pregnancy and during the nursing period. The female offspring received either pregnant mare's serum gonadotrophin (PMSG) or PMSG followed by human chorionic gonadotrophin (hCG) at 30 days of age. The ovaries of fetal ethanol-exposed animals responded greater to the exogeneous gonadotrophins with enhanced ovarian weights, increased numbers of ova shed, greater numbers of corpora lutea and antral follicles, and higher serum progesterone levels than in animals exposed to the control diets during gestation.
Key words. Fetal ethanol; ovary; progesterone.

Ethanol exposure during gestation results in a group of fetal abnormalities collectively referred to as the fetal alcohol syndrome [1]. Suppression of general body growth and brain development are major abnormalities found in both human [2, 3] and animal studies. The developing central nervous system is particularly susceptible to ethanol exposure [4, 5]; other experimental observations include increased incidence of stillbirths, decreased body weight of the offspring and smaller litter numbers [6, 7]. One specific area of the brain which has been demonstrated to be affected by fetal ethanol exposure is the sexually dimorphic nucleus of the preoptic area [8, 9], an area which contains neurons associated with regulation of reproductive function [10].

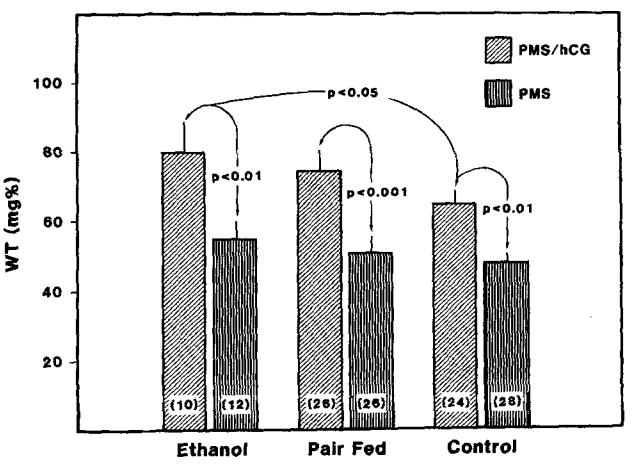
It has been demonstrated that chronic ethanol exposure in the non-pregnant female rat results in the reduction of ovarian function [11, 12] and that the effects of ethanol on reproduction are probably due to an ethanol-induced inhibition of hypothalamic LHRH release [13]. The objective of this study is to investigate the effects of in utero and postnatal ethanol exposure on reproductive function in the female rat by giving exogenous gonadotropic hormones to stimulate follicular development and induce ovulation.

Materials and methods. Adult pregnant Sprague-Dawley rats (Sasco, Omaha, NE, USA) were maintained on one of three dietary regimens. Each pregnant animal was provided either a nutritionally balanced ad libitum liquid diet containing ethanol (5%, v/v; Bio-Serve, Inc., Frenchtown, NJ, USA), a liquid diet in which maltose dextrin was isocalorically substituted for ethanol and pair fed with matched animals in the ethanol group, or a laboratory chow and water diet, ad libitum [8]. Animals were maintained on their respective diets from the first day of pregnancy and continued throughout the gestational and nursing periods. On the day of delivery, the offspring were sexed by anogenital distance and the male pups were removed from the litters. Female pups were allowed to remain with their respective mothers until the time of weaning after which all offspring were provided free access to laboratory chow and water.

Each female offspring rat received a s.c. injection of pregnant mares serum gonadotrophin (PMSG; 20 IU) on day 30 at 12.00 h. 56 h later, half of the offspring from each of the three dietary treatment groups received a s.c. injection of human chorionic gonadotrophin (hCG; 10 IU). 76 h after the initial injection of PMSG, each animal was weighed and killed by decapitation. Blood was collected and serum harvested. The uterus and ovaries were removed and each weighed to the nearest 0.1 mg. Ova were recovered from the isolated oviduct and counted. The ovaries were fixed, processed, serially sectioned and stained. The presence and numbers of corpora lutea and mature follicles were made by microscopic analysis. Serum progesterone was measured by radioimmunoassay (Diagnostic Products Inc., Los Angeles, CA, USA). All data were subjected to a one-way analysis of variance (ANOVA). When significance was indicated ($p < 0.05$), the data were further examined by a t-test for differences between means.

Results and discussion. Daily maternal ethanol consumption by the pregnant dams ranged from 6 to 8 g/kg b.wt. No gross physical deformities were observed in any of the offspring in any of the groups at birth. The average number of pups delivered per litter (12 ± 1) was reduced in the group that received ethanol during the gestational period (8 ± 2). Body weights of female rats at 30 days of age showed that the fetal ethanol-exposed animals weighed less (76.9 ± 6.5 g) than the offspring from mothers fed the laboratory chow diet during gestation (88.0 ± 2.5 g). Uterine weights were not significantly different with regard to gonadotrophic treatment or dietary exposure during the gestational period.

The figure illustrates the ovarian weights expressed relative to body weight (mg%). Ovarian weights of offspring from the ethanol and control groups given PMSG and hCG were significantly greater ($p < 0.01$) than ovarian weights from offspring given PMSG alone. The ovarian weight of the fetal ethanol-exposed rats given PMSG and hCG were significantly greater ($p < 0.05$) than the ovarian weights of the female rats derived from mothers which received the control diet during gestation. There were more ova shed from fetal ethanol-exposed offspring given PMSG and hCG than those from offspring given the gonadotrophins from mothers on either of the control diets (table). Serum progesterone levels in the fetal ethanol-exposed offspring given PMSG and hCG were higher ($p < 0.05$) than the hormone levels of the control offspring given both exogenous gonadotrophins (table).



Ovarian weight relative to body weight (g/100 g b.wt; mg%) of immature female rats exposed to ethanol or control diets following PMSG/hCG or PMSG administration. The vertical lines at the top of the bars represent the standard error of the mean (SEM). The numbers in parentheses indicate the observations in each group.

Effects of PMSG/hCG treatment on female rats exposed to the respective diets in utero.

Treatment	Serum progesterone (ng/ml)	Ova shed	Antral follicles	Corpora lutea
Ethanol	119.2 ± 4.6	46 ± 9	9 ± 1	17 ± 1
Pair fed	74.4 ± 11.5 [b]	38 ± 5	4 ± 1 [b]	10 ± 1 [b]
Control	89.8 ± 12.0 [a]	38 ± 5	6 ± 1	11 ± 1 [a]

[a] $p < 0.05$ vs ethanol; [b] $p < 0.01$ vs ethanol.

Furthermore, the table indicates that histological examination of the ovaries showed a greater number of corpora lutea present in the ovaries from animals exposed to ethanol in utero than in the female rats whose mothers received the pair fed diet ($p < 0.01$) or the control diet ($p < 0.05$) during pregnancy. There were also more antral follicles present in the ovaries in female rats subjected to ethanol in utero compared to animals treated with the pair fed diet or control diet during gestation.

The results indicate that the exogeneously administered gonadotrophins stimulated uterine growth equally in both the offspring from ethanol-exposed mothers and offspring from control mothers and that the ovaries of the offspring from each treatment are capable of responding to the exogenous gonadotrophins. However, administration of PMSG and hCG stimulated ovarian growth greater in fetal ethanol-exposed female rats than in offspring from the pair fed and control groups as evidenced by the enhanced ovarian weights and greater numbers of ova shed. The increase in ovarian weights in the ethanol animals were due to the large numbers of corpora lutea and antral follicles present in the ovary relative to those in ovaries from animals of control groups. The presence of the increased number of corpora lutea observed by histological examination corresponds to the increased number of total ova recovered and higher levels of serum progesterone measured in the ethanol-exposed offspring compared to the same parameters in offspring from control mothers. These data collectively indicate that fetal ethanol exposure may increase the sensitivity of the ovaries to the exogenous gonadotrophins.

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Secretion of the Dufour gland of the ant *Nothomyrmecia macrops* (Hymenoptera: Formicidae)

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Summary. The Dufour gland of the primitive Australian ant *Nothomyrmecia macrops* contains microgram quantities in total of linear alkanes and alkenes from C_{13} to C_{30} with heptadecene the major component (66%). In all, 50 substances, including methyl-branched hydrocarbons, aldehydes and acetates, α-farnesene and α-springene in low nanogram amounts, were identified by gas chromatography-mass spectrometry.
Key words. Ant; *Nothomyrmecia;* Dufour gland; hydrocarbons; farnesene; springene.

Nothomyrmecia macrops Clark is considered to be the most primitive living ant and is therefore interesting from many aspects, but it is extremely elusive and was probably also the species most sought by myrmecologists. Several expeditions have been organized to the region of Balladonia in Western Australia, where the first two specimens were collected [1] in 1931. All but the first were unsuccessful in finding this "holy grail to ant specialists" [2], until it was rediscovered in 1977 at Poochera, South Australia by a CSIRO field party [3].

Because of its primitive behaviour and some rather peculiar anatomical features [3], *N. macrops* has been placed in the subfamily of Nothomyrmeciinae, in which it is the only species [4]. Since its rediscovery, it has been the subject of several studies, dealing with its general anatomy and phylogenetic position [3], its behaviour [5], genetics [6], sting morphology [7], and, in connection with the present work, its gland ultrastructure [8]. However, until now, no investigations have been made on the chemical secretions of this species. During a recent field trip to Poochera, foraging workers of *N. macrops* were collected and various tissues prepared for chemical analysis.

The Dufour gland is a bulbous organ attached to the poison apparatus in all aculeate Hymenoptera. In all ant species that have been studied, it is filled with an oily mixture of organic substances [9]. Its primary purpose is unknown, though in individual cases it has been shown to contain a trail pheromone, a home range or territorial marking pheromone, and to have other pheromonal properties. Hölldobler and Taylor have already shown, in a simple behavioural test, that *N. macrops* workers were alerted by, and attracted to, the secretion of their Dufour glands [5].