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## Reproductive Competence in the Golden Hamster Following Prenatal Alcohol Exposure

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REPRODUCTIVE COMPETENCE IN THE GOLDEN HAMSTER FOLLOWING  
PRENATAL ALCOHOL EXPOSURE

by

JENNIFER JAYNE SWIATEK

A Thesis Submitted to the Faculty of the Graduate School of  
Loyola University of Chicago in Partial Fulfillment of the  
Requirements for the Degree of  
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## VITA

The author, Jennifer Jayne Swiatek, is the daughter of James M. and Jayne B. Swiatek. She was born on November 20, 1963 in Chicago, Illinois.

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## TABLE OF CONTENTS

	Page
ACKNOWLEDGMENTS.....	ii
VITA.....	iii
LIST OF TABLES.....	iv
LIST OF FIGURES.....	v
Chapter	
I. INTRODUCTION.....	1
II. REVIEW OF RELATED LITERATURE.....	3
The History of Fetal Alcohol Syndrome.....	3
What is Fetal Alcohol Syndrome?.....	4
Human Studies of Prenatal Alcohol Exposure... ..	5
The Use of an Animal Model.....	6
The Golden Hamster as an Animal Model.....	7
Administration of the Ethanol.....	10
The Effect of Alcohol Exposure on the Reproductive Function of Laboratory Animals.....	11
The Effect of <u>In Utero</u> Alcohol Exposure on Female Reproduction in Laboratory Animals....	13
III. MATERIALS AND METHODS.....	16
Experiment I.....	16
Prepubertal Autopsy.....	19
Postpubertal Autopsy.....	20
Reproductive Competence in Adult Females Exposed to Alcohol <u>In Utero</u> .....	20
Experiment II.....	21
Determination of Blood Alcohol Levels.....	23
Statistical Analysis.....	24
IV. RESULTS.....	26
Experiment I.....	26
Experiment II.....	31
Blood Ethanol Levels.....	34

V. DISCUSSION..... 37

REFERENCES..... 70

## LIST OF TABLES

Table	Page
I. The Total Number of Pups Present at Parturition, Weaning, and the Sex of the Offspring of Each Gestational Treatment Group (5% Ethanol Diet).....	49
II. Body Measurements of the Offspring of the Gestational Treatment Groups at 21 days of Age (5% Ethanol Diet).....	50
III. Age of the Female Offspring at the First and Third Estrus of the Gestational Treatment Groups (Mean $\pm$ SEM).....	51
IV. Body Measurements of the Offspring of the Gestational Treatment Groups at 28 Days of Age (5% Ethanol Diet).....	52
V. Body Measurements of the Offspring of the Gestational Treatment Groups at 60 Days of Age (5% Ethanol Diet).....	53
VI. The Total Number and Sex of Offspring Born to Mothers of Which were Prenatally Exposed to a Treatment Group (5% Ethanol Diet).....	54
VII. The Total Number and Sex of the Offspring of Each Gestational Treatment Group (15% Ethanol Diet).....	55
VIII. The Number of Pups Surviving Until Weaning (15% Ethanol Diet).....	56
IX. The Number and Weights of Fetuses Which Were Exposed to Treatment <u>In Utero</u> and Autopsied on Day 13 of Gestation.....	57

## LIST OF FIGURES

Figure	Page
1. The Percent Change in Body Weight of the Treatment Group Mothers Throughout Gestation (5% Ethanol Diet).....	58
2. The Amount of Diet (ml) Consumed by the 5% Ethanol Mothers During Gestation.....	60
3. A Comparision of the Growth Curves of the Offspring of Each Gestational Treatment Group For Five Consecutive Weeks.....	62
4. The Percent Change in Body Weight of the Treatment Group Mothers Throughout Gestation (15% Ethanol Diet).....	64
5. The Amount of Diet (ml) Consumed by the 15% Ethanol Mothers During Gestation.....	66
6. A Comparision of the Amount of Diet Consumed by the 5% Ethanol Mothers and 15% Ethanol Mothers During Gestation... ..	68



## CHAPTER I

### INTRODUCTION

Fetal alcohol syndrome (FAS) is a constellation of abnormalities seen in some offspring of chronic alcoholic women. Although a great deal of research has been performed over the last decade on the study of prenatal alcohol exposure, the impact of fetal alcohol exposure on reproductive competence is unknown. Colangelo and Jones (1982) report that neural and endocrine systems are two of the organ systems that are adversely affected by fetal alcohol exposure. Because normal reproductive function relies on normal neuroendocrine function, both neonatally and in adulthood, this suggests that alcohol-exposed animals may have impaired reproductive function.

While studying the action of alcohol on the fetus, a number of variables must be controlled. Some of the significant variables are: dose of alcohol ingested, impact of social variables, nutrition, and the use of other drugs. These variables generally limit epidemiological and clinical studies of prenatal alcohol effects in humans and thus an appropriate animal model is necessary to control the confounding variables (Boggan, 1982; Riley and Meyer, 1984; Weinberg, 1984). Although researchers have used the rat and

mouse as animal models for FAS, the results have produced inconsistent data (Mankes et al., 1982). The golden hamster has been chosen as the animal model for this study because of its regular four day estrus cycle and a relatively short gestation period of sixteen days (Orsini, 1961; Lisk, 1985). Additionally, the golden hamster will readily consume large quantities of sweetened ethanol in concentrations up to 40% (Arvola and Forsander, 1961; DiBattista, 1986). This thesis proposes that ethanol will have an adverse effect on pregnancy of the adult female golden hamster both with regard to behavior and fecundity. Also, it proposes that maternal consumption of ethanol will have adverse effects on the reproductive competence of the female offspring when adult (i.e., the ability to mate with normal males, conceive, and carry litters to term).

## CHAPTER II

### REVIEW OF LITERATURE

#### The History of Fetal Alcohol Syndrome

Fetal alcohol syndrome (FAS) refers to a specific pattern of abnormalities found in children born to alcoholic women. Although FAS is relatively rare in the general population, about 1.1 per 1,000 live births, it occurs considerably more often among alcoholics with 25 per 1,000 live births (Abel, 1985). The history of FAS can be traced back to Biblical as well as Greek and Roman times where in the literature there is reference to the harmful effects of drinking during pregnancy (Russell, 1982). In 1889, the first study of the relationship between maternal alcoholism and fetal outcome was reported (Abel, 1985). FAS was then "rediscovered" by Jones and Smith in 1973 which resulted in a great deal of research being performed in order to understand the effects of prenatal alcohol exposure. These studies have led to greater recognition of the need to develop methods of preventing alcoholism and alcohol abuse in women, especially those in their child-bearing years, in order to improve the health of both the mothers and their offspring (Russell, 1982).

### What is Fetal Alcohol Syndrome?

FAS is difficult to define, because like most syndromes there is no single feature (i.e., biochemical, chromosomal, or pathologic change) that can be considered a specific characteristic of fetal alcohol syndrome (Russell, 1982). What is currently necessary for diagnosis is recognition of a constellation of morphological and behavioral features in the offspring of women who had consumed excessive amounts of alcohol during their pregnancies. Jones and Smith (1973) and Clarren and Smith (1978) described this constellation in humans as including the following features:

1. Prenatal and postnatal growth retardation (including intrauterine growth retardation and congenital heart defects) and failure to thrive.
2. Defects of the central nervous system (CNS), including microcephally (small head size) and mental retardation.
3. Congenital anomalies.

The anomalies combine to produce distinctive craniofacial features such as; narrow receding forehead, short palpebral fissure (length of eye slits), low nasal bridge, short nose, narrow upper lip and flat midface. Although a few of these features may be found in the normal person, FAS is diagnosed when a significant number of these characteristics are iden-

tified (Streissguth et al., 1980; Majewski and Goecke, 1982).

#### Human Studies of Prenatal Alcohol Exposure

The mechanism of impairment of prenatal exposure to alcohol and related factors have not yet been found. In humans, the study of FAS can be limited by the choice of design experimental. Wilsnak (1982) determined that the clinical-retrospective design of most studies on reproductive dysfunction of alcoholic women does not include inferences about whether reported difficulties were antecedents or consequences of women's alcohol use. The epidemiological studies overcome many limitations of clinical studies, but they do not address the role of obstetrical-gynecological problems as antecedents and possible precipitants of women's drinking problems. Researchers have found no correlation between amounts of daily intake of alcohol during pregnancy and birth weight or duration of gestation. But the average amounts of daily alcohol consumption in grams pure alcohol are correlated with the degree of FAS in the progeny (Majewski & Goecke, 1982). Also, in human studies the prognosis of FAS depends on the persistence of anomalies which may account for the defects observed and the ability to detect such anomalies. Therefore, transient effects produced by alcohol in utero would not be detected by examining the newborn. Another limitation is that the subjects of these studies may not be a representative sample

(Russell, 1982). While studying the action of alcohol on the fetus, a number of variables must be controlled, such as the use of drugs, smoking and poor nutrition. These variables generally limit epidemiological and clinical studies and thus an appropriate animal model is necessary to control the confounding variables (Abel, 1982; Boggan, 1982; Riley and Meyer, 1984; Weinberg, 1984).

### The Use of an Animal Model

Some animal models have characteristics similar to humans and thus provide the researcher with the option of controlling and manipulating variables which are difficult to control in human studies. The test animal should also absorb, metabolize, and eliminate drugs in a manner similar to that seen in humans. However, no one species can accommodate all of the criteria (Riley and Meyer, 1984). Thus, the search for appropriate animal models for the study of alcoholism has been ongoing. In choosing an appropriate animal model, numerous factors need to be taken into consideration such as the biochemistry, growth and development, and behavior of a particular species. Other factors that need to be taken into consideration are the availability of the animals themselves, housing facilities, funds to care for the animals, and experience in dealing with the animals (Boggan, 1982; Riley and Meyer, 1984).

Although researchers have used the rat and mouse as two predominant animal models for FAS, the results have produced inconsistent data (Mankes et al., 1982). Mice also have an additional disadvantage by possessing a higher rate of spontaneous malformations. Other species have been used, dog (Ellis and Pick, 1976), pig (Dexter et al., 1980), and monkey (Golub, 1979). The major disadvantages are size, cost, seasonal breeding patterns (dogs and pigs) and small litter size.

#### The Golden Hamster as an Animal Model

There are four major reasons why the golden hamster is a particularly attractive animal model in which to perform these experiments. One reason is that hamsters have relatively few spontaneous diseases due to susceptibility to the many introduced pathogenic agents as compared to other laboratory rodents. Further golden hamsters have been found to have less than a 0.1% spontaneous malformation rate (Ferm, 1967).

A second reason is that golden hamsters will preferentially consume ethanol solutions rather than water at concentrations of up to 25% and will voluntarily consume substantial amounts of ethanol even at a concentration of up to 40% (Kulkosky, 1978; DiBattista, 1986). Despite this remarkable rate of consumption and the attainment of high blood ethanol levels, prolonged ethanol intake by hamsters

does not produce either the behavioral symptoms of tolerance and dependence or the physiological effects upon the liver typically observed in the other species (McMillan et al., 1977; Harris et al., 1979; DiBattista, 1986). The volume of liquid consumed decreases as ethanol concentration rises, so that the ethanol intake remains fairly constant at concentrations from 10-40% (McMillan et al., 1977). It has also been shown that as the ethanol concentration is increased, the percentage of calories consumed as ethanol also increased. At its highest concentration, ethanol comprised over half the total caloric intake (McCoy et al., 1981). The activity of alcohol dehydrogenase (ADH), located almost entirely in the liver, has been shown to be the most important rate determining factor in the oxidative metabolism of ethanol (Kulkosky and Cornell, 1979). McCoy et al. (1981) suggest that the differences in alcohol-metabolizing abilities among the various animal species could provide an explanation for their different levels of alcohol consumption. The fact that hamster liver postmicrosomal supernatants have three times the activity of ADH of similar preparations from rats suggests that greater ethanol metabolism could be a factor associated with the hamsters' higher ethanol consumption. Also, that the rate of ethanol disappearance from hamsters lies between the rates for rats and mice, consistent with the concept that



the ethanol-elimination rate correlates positively with basal metabolic rate.

A third reason for the selection of the golden hamster as an animal model is its reproductive physiology and behavior. The golden hamster is a "long-day" seasonal breeder which is reproductively functional when housed under a photoperiod of 14L:10D (Van Hoosier and Ladiges, 1984). In the female golden hamster, the first ovulation occurs between 26 and 30 days after birth (Greenwald and Pepler, 1968; Lisk, 1985) while the first behavioral estrus is seen from 26-43 days of age (Diamond and Yanagimachi, 1970). Once the estrus cycle of the hamster is established it results in a four day estrus cycle which can be determined with accuracy. Estrus occurs in the female between six and eight weeks of age when she weighs 90-100 grams (Van Hoosier and Ladiges, 1984; Lisk, 1985). On the day of ovulation, there is a postovulatory vaginal discharge that is readily recognizable because of its copious nature, tacky consistency, and strong odor. This marks the day of estrus or Day 1 of the hamster 4-day estrous cycle (Orsini, 1961). Thus the estrous cycle of the hamster is particularly easy to follow by monitoring the estrous discharge.

The fourth reason for the selection of the golden hamster is its rapid development and short life cycle. The hamster has a gestation period of 16 days which is the shortest of any eutherian mammal (Lisk, 1985). When exposed

to a 14L:10D cycle, hamsters deliver between 4-12 pups, with 6-8 being the most common (Van Hoosier and Ladiges, 1984).

#### Administration of the Ethanol

Ideally, potential teratogens should be administered to animals by the same route that exposure occurs in humans and for alcohol the obvious choice is the oral route (Riley and Meyer, 1984). The study of FAS in the laboratory is hampered by the fact that most animals will not voluntarily consume sufficient quantities of ethanol. Investigators studying FAS use three main routes of ethanol administration. One is by injection of ethanol which has the advantage of easy administration and the dosage of ethanol can be controlled. The major disadvantage is that this route does not simulate human use. A second avenue is by intubation which has the advantage that it simulates human route and method of intake and also allows for the administration of relatively large volumes of ethanol. The disadvantage is that it does not simulate human use and that the tube may stress and/or injure the animal. Liquid diet has the advantage that it simulates human route and method of intake and unlike injection procedures, steady blood alcohol levels can be achieved. The disadvantage is that given as a sole food source, intake may be less than normal and there is a question of the nutritional adequacy of diets

(Leichter and Lee, 1982; Riley and Meyer 1984; Boggan, 1982).

The Effect of Alcohol Exposure on the Reproductive Function of Laboratory Animals

The direct effect of ethanol consumption on reproductive function has been examined in various animal models. Mello et al. (1983) showed that alcohol, rather than some other factor such as malnutrition or liver disease, was the main reason for disruption of menstrual cycle regularity in the monkey. The study also suggests that alcohol produces a toxic effect on the ovary which somehow inhibits menstruation in the treated animals. Several investigators have studied the effect of alcohol on ovarian function using the rat as an animal model. Van Thiel et al. (1978) and Gavaler et al. (1980) concluded that chronic alcohol treatment results in ovarian atrophy (i.e., loss of ovarian mass) in the exposed animals. They also concluded that the histologic appearance of the fallopian tubes and uteri of the treated animals were similar to those seen in ovariectomized female rats which had markedly reduced tissue mass compared to control animals. Other studies, however, have shown that alcohol exposure only suppresses ovarian function rather than resulting in ovarian failure. Bo et al. (1982) and Krueger et al. (1982) proposed that ovarian function was suppressed by the treatment with alcohol but was not abolished because the alcohol-consuming rats were

still capable of breeding and delivering viable offspring. These studies also showed that alcohol consumption caused a delay in vaginal patency and irregular estrous cycles. Further, the lengths of the estrous cycles were significantly greater than those of the controls. Therefore, the data concerning the effect of ethanol on ovarian morphology and function are somewhat conflicting.

Other investigators have studied the effect of ethanol on the hypothalamic-pituitary-gonadal axis. Normal follicular function has been found to be dependent on gonadotropins, follicle stimulating hormone (FSH) and luteinizing hormone (LH), which are secreted by the adenohypophysis. These gonadotropins are necessary to stimulate ovarian changes and the release of steroids (especially estrogens) which result in ovulation. Therefore normal follicular development only occurs when both FSH and LH are present (Schwartz, 1969). The mechanism of action by which ethanol alters the release of certain pituitary hormones is not well understood. Dees et al. (1985) suggest that decreased LH levels produced by ethanol may be due to a decrease in the release rates of luteinizing hormone releasing hormone (LHRH) from the hypothalamus. They also showed that animals which were given ethanol had decreased plasma concentrations of LH, without noticeable changes in the plasma concentrations of FSH. They concluded that ethanol inhibits the release of the neurohormone LHRH from

the hypothalamus which results in decreased pulsatile secretion of LH from the pituitary. Therefore, ethanol may inhibit the secretory activity of LHRH producing neurons without affecting the secretory activity of hypothetical follicle stimulating hormone releasing factor (FSHRF) neurons which have yet to be identified. Other investigators, however, have concluded that LH levels of alcohol-consuming animals are unchanged and therefore the alcohol acts as a direct gonadal toxin and not at the hypothalamic-pituitary-gonadal axis. They showed that moderate to low circulating levels of ethanol disrupted the normal estrous cycle without concomitant elevation in plasma LH levels (Eskay et al., 1981).

Therefore, although several studies have focused on the effect of alcohol on female reproduction, inconsistent data between investigators have lead to few conclusions about the direct effect of ethanol consumption on reproductive function.

#### The Effect of In Utero Alcohol Exposure on Female Reproduction in Laboratory Animals

Fetal alcohol exposure has been shown to affect many organ systems including the endocrine and neural systems (Streissguth et al., 1980; Colangelo and Jones, 1982). Because normal reproductive function is intricately tied to normal neuroendocrine function, both neonatally and in adulthood, these studies suggest the possibility of impaired

reproductive function in animals exposed to alcohol. Few studies have examined the effect of in utero alcohol exposure on female reproduction. Hard et al. (1985) suggest that prenatal alcohol exposure acts as an adverse environmental stimulus which interferes with the normal course of the development of the central nervous structures regulating reproductive function. They showed that rats prenatally exposed to alcohol displayed a later onset of regular behavioral estrous cycles. Other investigators have shown that sexual maturation, measured by vaginal opening, is delayed in female mice prenatally exposed to alcohol (Boggan et al., 1979). However, a study using rats showed no difference in vaginal opening, the appearance of the first vaginal estrus, nor the onset of regular vaginal estrous cycles (Hard et al., 1985).

Investigators have also begun to study the hypothalamic-pituitary-gonadal axis in prenatally exposed animals. Handa et al. (1985) reported an alteration in the adult patterns of LH secretion in female rats which were exposed to alcohol during the last week of fetal life. They believe that some of the central mechanisms controlling pituitary LH secretion are altered by alcohol, although this mechanism has not been identified. Esquifino and coworkers (1986) have shown an increase in plasma prolactin coupled with a decrease in plasma LH at the time of vaginal opening in female rats prenatally exposed ethanol. They conclude

that prenatal exposure to alcohol causes a delay in sexual maturation and produces hormonal alterations which persist into adulthood.

Therefore, although some investigation of prenatal alcohol exposure has been documented, inconsistent data between laboratories have revealed little information on the effect of ethanol exposure in utero on adult reproductive function.

## CHAPTER III

### MATERIALS AND METHODS

Adult female golden hamsters were obtained from Charles-River Breeding Laboratories. On the day of arrival, the animals were each marked by ear punch, weighed, and housed six hamsters to a cage. They were maintained under a 16L:8D photoperiod (lights on from 0500-2100h) with temperature and humidity regulated. All animals were given free access to Purina Lab Chow and tap water ad libitum. After a one week adaptation period, estrous cycles were monitored daily between 0700 and 0900h by examination of vaginal discharge until three complete estrous cycles were recorded (Orsini, 1961). Only the females showing a regular four day estrous cycle were included in the study.

#### EXPERIMENT I

On the day of proestrus, a female was placed in a polypropylene cage with a sexually experienced adult male hamster. Mating was confirmed by the presence of sperm in the vaginal smear the next morning. The male was removed and the female remained alone in the cage throughout the 16 day gestation period. The female hamsters were randomly divided into three treatment groups (pellet diet, liquid



control diet, and ethanol diet) on the first day of pregnancy. Day one of pregnancy was determined by a sperm positive vaginal smear.

To study the effects of ethanol exposure in utero, one group (N=9) received free access to a 5% ethanol-containing liquid diet (35.5% calories derived from ethanol, 12% fat, 28.1% protein, 24.4% carbohydrate) ad libitum formulated for pregnant hamsters (Bio-Serv, Inc. #1878, Frenchtown, N.J.) during gestation. A second group of females was pair-fed to the first group (N=9). In order to assure identical conditions before manipulation of the diet, each female in this group was paired to a hamster of similar weight and age in the ethanol group. These control animals received an isocaloric liquid diet in the amount consumed by its partner on the same day of gestation. The liquid control diet (Bio-Serv, Inc. #1879, Frenchtown, N.J.) was of identical composition to that of the ethanol diet with the exception that maltose-dextrin was substituted isocalorically for the ethanol derived calories (12% fat, 28.1% protein, 59.9% carbohydrate derived calories). The control liquid diet group is important in order to distinguish between effects due to ethanol exposure rather than nutritional status of the mother (Goad et al., 1984; Riley and Meyer, 1984). In addition to the liquid diet, tap water was available in separate bottles at all times. The liquid diets were kept refrigerated and fresh diet was presented daily in hamster

water bottles with sip tubes containing ball bearings (Great Lake Pet Supply, Franklin Park, IL). The bottles containing the liquid diet were placed to the left of the water bottle relative to the hamster, as hamsters show a preference for bottles positioned to their left (Slighter, 1970). A third group (N=9) of females (controls) were given ad libitum access to a lab chow pellet diet and tap water throughout gestation.

The hamsters were fed and weighed daily at 0700-0900h. Throughout gestation, the females given the ethanol diet were allowed free access to a recorded amount of diet. The next morning, the amount consumed was found by subtracting the amount given from the amount remaining. The amount consumed by the animal fed the ethanol diet was then given to its control liquid diet partner on the same day of gestation. In all the dams, the following variables were measured: 1) daily weight (g) during gestation 2) length of gestation (days) 3) number of live/dead pups at parturition in each litter.

The day before parturition, the hamsters were given shredded paper towel for nesting material. On the day of parturition, the number of pups was determined and the dams receiving liquid diets were switched to ad libitum lab chow pellets. Twenty one days after birth, the pups were weaned, sexed, marked by ear punch, and the following measurements were taken on the female offspring: body weight (g),

anogenital distance (in), tail length (in), diameter of the head (in), length of palpebral fissure (eye slit) (in), and appendage abnormalities if present. The measurements performed in inches were recorded to the nearest .001" using a caliper obtained from Bel-Art Products catalog no. 13416 (Pequannock, N.J.).

#### Prepubertal Autopsy

On day 28 after birth, between 0830-0930h, a random selection of female pups (N=6) from each treatment group (ethanol, liquid diet and pellet) whose treatment mothers were paired in the above procedure were removed from their cage and immediately decapitated by guillotine to eliminate the effects of stress. The following measurements were performed: body weight (g), brain weight (mg), pituitary weight (mg), adrenal weight (mg), the weight of ovaries + one oviduct (mg), the weight of the uterus when wet (mg), and the weight of the uterus after being dried (mg). Blood was also collected from the trunk and stored in the refrigerator until the next day when it was centrifuged. The serum was stored at -70C for later analysis by RIA to quantitate serum estradiol and progesterone. The ovaries were fixed in Bouin's solution for one day. The next day, the Bouin's solution was removed and the ovaries were rinsed quickly with distilled water and then 70% ethanol was added. During the proceeding days, the ethanol solution was removed

and replaced by fresh 70% ethanol solution until the solution containing the ovaries was colorless (approximately 30-45 days).

#### Postpubertal Autopsy

A second group of female offspring (N=6) was autopsied after 60 days of age on the morning (0830-0930h) of estrus. The same procedure and measurements were performed as described above for the 28 day autopsied animals. Blood was also collected, centrifuged and stored for future analysis and the ovaries were fixed in Bouin's solution.

#### Reproductive Competence in Adult Females Exposed to Alcohol In Utero

The remaining female offspring were weighed on days 21, 28, 35, 42, 49 after birth in order to compare growth curves for each treatment group. Beginning on day 26 after birth, all female offspring were monitored daily for estrous discharge until they had shown three complete estrous cycles as an indication of puberty onset and regularity of estrous cycles (Orsini, 1961).

Some female offspring of each treatment group (N=10) were mated when adult to normal, sexually experienced males on the night of proestrus. The next morning, they were tested for sperm in the vaginal discharge. Sperm positive females were allowed to carry their pregnancies to term.

The animals were fed daily an ad libitum chow diet and tap water with the weights of the mothers also being recorded throughout gestation. On the day of parturition, the number of live/dead pups was recorded and the sex of each determined first by gross comparison of the anogenital distance of each (males have a longer anogenital distance) and then positive identification by autopsy (presence of testes indicating male and absence of testes and presence of uterus indicating female).

#### EXPERIMENT II

The exact procedure as experiment I was used with the exception that a 15% ethanol liquid diet was administered and an isocaloric liquid control diet to that of the 15% ethanol was given to the control animals. Because the ethanol diet for pregnant hamsters was not available, the diet (Bio Serve, #1965) was modified by adding to it an additional 0.3% vitamin mix, 0.6% fiber, 0.02% chloride choline, and 0.6% salt. The modification was performed to ensure for nutritional adequacy of the ethanol diet and thus, based on the percentage composition of experiment I, the diet used in experiment II had 67% calories derived from ethanol, 21% from protein, 3.5% from carbohydrate and 9% from fat. The added percentages of the above components were also added to the liquid control diet in order to ensure caloric and nutritional equality between the ethanol

and the liquid diets. Due to the high concentration of ethanol, the ethanol animals were given a three day adaptation period where on days 1 and 2 of gestation they were given a  $1/3$  ethanol diet mixed with a  $2/3$  liquid control diet. On day 3 the ethanol animal was then given  $2/3$  ethanol diet mixed with a  $1/3$  liquid control diet. On the remaining days of gestation (days 4-16) the animals received the full 15% ethanol diet. The control liquid diet hamster was then given the same amount of control diet as its paired ethanol treatment animal on the same day of gestation. On the day of parturition, the liquid diets were removed and the animals were given standard lab chow. The number of live/dead pups was also recorded. All litters were culled to six pups (three males and three females) in order to rule out any effects that may be due to litter size. Some of the treatment mothers were allowed to nurse their own pups till weaning. Other treatment mothers were paired by weight to normal mothers (these females were given standard lab chow) on the same day of gestation. On the day of parturition the offspring of the treatment groups were cross-fostered (the six pups from the treatment groups were given to the normal mothers and the six pups from the normal mothers were given to the treatment mothers). This cross-fostering experiment separates the prenatal effects due to ethanol from postnatal maternal effects. Twenty one days

after birth, the pups were weaned from their mothers by the same procedure as outlined in experiment I.

#### Determination of Blood Alcohol Levels

Female virgin hamsters were mated on the day of proestrus and the identification of sperm the next day indicated day 1 of pregnancy. The mothers were then divided into two treatment groups. One group was given the 5% ethanol diet as indicated in experiment I, and the other group was given the 15% ethanol treatment as described in experiment II. Several pregnant animals consuming a pellet diet were also included in the study as control animals. All of the hamsters were allowed to carry their pregnancies till the 13th day of gestation (2400h) when the animals were sacrificed by decapitation and the trunk blood was collected. The autopsy day was determined by data produced by experiment I and II which indicated that the greatest amount of ethanol consumed was during the evening hours of day 13. Taylor et al. (1982) had shown in rats that blood samples obtained 3 hours after lights-out produced the highest blood ethanol readings. The animals were then autopsied and the following observations were recorded: the number of fetuses present, the weight of the fetuses, and the number of reabsorption sites. Plasma alcohol concentration was determined by the alcohol dehydrogenase method (Sigma Diagnostic kit #332-A, St. Louis, MO) and a

Bausch and Lomb Spectronic 21 was used to determine the absorbance. Each sample was run in duplicate.

### STATISTICAL ANALYSIS

The statistical tests that were used to analyze the data from these experiments were performed using the Systat Program (Evanston, IL).

In experiment I, all of the data were analyzed by one way analysis of variance with significance between the means determined by either the Tukey's test or Duncan's test.

In experiment II, data regarding the body weights of the treatment group mothers, the amount of diet consumed by the 15% ethanol-treated animals, and the number of pups at parturition were all analyzed by one way analysis of variance and Tukey's test. Data regarding the number of offspring surviving until weaning were first transformed as  $\sqrt{y + 0.5}$  (Sokol and Rohlf, 1981). A two way analysis of variance was performed on the transformed data to determine the effect of treatment X cross-fostering or no cross-fostering. A one way analysis of variance was performed on the transformed data to determine the maternal effect on pup survival.

When comparing the amount of diet consumed by the 5% ethanol mothers to the 15% ethanol mothers, a two way analysis of variance was used. The data from the autopsy at day 13 of gestation was analyzed by analysis of variance



with significance between the means determined by Tukey's test. In all the above statistical tests, significance was accepted at  $p < 0.05$ .

## CHAPTER IV

### RESULTS

#### EXPERIMENT I

The body weights of the treatment group mothers (pellet diet, liquid control diet, ethanol diet) were recorded daily throughout gestation (N=9/group). These data were then converted into percent change in body weight and can be seen in Figure 1. After day 7, all treatment groups had an increase in body weight. However, no significant difference in body weight between the groups was seen until days 13 [ $F(2,24)=4.10$ ,  $p<0.05$ ], 14 [ $F(2,24)=3.875$ ,  $p<0.05$ ], and 15 [ $F(2,24)=3.46$ ,  $p<0.05$ ] of gestation as revealed by analysis of variance. On day 13, there was a significant difference in the percent change in body weight of the animals given the ethanol diet compared to the mothers given the pellet diet ( $p<0.05$ ). On day 14, a significant difference in percent change of body weight was seen between the ethanol and liquid diet group mothers when compared to the pellet mothers ( $p<0.05$ ). On day 15, a significant difference in the percent change in body weight was seen of the ethanol treated animals compared to the pellet group ( $p<0.05$ ). All of the mothers produced pups on day 16 of gestation.

The amount of diet consumed by the ethanol mothers was recorded during each day of gestation and can be seen in Figure 2. Analysis of variance showed that there was a significant difference in the daily amount of diet consumed [ $F(14,106)=2.79$ ,  $p<0.001$ ]. There was a significant increase in the amount of diet consumed during days 13 and 14 of gestation compared to a few of the earlier days of gestation.

The total number of pups present at parturition and on the day of weaning for the three treatment groups is shown in Table I. This table also shows the number of male and female pups present on the day of weaning. The data were analyzed by analysis of variance and showed no significant differences in the number of pups born [ $F(2,31)=1.76$ ,  $p>0.10$ ], and the number of male pups present at the time of weaning [ $F(2,31)=2.62$ ,  $p>0.05$ ]. A significant difference was noted in the number of ethanol pups remaining at the time of weaning when compared to the pellet group [ $F(2,31)=7.01$ ,  $p<0.005$ ]. A significant difference was also seen in the number of ethanol female pups remaining at the time of weaning [ $F(2,31)=6.92$ ,  $p<0.005$ ]. Analysis by Tukey's test showed that the ethanol group had a significant decrease in both the total number of pups ( $p<0.005$ ), as well as number of female pups ( $p<0.005$ ) remaining at the time of weaning compared to the pellet group.

When the female pups from the treatment group mothers were weaned several variables were measured as outlined in Table II. The data were recorded and analyzed by analysis of variance and showed that there were no significant differences in the following body measurements: ano-genital distance [ $F(2,116)=0.150$ ,  $p>0.75$ ], tail length [ $F(2,116)=0.687$ ,  $p>0.50$ ], the distance between the eyes [ $F(2,116)=1.57$ ,  $p>0.10$ ], eye slit [ $F(2,116)=0.633$ ,  $p>0.50$ ]. A significant difference was seen in the body weight of the offspring [ $F(2,116)=17.46$ ,  $p<0.001$ ]. Analysis by Tukey's test showed that both the liquid diet ( $p<0.001$ ) and ethanol group ( $p<0.001$ ) were significantly larger than those of the pellet group. A significant difference was also seen in the width of the head [ $F(2,116)=3.51$ ,  $p<0.05$ ]. Analysis by Duncan's test showed that the liquid diet offspring had a significantly smaller head size compared to both pellet ( $p<0.05$ ) and ethanol ( $p<0.05$ ) groups.

Beginning at 26 days after birth, all the female offspring were checked daily for the postovulatory vaginal discharge. Once the first day of estrus was recorded, the animals continued to be checked daily until three complete estrous cycles were recorded. The data in Table III show the age of the first and third of estrus for the offspring of the treatment group mothers. The data were analyzed by analysis of variance and showed no significant difference in

the onset of estrus [ $F(2,98)=2.21$ ,  $p>0.10$ ] or the observed third day of estrus [ $F(2,98)=1.20$ ,  $p>0.25$ ].

Data obtained at the prepubertal autopsy of the female offspring from the treatment group mothers are shown in Table IV. The data were analyzed by analysis of variance and showed no significant difference in the following variables: body weight [ $F(2,13)=3.59$ ,  $p>0.05$ ], brain weight [ $F(2,13)=3.42$ ,  $p>0.05$ ], pituitary weight [ $F(2,13)=0.930$ ,  $p>0.75$ ], adrenal weight [ $F(2,13)=0.370$ ,  $p>0.50$ ], uterus when wet [ $F(2,13)=3.31$ ,  $p>0.05$ ], uterus when dry [ $F(2,13)=1.06$ ,  $p>0.25$ ]. A significant increase was noted in the weight of the ovaries + one oviduct [ $F(2,13)=6.66$ ,  $p<0.001$ ]. The value for the liquid diet group offspring was significantly greater than both the ethanol ( $p<0.001$ ) and pellet groups ( $p<0.001$ ). In order to determine whether the body weight of the animals may have some impact on the results, the above data were analyzed per 100 grams of body weight. This analysis showed that body weight did not have any effect as to whether the variables were found to be significant or not significant by analysis of variance.

Data obtained at the postpubertal autopsy of female offspring of treatment group mothers are shown in Table V. These animals were autopsied on the day of estrus. The data were analyzed by analysis of variance and showed no significant difference in body weight [ $F(2,15)=0.908$ ,  $p>0.25$ ], brain weight [ $F(2,15)=1.12$ ,  $p>0.25$ ], pituitary

weight [ $F(2,15)=1.11$ ,  $p>0.25$ ], ovaries + one oviduct [ $F(2,15)=0.882$ ,  $p>0.25$ ]. Analysis of variance showed a significant difference in the uterine dry weight [ $F(2,15)=3.87$ ,  $p<0.05$ ]. Analysis by Duncan's test showed that both liquid diet ( $p<0.05$ ) and ethanol groups ( $p<0.05$ ) had a significant decrease in uterine dry weights compared to the pellet group. A significant difference was noted in adrenal weight [ $F(2,15)=0.504$ ,  $p<0.025$ ]. There was a significant increase in adrenal weight of the liquid diet group when compared to both the ethanol and pellet diet groups ( $p<0.025$ ). In order to determine whether the body weight of the animals may have some impact on the results, the above data were analyzed per 100 grams of body weight. This analysis showed that body weight did have an effect on the variables except for adrenal weight of which the following Mean  $\pm$  SEM (mg) were found: pellet group  $14.79 \pm 1.17$ , liquid diet group  $17.41 \pm 1.71$ , and ethanol diet group  $14.87 \pm 1.34$ , resulting in no significant difference between the groups [ $F(2,15)=1.10$ ,  $p>0.25$ ].

The offspring of the treatment group mothers were weighed for five consecutive weeks beginning on the day of weaning and the growth curves can be seen in Figure 3. At 21 days after birth, there was a significant difference in body weight [ $F(2,100)=12.68$ ,  $p<0.001$ ]. Both the ethanol ( $p<0.05$ ) and liquid diet ( $p<0.05$ ) pups were significantly heavier compared to the pellet pups. There was also a

significant difference in body weight on days 28 [ $F(2,100)=7.102$ ,  $p<0.005$ ] and 35 [ $F(2,100)=4.84$ ,  $p<0.010$ ]. The liquid diet group pups were significantly heavier compared to both the pellet group ( $p<0.010$ ) and the ethanol pups ( $p<0.010$ ). The remaining two days, 42 and 49 days of age, show no significant difference in the body weights of the treatment group offspring.

At 60 days after birth, several of the females ( $N=9/\text{group}$ ) exposed to treatment in utero were mated with sexually experienced males and allowed to carry their pregnancies to term. On the day of parturition, the numbers of male and female pups were recorded as shown in Table VI. The data were analyzed by analysis of variance and showed that there was no significant difference in the number of male offspring [ $F(2,24)=0.200$ ,  $p>0.75$ ]. A significant decrease, however, was seen both in the number of females [ $F(2,24)=5.03$ ,  $p<0.025$ ] and in the total number of offspring [ $F(2,24)=1.331$ ,  $p<0.025$ ]. These values were significantly lower in the ethanol-exposed females when compared to either the pellet or liquid diet control groups ( $p<0.025$ ).

## EXPERIMENT II

The body weights of the treatment group mothers were recorded daily throughout gestation. These data were then converted into percent change in body weight and can be seen in Figure 4. During the first 4 days of gestation, there

were no significant differences between the groups. The remaining days of gestation show a significant difference in the percent change in body weight: day 5 [ $F(2,26)=3.92$ ,  $p<0.05$ ], day 6 [ $F(2,26)=6.17$ ,  $p<0.010$ ], day 7 [ $F(2,26)=9.38$ ,  $p<0.005$ ], day 8 [ $F(2,26)=16.11$ ,  $p<0.001$ ], day 9 [ $F(2,26)=12.83$ ,  $p<0.001$ ], day 10 [ $F(2,26)=25.42$ ,  $p<0.001$ ], day 11, [ $F(2,26)=33.94$ ,  $p<0.001$ ], day 12 [ $F(2,26)=31.88$ ,  $p<0.001$ ], day 13 [ $F(2,26)=38.73$ ,  $p<0.001$ ], day 14 [ $F(2,26)=41.55$ ,  $p<0.001$ ], day 15 [ $F(2,26)=45.23$ ,  $p<0.001$ ]. These values were significantly lower in the ethanol group when compared to either the pellet or liquid diet control groups ( $p<0.001$ ).

The amount of diet consumed by the ethanol mothers was recorded during each day of gestation and can be seen in Figure 5. Analysis of variance showed no significant difference in the daily amount of diet consumed [ $F(14,120)=1.40$ ,  $p>0.15$ ].

Figure 6 compares the amount of diet consumed by the 5% ethanol mothers to the 15% ethanol mothers during gestation. A two way analysis of variance showed that for days 1-8 of gestation, the 5% ethanol mothers consumed a significantly greater amount of diet compared to the 15% ethanol mothers [ $F(1,7)=91.1$ ,  $p<0.001$ ]. There was neither a significant effect due to day [ $F(1,7)=0.791$ ,  $p>0.50$ ] nor a significant interaction (treatment X day) [ $F(1,7)=0.56$ ,  $p>0.75$ ]. When days 9-15 of gestation were compared, the 5%



ethanol diet mothers consumed a significantly greater amount of diet compared to the mothers receiving the 15% ethanol diet [ $F(1,6)=292.686$ ,  $p<0.001$ ]. Again, there was no significant difference in daily consumption [ $F(1,6)=1.71$ ,  $p>0.10$ ], and also no significant interaction (treatment X day) [ $F(1,6)=2.0$ ,  $p>0.05$ ].

On the day of parturition, all of the litters were culled to six pups each. Some of the pups remained with their biological mothers and others were cross-fostered. The numbers of males and females and weights of the culled pups are shown in Table VII. The data were analyzed by analysis of variance and showed no significant difference in the number of males [ $F(2,22)=3.16$ ,  $p>0.05$ ] nor in the number of females [ $F(2,22)=2.13$ ,  $p>0.10$ ]. A significant difference was seen in the total number of offspring [ $F(2,22)=2.69$ ,  $p<0.05$ ]. Mothers who consumed ethanol during gestation had fewer offspring than those on a pellet diet ( $p<0.05$ ). A significant difference was also seen in the body weight of the culled pups [ $F(2,84)=84.56$ ,  $p<0.001$ ]. The body weight of the ethanol pups was significantly less than that of either the pellet group offspring or liquid diet group ( $p<0.05$ ).

The pups were weaned from their mothers 21 days after birth and the number of pups present was recorded. Table VIII shows the low number of surviving ethanol offspring both in the cross-fostered group and in the groups raised

with their biological mothers. Therefore, the data were first transformed using the method described by Sokol and Rohlf (1981) and then tested by a two way analysis of variance. The data showed that whether the pups were cross-fostered or left with their biological mothers was not a significant factor on the total number of pups at weaning [ $F(1,21)=0.077$ ,  $p>0.75$ ]. Gestational treatment had a significant effect on the number of pups when compared [ $F(2,21)=21.79$ ,  $p<0.001$ ]. There was no significant difference between the interaction (diet X cross-fostering or no cross-fostering) [ $F(2,21)=2.052$ ,  $p>0.10$ ]. Possible maternal effects on the survival of the pups were determined by comparing, treatment mothers given control offspring. Analysis of variance was performed on the transformed data and showed a significant effect of treatment [ $F(2,6)=5.81$ ,  $p<0.05$ ]. Analysis by Tukey's test showed that there was a significant decrease in the number of surviving pups in the litters cross-fostered to ethanol mothers compared to both liquid diet ( $p<0.05$ ) and pellet ( $p<0.05$ ) control mothers.

#### BLOOD ETHANOL LEVELS

In order to determine the blood ethanol level of the pregnant hamsters consuming ethanol during pregnancy, hamsters were given either a 5% or 15% ethanol diet on day 1

of gestation. At 2400h on the 13th day of gestation, the females were autopsied and the trunk blood collected.

The blood alcohol levels of the three treatment groups were analyzed the next day. All of the pellet group mothers (N=7) had levels below 10 ng/dl (0.01%) and were recorded as negative for the presence of ethanol. The blood ethanol levels of the 5% ethanol diet (N=5) were less than 10 ng/dl (0.01%) and were also recorded as negative for the presence of ethanol. The 15% ethanol diet mothers (N=4) produced the following levels: 240 ng/dl (0.240%), 125 ng/dl (0.125%), 75 ng/dl (0.075%), and 17.5 ng/dl (0.018%).

Table IX depicts the results of the autopsy performed to determine the number and weight of the fetuses and the appearance of any gross malformations of the fetuses on day 13 of gestation. Analysis of variance showed a significant difference in fetal weight [ $F(2,131)=11.94$ ,  $p<0.001$ ]. Analysis by Tukey's test showed that both the 5% fetuses ( $p<0.001$ ) and the 15% fetuses ( $p<0.001$ ) were significantly lighter compared to the pellet group. Further, the 15% ethanol fetuses ( $p<0.001$ ) were found to be significantly lighter than the fetuses exposed to 5% ethanol diet. A significant difference was also seen in the number of fetuses [ $F(2,131)=13.19$ ,  $p<0.001$ ]. There were significantly more fetuses in the 15% ethanol group compared to both pellet ( $p<0.001$ ) and 5% ethanol ( $p<0.001$ ) groups.

The number of reabsorption sites was also recorded for each group. The pellet group had 2 reabsorption sites, the 5% ethanol group had 2 reabsorption sites and the 15% ethanol group had 3 reabsorption sites. All of the pellet and 5% diet fetuses were found to be grossly normal. Two of the mothers in the 15% ethanol diet group which had the highest blood ethanol levels produced three fetuses with exencephaly. The mother with the recorded 240 ng/dl (0.240%) blood level of ethanol had two fetuses with exencephaly and the mother with the 125 ng/dl (0.125%) blood ethanol level had one fetus with exencephaly.

## CHAPTER V

### DISCUSSION

The inability to control certain variables in the study of Fetal Alcohol Syndrome (FAS) in humans has somewhat limited this area of research. Thus the search for an appropriate animal model has been ongoing. The goals of this study were (1) to explore the possibility that the golden hamster can be used as an animal model for FAS and if so, then (2) to study the reproductive competence of female offspring which were exposed to alcohol in utero. This study has shown that adult female offspring exposed to alcohol in utero have no delay in the onset of puberty or irregularity of estrous cycles, but when adult produce significantly fewer pups per litter compared to control animals. It has also shown that the golden hamster is a viable model for studying the effects of fetal alcohol exposure.

A 5% ethanol diet was used in experiment I because numerous studies using the rat and mouse as animal models have shown that this concentration produced defects similar to gross malformations seen in FAS children (Chernoff, 1977; Clarren & Smith, 1978; Sulik et al., 1981). The ethanol content of the diet was increased to 15% in experiment II so

that any slight abnormalities found in experiment I would be enhanced, indicating that the deviations are due to the ethanol exposure.

The effect of the ethanol diet on the mother during gestation was monitored by the percent change of body weight compared to the control groups (Fig. 1). In experiment I, the body weights of the treatment group mothers showed no significant differences until days 13, 14, and 15 of gestation when the mothers consuming the ethanol diet gained less weight than the mothers consuming the pellet diet. Mothers consuming 15% ethanol (Fig. 4) gained significantly less weight compared to both pellet and liquid diet control groups from day 4 of gestation until parturition. Because malnutrition often accompanies excessive alcohol consumption, it is necessary to know whether the effects seen in the offspring are due to maternal ethanol consumption or maternal malnutrition. Rats given an isocaloric liquid diet and pair-fed to an ethanol group during pregnancy had body weights which were significantly less than animals on a pellet diet at parturition (Detering et al., 1979; Bo et al., 1982). However, this decreased body weight did not produce the abnormalities in offspring seen in the ethanol group offspring. Thus the changes seen in the alcohol treated rats were not due to caloric deprivation, but due to the effect of ethanol. This reported diminished body weight is in contrast to the results reported by Gallo and Weinberg

(1982). Both alcohol and pair-fed control liquid diet females exhibited weight gain similar to those of pellet controls during gestation in that study.

Because all of the treatment group mothers in both the 5% and 15% diet had an increase in body weight during gestation, the difference in body weight between the groups may be due to either the number or the weight of the fetuses. During the last few days of gestation, the fetuses gain a large amount of weight compared to the first 2/3 of gestation. Abel (1979) concluded that the effect of alcohol is greatest during the last third of gestation and that prenatal growth retardation is the most common effect of FAS. Because there was no significant difference in the number of pups born between the three treatment groups, it is possible that the low weight gain of the alcohol mother during the last third of gestation may be due to low fetal weight. Because the pups in this study were not weighed at parturition this theory can not be supported or rejected. The effect may also be due to the mother herself not gaining as much weight, and although this theory was not tested, it is possible that the ethanol mother may have less water retention compared to the control groups. These results are in contrast to other studies using the rat and mouse as animal models. A decrease in both litter size and pup weight in offspring of mothers consuming 5% ethanol diets during gestation has been reported (Abel, 1979; Streissguth

et al., 1980; Abel and Dintcheff, 1984; Witek-Janusek, 1986).

The difference between these studies and the effect of the 5% ethanol diet in hamsters reported here may be in the fact that the golden hamster has a faster ethanol clearance rate. Two liver enzyme systems, alcohol dehydrogenase (ADH) and microsomal ethanol oxidizing system (MEOS) are responsible for ethanol metabolism in man and have also been found in the hamster (Kulkosky and Cornell, 1979; Chernoff, 1980). The hamster has an ethanol metabolizing capability three times that of the rat, and therefore, the hamster is able to consume larger quantities of ethanol (McCoy et al., 1981). In rats, the fetus lacks the enzymes required to metabolize ethanol and relies on the dam to effect such metabolism, therefore, the level of ethanol in the circulation may be higher on occasion than in the dam (Holmes and Masters, 1978; Abel, 1979). Chernoff (1977) and Bo et al. (1982) showed that mice exposed to a 5% ethanol diet produce gross abnormalities seen in FAS but this study has shown that this concentration does not produce any gross malformations in the offspring exposed to the diet in utero. However, as will be discussed later, the female offspring exposed to alcohol in utero produce significantly fewer offspring when adult. The increase in ethanol concentration to 15% was high enough so that even with the high activity of ADH, high blood ethanol levels were recorded. Some of



the blood ethanol levels attained using the hamster as an animal model were similar to those attained by other investigators using different animal models and have produced FAS characteristics in the offspring. This topic will be discussed further in a later section. Two of 5 hamsters had high blood ethanol levels and produced fetuses with gross malformations. Therefore, 15% ethanol diet was in high enough concentration and was not fully cleared by alcohol dehydrogenase in the liver. Harris et al. (1978) reported ADH clearance was the limiting factor in the use of the hamster compared to other animal models.

When the ethanol concentration was increased to 15% ethanol diet, both the number and weights of the alcohol-exposed pups were significantly less than those of controls. An autopsy of mothers (Table IX) on day 13 of gestation showed that mothers in the 5% ethanol group had fetuses with significantly reduced body weights, although the number of fetuses was not significantly different compared to pellet diet. Mothers given the 15% ethanol had significantly fewer pups per litter and there was a significant reduction in fetal weight. The results from the autopsy and those from the day of parturition (experiment II) suggest that the 5% ethanol diet affects only the body weight of the pups where as the increase in alcohol concentration to 15% ethanol produces both lower pup weight and reduced litter size.

There was no difference between groups in the length of gestation on either 5% or 15% ethanol diets. This is in contrast to results showing a significant increase in gestation length due to ethanol in the rat (Stressguth et al., 1980; Gallo and Weinberg, 1982). Bond et al. (1982) also concluded that in rats, the increase in gestational length causes increased mortality and thus lower litter size.

Although there was no significant difference between the number of pups at birth in experiment I, there was a significant decrease in the number of pups which were exposed to ethanol in utero at the time of weaning compared to the control groups (Table II). There were also significantly fewer female offspring from the 5% ethanol treated mothers compared to the pellet group. Because the treatment group pups were not cross-fostered to 'surrogate' mothers, it is unknown as to whether the effect is due to maternal behavior (Abel et al., 1981) or that whether in utero alcohol exposure has a direct effect on the overall health of the pups. When hamsters are stressed, they cannibalize their offspring (Lisk, 1985). Because of this characteristic, it is difficult to record such things as the number of stillbirths, the number of pups dying soon after birth, or the number of pups with gross malformations. The results indicate that pups exposed to 15% ethanol diet in utero are too weak to survive since offspring survival was

reduced regardless of cross-fostering. Maternal effects may also be a factor since the survival rate of normal pups (the offspring of the cross-fostered mothers) was decreased in litters cross-fostered at birth to dams who had consumed 15% ethanol during gestation.

On day 21 after birth, various measurements were performed in order to identify FAS abnormalities in the offspring of the 5% treatment mothers (Table II). There were no significant differences in the anogenital distance, tail length, the distance between the eyes, the palpebral fissure of the ethanol pups compared to the pellet and liquid diet pups. These results are in contrast to studies performed on mice which have shown short palpebral fissures and increased distance between the eyes of mice which were injected (intraperitoneal) with 25% ethanol on day 7 of gestation (Sulik et al., 1981). Although no gross morphological changes were noted in this study, neurochemical, hormonal, and neural anomalies may still have occurred as a consequence of ethanol exposure (Stressguth et al., 1980).

Autopsies on the female offspring on days 28 (Table IV) and 60 after birth (Table V) were performed to examine any internal malformations due to treatment in utero. A significant difference was found during the prepubertal autopsy in the weight of the ovaries + one oviduct of the liquid diet offspring compared to the pellet or ethanol

groups. This difference was not due to the slightly greater body weight of that group and was not seen when animals were autopsied at 60 days of age. The postpubertal autopsy showed a significant difference in adrenal weight of the liquid diet offspring compared to pellet and ethanol groups. This higher adrenal weight may reflect a somewhat higher body weight of the liquid diet offspring.

At 21 days after birth, both the liquid diet and ethanol offspring had significantly higher body weights compared to controls (Fig. 3). By 42 days after birth, the significant difference in body weight was diminished as the pellet pups caught up to the weights of the liquid diet and ethanol pups. Abel and Dintcheff (1986) reported permanent decreases in the body weights of rat pups exposed to alcohol in utero compared to control animals. The effect on body weight is irreversible as these ethanol-exposed pups are unable to 'catch up' in body weight after birth. Because the litters were not culled and there were fewer pups in the litters of 5% ethanol mothers, the difference in the two studies may be due to a secondary effect of litter size.

The effect of the ethanol diet on the reproductive competence of the female offspring was first measured by observing the onset of puberty. Once the first day of estrus was recorded, the estrus cycle was checked until three complete cycles were observed. Table III shows that there was no significant difference between the three groups

for either the onset of puberty or the regularity of the estrus cycle. Hard et al. (1985) have shown that in rats exposed to ethanol in utero, there is normal onset of first vaginal estrus and regular estrous cycles; however, there was a later onset of behavioral estrus.

The ability of the offspring to mate and conceive was tested by mating the female offspring (60 days after birth) to sexually experienced males. On the day of parturition, the in utero exposed ethanol group gave birth to fewer pups per litter compared to both the pellet and liquid diet control groups. Therefore, although the prenatally exposed ethanol offspring showed no difference in the appearance of the first estrous cycle or in the regularity of the estrous cycle, they produce significantly lower number of offspring when adult. There may be a number of reasons for this effect. In utero alcohol exposure may cause either a reduction in the number of eggs ovulated or the viability of the eggs which are ovulated. Because alcohol exposure can produce neurological defects as well as behavioral defects, it is possible that neuroendocrine function is defective in these females. Since these theories were not studied in this experiment, they cannot be ruled out or supported by these data as possible explanations for the reduced litter size. These results are in contrast to prenatally exposed rat offspring which were mated when adults and gave birth to normal number of pups (Hard et al., 1985).

A comparison was made in the blood ethanol levels of the female offspring receiving 5% or 15% ethanol diet. The blood ethanol levels of the mothers were tested on the 13th day of gestation, 3 hours after lights-out. In rats consuming a 5.5% ethanol diet, blood ethanol levels of  $126.9 \pm 21.1$  ng/dl (0.127%) are found to be the highest at this time during gestation (Taylor et al., 1982). Detering et al. (1979) have found that maternal blood ethanol levels in rats consuming a 5.5% ethanol diet were either low or non-detectable in the dams at parturition. In contrast to Taylor et al. (1982) the 5% ethanol diet used in this study produced blood ethanol levels less than 0.01% on day 13 of gestation and thus were recorded as negative for the presence of alcohol. This is probably due to the high clearance rate of alcohol by the hamster as discussed earlier. A wide range of blood ethanol levels in pregnant hamsters consuming 15% ethanol diet [240 ng/dl (0.240%) to 17.5 ng/dl (0.018%)] was seen in this experiment. It has been reported that the blood ethanol level of both human and animal fetuses exposed to alcohol in utero are at least as high as that in the mother (Streissguth, et al., 1980).

When the blood ethanol levels were tested in both the 5% and 15% ethanol groups, the mothers were autopsied and the fetuses examined. All fetuses of mothers consuming 5% ethanol were grossly normal. The 15% ethanol diet mothers who had blood ethanol levels of 210 ng/dl (0.245%) and 125

ng/dl (0.13%) produced 2 fetuses and 1 fetus with exencephaly respectively. Thus the 15% ethanol diet was in high enough concentration to produce abnormalities seen in FAS. While the blood ethanol levels were only tested on this one particular day and time of gestation, it does show that hamsters can attain blood ethanol levels that are teratogenic.

In summary, the golden hamster is a viable model for FAS because hamsters can obtain much higher blood ethanol levels than previously reported for rats in free selection situations and also they do not produce obvious signs of physical dependence upon ethanol (Kulkosky, 1978). The hamster can attain high enough blood alcohol levels to produce FAS characteristics such as decreased number of fetuses and decreased fetal weight.

The hamster also has a short gestation length of 16 days which is not affected by the administration of alcohol in contrast to the rat and mouse. This study has also shown that a 5% ethanol containing liquid diet has no effect on the onset of puberty in the female offspring, but these same animals when adult produce fewer offspring. For future studies, maternal behavior such as cannibalism needs to be controlled in order to increase the chances of pup survival. Also the ethanol concentration in the diet should be between 5% and 15% ethanol diet in order to produce measurable blood

ethanol levels in the mothers and at the same time assure for pup survival.



TABLE I  
5% Ethanol Diet

The total number of pups present at parturition, weaning, and the sex of the offspring of each gestational treatment group (Mean  $\pm$  SEM)

Treatment Group	# Pups @ birth	# Pups @ weaning	# Male pups	# Female Pups
Pellet (N=8)	12.1 $\pm$ 0.8	10.1 $\pm$ 0.8	4.9 $\pm$ 0.7	5.3 $\pm$ 0.8
Liqd (N=14)	9.9 $\pm$ 0.8	7.9 $\pm$ 0.8	4.2 $\pm$ 0.6	3.7 $\pm$ 0.4
Etoh (N=12)	10.4 $\pm$ 0.8	4.8 $\pm$ 1.1*	2.6 $\pm$ 0.7	2.2 $\pm$ 0.6*

\*p<0.05 compared to pellet control group.

Liqd = Liquid diet control group.

Etoh = Ethanol diet group.

TABLE II

## 5% Ethanol Diet

Body measurements of the offspring of the gestational treatment groups at 21 days of age (Mean  $\pm$  SEM)

Variables	Treatment group		
	Pellet (N=41)	Liqd (N=51)	Etoh (N=27)
Weight (g)	34.7 $\pm$ 0.9	40.7 $\pm$ 0.6*	39.0 $\pm$ 0.9*
Anogenital dist. (in)	0.17 $\pm$ 0.003	0.18 $\pm$ 0.003	0.18 $\pm$ 0.004
Tail length (in)	0.47 $\pm$ 0.01	0.45 $\pm$ 0.01	0.46 $\pm$ 0.01
Head width (in)	0.69 $\pm$ 0.01	0.65 $\pm$ 0.01**	0.69 $\pm$ 0.01
Eye dist. (in)	0.39 $\pm$ 0.01	0.38 $\pm$ 0.003	0.39 $\pm$ 0.005
Eye slit (in)	0.20 $\pm$ 0.003	0.20 $\pm$ 0.002	0.21 $\pm$ 0.003

\*p<0.05 compared to pellet control group.

\*\*p<0.05 compared to both pellet control and ethanol groups.

Liqd = Liquid diet control group.

Etoh = Ethanol diet group.

TABLE III

## 5% Ethanol Diet

Age of the female offspring at the first and third estrus  
of the gestational treatment groups (Mean  $\pm$  SEM)

Treatment Group	First estrus (day)	Third estrus (day)
Pellet (N=37)	34.1 $\pm$ 0.6	42.7 $\pm$ 0.6
Liqd (N=43)	35.0 $\pm$ 0.4	43.0 $\pm$ 0.4
Etoh (N=21)	33.2 $\pm$ 0.7	41.7 $\pm$ 0.7

Liqd = Liquid diet control group.

Etoh = Ethanol diet group.

TABLE IV

## 5% Ethanol Diet

Body measurements of the offspring of the gestational treatment groups at 28 days of age (Mean  $\pm$  SEM)

Variables	Treatment group		
	Pellet (N=6)	Liqd (N=6)	Etoh (N=5)
Body weight (g)	58.0 $\pm$ 2.3	65.5 $\pm$ 2.1	63.3 $\pm$ 1.4
Brain weight (mg)	813.1 $\pm$ 28.6	880.5 $\pm$ 28.7	783.3 $\pm$ 26.7
Pituitary weight (mg)	2.3 $\pm$ 0.3	3.0 $\pm$ 0.3	2.6 $\pm$ 0.4
Adrenal weight (mg)	6.5 $\pm$ 1.2	7.4 $\pm$ 0.6	7.1 $\pm$ 0.5
Ovaries + one oviduct weight (mg)	21.7 $\pm$ 0.7	34.5 $\pm$ 3.0*	23.6 $\pm$ 2.7
Uterus wet weight (mg)	49.8 $\pm$ 6.8	76.2 $\pm$ 9.6	56.0 $\pm$ 4.7
Uterus dry weight (mg)	8.7 $\pm$ 1.2	10.8 $\pm$ 1.2	8.9 $\pm$ 0.9

\*p<0.05 compared to both pellet control and ethanol groups.

Liqd = Liquid diet control group.

Etoh = Ethanol diet group.

TABLE V

## 5% Ethanol Diet

Body measurements of the offspring of the gestational treatment groups at 60 days of age (Mean  $\pm$  SEM)

Variables	Treatment groups		
	Pellet (N=6)	Liqd (N=6)	Etoh (N=6)
Body weight (g)	119.0 $\pm$ 4.8	127.7 $\pm$ 7.6	117.8 $\pm$ 3.8
Brain weight (mg)	1016.2 $\pm$ 23.1	969.1 $\pm$ 20.6	979.0 $\pm$ 26.2
Pituitary weight (mg)	6.2 $\pm$ 0.4	6.1 $\pm$ 0.5	5.4 $\pm$ 0.3
Adrenal weight (mg)	17.3 $\pm$ 0.7	21.8 $\pm$ 1.3*	17.4 $\pm$ 1.3
Ovaries + one oviduct weight (mg)	52.9 $\pm$ 2.0	56.8 $\pm$ 2.6	52.0 $\pm$ 3.4
Uterus wet (mg)	334.6 $\pm$ 9.8	316.9 $\pm$ 8.5	318.6 $\pm$ 9.5
Uterus dry (mg)	55.4 $\pm$ 1.5**	49.6 $\pm$ 1.5	49.9 $\pm$ 2.0

\*p<0.05 compared to both pellet control and ethanol groups.

\*\*p<0.05 compared to both liquid diet and ethanol groups.

Liqd = Liquid diet control group.

Etoh = Ethanol diet group.

TABLE VI

## 5% Ethanol Diet

The total number and sex of offspring born to mothers of which were prenatally exposed to a treatment group (Mean  $\pm$  SEM)

Treatment Group	# Males	# Females	Total # offspring
Pellet (N=8)	6.8 $\pm$ 0.5	7.9 $\pm$ 0.7	14.6 $\pm$ 0.5
Liqd (N=9)	6.6 $\pm$ 0.6	7.2 $\pm$ 0.9	13.7 $\pm$ 1.0
Etoh (N=10)	6.2 $\pm$ 0.7	4.7 $\pm$ 0.7*	10.9 $\pm$ 1.1*

\*p<0.05 compared to pellet control group.

Liqd = Liquid diet control group.

Etoh = Ethanol diet group.

TABLE VII

## 15% Ethanol Diet

The total number and sex of offspring found on the day of parturition in each gestational treatment group (Mean  $\pm$  SEM)

Treatment group	# Males	# Females	Total # offspring	Pup weight (g)
Pellet (N=8)	5.3 $\pm$ 0.4	5.9 $\pm$ 0.6	11.2 $\pm$ 0.7	2.6 $\pm$ 0.03
Liqd (N=8)	4.3 $\pm$ 0.4	4.5 $\pm$ 0.5	8.8 $\pm$ 0.7	2.5 $\pm$ 0.03
Etoh (N=8)	3.9 $\pm$ 0.5	4.6 $\pm$ 0.5	8.5 $\pm$ 0.9**	1.8 $\pm$ 0.06*

\*p<0.05 compared to pellet and liquid diet groups.

\*\*p<0.05 compared to pellet control group.

Liqd = Liquid diet control group.

Etoh = Ethanol diet group.

TABLE VIII

## 15% Ethanol Diet

The number of pups surviving until weaning

Variable	Treatment		
	Pellet	Liqd	Etoh
Not cross-fostered	6,5,5,6,6	0,4,6,6,6	0,0,0,0,4*
Cross-fostered:			
Treatment pups and control mom	6,6,6	5,5,5	0,0,0
Treatment mom and control pups	6,6,6	3,5,5	0,1,4**

\*p&lt;0.001 compared to pellet control and liquid diet groups.

\*\*p&lt;0.05 compared to pellet control group.

Liqd = Liquid diet control group.

Etoh = Ethanol diet group.



TABLE IX

The number and weights of fetuses which were exposed to treatment in utero and autopsied on day 13 of gestation  
(Mean  $\pm$  SEM)

Treatment Group	Fetal Weight (g)	# of Fetuses
Pellet (N=7)	0.67 $\pm$ 0.02	10.71 $\pm$ 0.08
5% Etoh (N=5)	0.61 $\pm$ 0.02*	11.33 $\pm$ 0.41
15% Etoh (N=4)	0.55 $\pm$ 0.01* <sup>a</sup>	12.89 $\pm$ 0.18**

\*p<0.05 compared to pellet control group.

\*\*p<0.05 compared to both pellet and 5% ethanol diet groups.

<sup>a</sup>p<0.05 compared to 5% ethanol group.

Etoh = ethanol diet.

Figure 1. The percent change in body weight of treatment group mothers throughout the 16 days of gestation. The treatment group mothers were given either pellet diet ( $\square$ ), liquid control diet ( $\circ$ ), or a 5% ethanol diet ( $\Delta$ ) beginning on day 1 of gestation with their body weights recorded daily. Each value represents the average of 9 hamsters  $\pm$  SEM. Significant differences: \* $p < 0.05$  ethanol diet compared to pellet control group, \*\* $p < 0.05$  ethanol and liquid diet compared to pellet group.

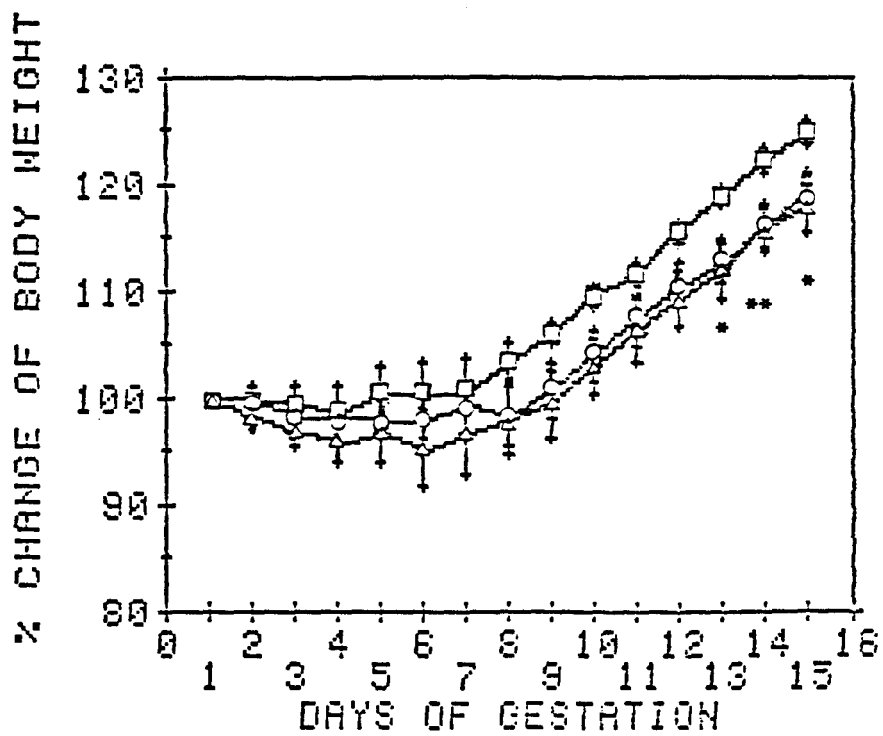


Figure 2. The Mean  $\pm$  SEM (in ml) of 5% ethanol diet consumed by the treated mothers throughout the 16 days of gestation (N=9).

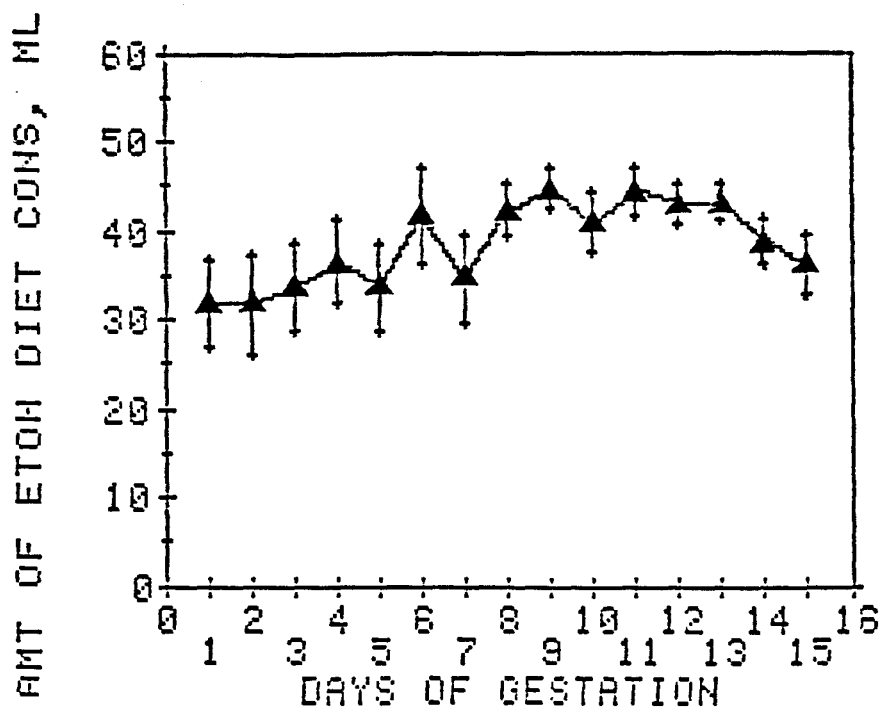


Figure 3. Body weight of female offspring from the three treatment groups; pellet control diet [N=37] ( $\square$ ), liquid control diet [N=45] ( $\circ$ ), and ethanol diet [N=21] ( $\triangle$ ), from 21-49 days of age. Each value represents the Mean  $\pm$  SEM; Significant differences: \* $p < 0.001$  liquid diet and ethanol pups compared to pellet control pups, \*\* $p < 0.010$  liquid diet pups compared to ethanol and pellet control pups.

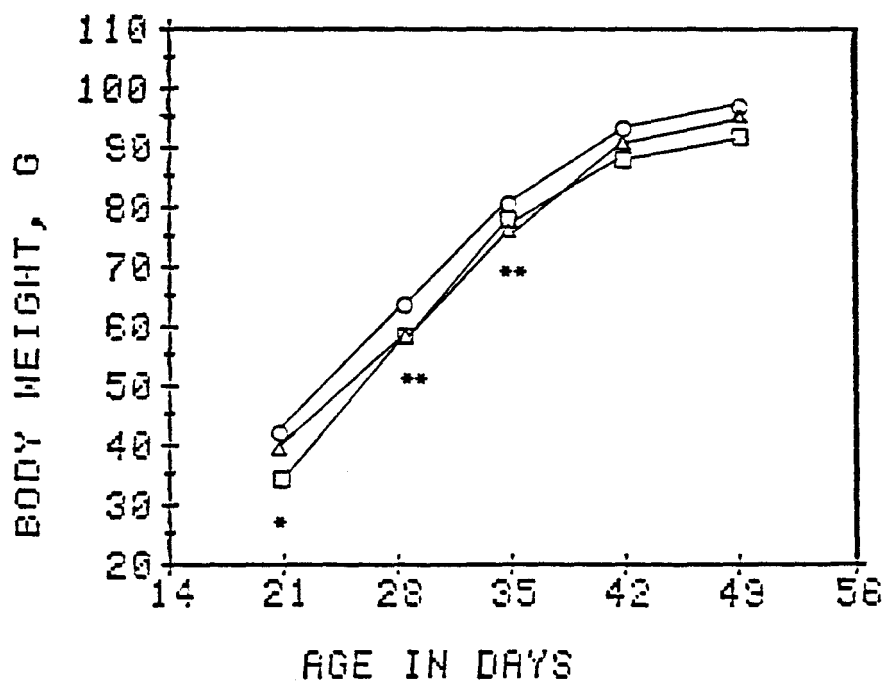


Figure 4. The percent change in body weight of treatment group mothers throughout the 16 days of gestation. The pregnant mothers were given either pellet diet ( $\square$ ), liquid control diet ( $\circ$ ), or 15% ethanol diet ( $\triangle$ ) beginning on day 1 of gestation (N=8-9). Each value represents the Mean  $\pm$  SEM. Significant differences: \* $p < 0.05$  compared to liquid diet control and pellet control groups.



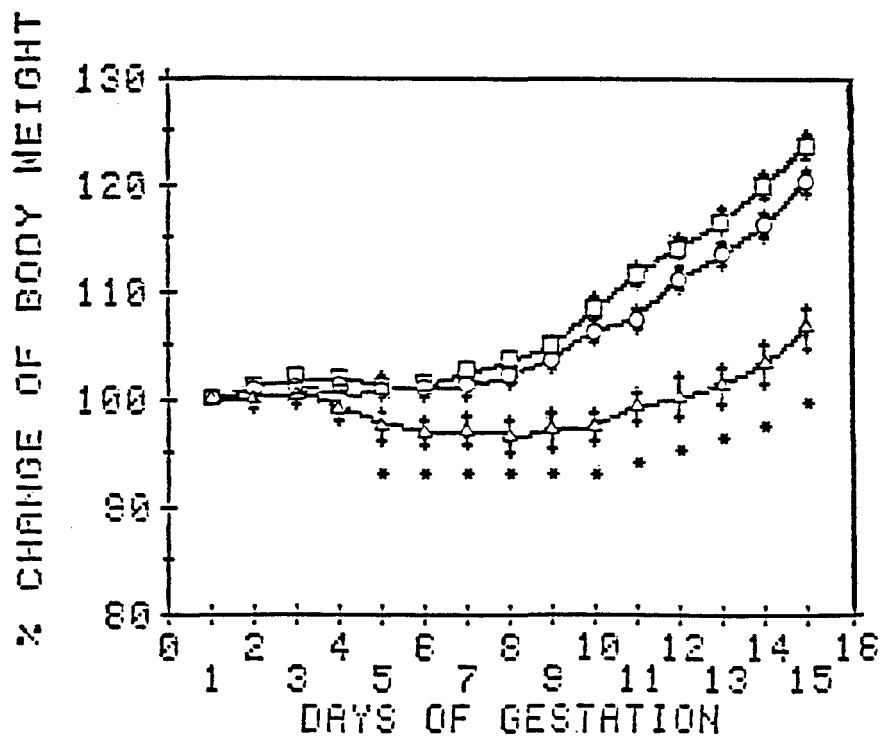


Figure 5. The Mean  $\pm$  SEM (in ml) of 15% ethanol diet consumed by the treated mothers throughout the 16 days of gestation (N=8).

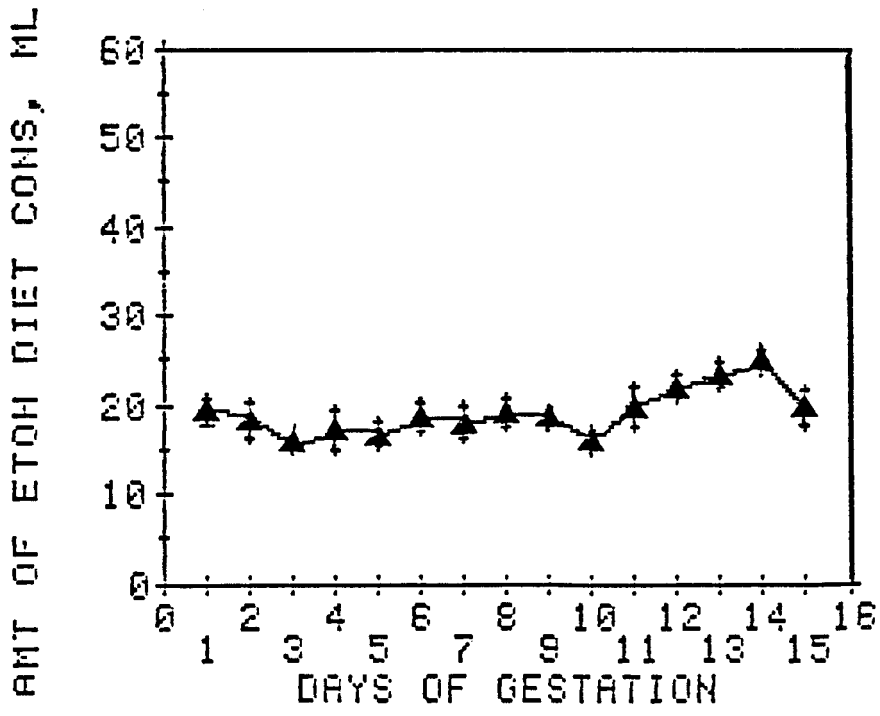
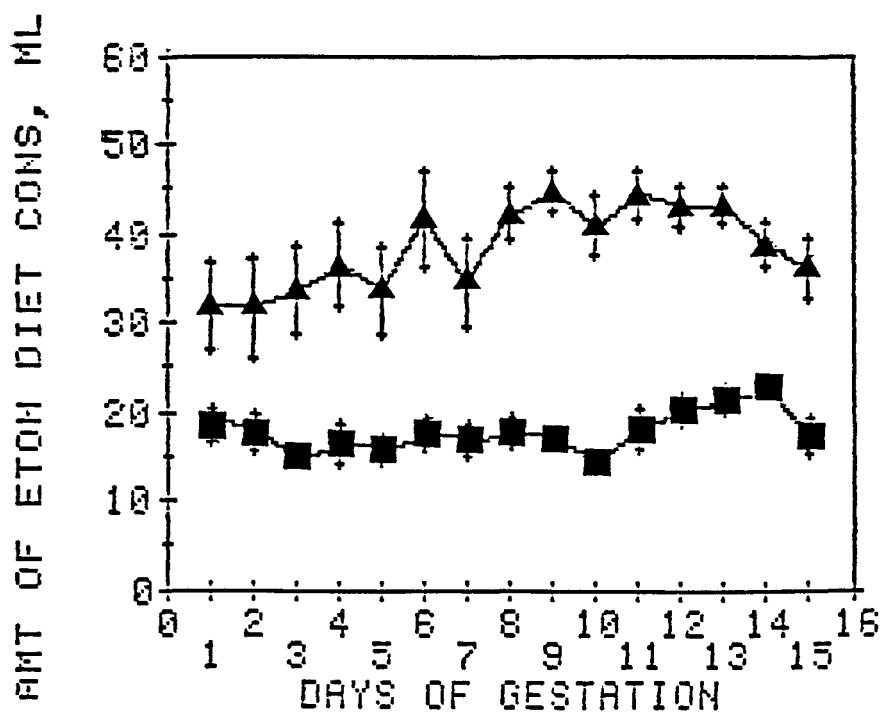


Figure 6. The Mean  $\pm$  SEM (in ml) of both the 5% ethanol mothers ( $\blacktriangle$ ) and the 15% ethanol mothers ( $\blacksquare$ ) throughout the 16 days of gestation (N=8-9).



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The thesis is therefore accepted in partial fulfillment of the requirements for the degree of Master of Science.

April 9, 1987

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