



## Effect of Maternal Alcohol Consumption on Epididymal Growth in Neonatal Mice

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### SUMMARY

A study was conducted to determine the effect of maternal alcohol consumption on the growth of epididymis in neonatal mice. Three groups of adult female mice were used. The pups of group 1 served as control while the pups of groups 2 and 3 were given 30% ethanol (v/v) during pregnancy and during pregnancy and lactation respectively. At 3, 4, 5 and 6 weeks of age, 10 male pups were randomly selected from each of the three groups and sacrificed. After sacrifice, the epididymis were dissected out and their weights determined. The results of the study showed significant decrease in the weights and growth rates of the epididymis of the pups exposed to alcohol when compared with the controls. The study has therefore demonstrated that maternal alcohol intake during pregnancy and during pregnancy and lactation affects the growth of epididymis of the neonates and that the epididymis of the neonates exposed to alcohol attempted catch-up growth with the controls

**KEY WORDS:** Maternal alcohol consumption, Epididymis, Growth, Neonatal mice.

### INTRODUCTION

It has been established that alcohol has a potential teratogenic effects (Jones and Smith, 1973). Morrison and Maykut, (1979) described a pattern of congenital abnormalities in children born to women with severe alcoholism. These reports emphasized retardation of weight and length of such infants at birth and noted the lack of catch-up growth in them. Human and non-human primate studies on brain structure and function now strongly suggest that consumption of alcohol during pregnancy can affect fetal brain structure and function (Clarren *et al.*, 1985). As a result of this effect, the hypothalamic-pituitary-gonadal axis regulation

which plays a critical role in control of reproduction (Grober *et al.*, 1998) could be disrupted. The hormone from this axis plays a critical role in stimulating the growth and activity of the reproductive system (Fugii, 1976).

The effects of maternal alcohol intake on the growth and development of some parts of the body in both human and experimental animals have been reported (Lee and Leichter, 1980; Ihemelandu, 1984; Clarren *et al.*, 1985; Piqueras-Renau *et al.*, 1987; Nwaogu and Ihemelandu, 1999a, b). In the reproductive system, reduced growth have been reported on the testes (Onu and Ezeasor, 2001; Onu *et al.*, 2002a, b; Fakoya and Caxton-Martins, 2004) and on the penis (Onu *et al.*, 2004). The possible effect of alcohol intake during pregnancy and during pregnancy and lactation on the growth of epididymis has not been elucidated, hence this study.

### Materials and methods

The experimental procedures employed in this study were similar to those of Lee and Leichter (1980). Thirty six (36) virgin female and 9 immature male mice used in this study were randomly selected at the weaning age of 21 days from a colony of locally in-bred mice. The mice were maintained for research in the animal house of the Faculty of Veterinary Medicine, University of Nigeria, Nsukka. The virgin female mice were divided into three groups of 12 each while the immature males were kept separately. The mice were housed separately in cages with screened tops and were acclimatized for three weeks before the beginning of the study. All the female mice in the three groups and the males were provided water and commercial diet (Guinea Feed produced by Bendel Feed and Flour Mills, Nigeria, PLC, Ewu, Delta State) *ad*

*libitum* until they were six weeks of age. At the beginning of the 7<sup>th</sup> week of age, the female mice in group 2 and 3 were given 10% ethanol (v/v) in drinking water for 2 weeks and 20% ethanol (v/v) for another 1 week. The female mice in the three groups were then bred overnight by introducing 1 male mouse into a cage housing 4 female mice. Day 1 of pregnancy was presumed after observation of vaginal plug the following morning. Following diagnosis of pregnancy, the female mice in groups 2 and 3 were given 30% ethanol (v/v) till delivery. After delivery, the alcohol for group 2 (alcohol exposure during pregnancy) was replaced with drinking water while group 3 (alcohol exposure during pregnancy and lactation) continued to receive the 30% ethanol (v/v) in drinking water until their pups were weaned at 21 days of age. At the age of 3, 4, 5 and 6 weeks, 10 male pups were randomly selected from groups 1, 2 and 3 and sacrificed. A total of 120 pups were used in the study.

**Quantitative measurements**

After the sacrifice, the two epididymis were dissected out, trimmed of extraneous tissue and weighed using Metler's Analytical Balance (BFN-390). After this, the relative growth rates of the organ were calculated thus: the mean of logarithms of the weights at week 3 was calculated and then used to divide the logarithms of their respective weights at week 4, 5 and 6 (Nwaogu and Ihemelandu, 1999a).

**Statistical analysis**

Means and standard errors were calculated for each group. One-way analysis of variance (ANOVA) was used to examine whether there were significant differences in the parameters measured amongst the pups of the three groups. Following the observation that there were significant differences amongst the three groups, Duncan's Multiple Range Test (DMRT) (Duncan, 1955) was used to determine which groups differed. For the statistical test,  $p < 0.05$  and  $p < 0.01$  were considered statistically significant.

**RESULTS**

**Epididymal Weights**

Comparison of the epididymal weights between the control and the two alcohol-exposed groups using ANOVA showed that there was significant

difference ( $p < 0.01$ ) amongst the three groups throughout the duration of the study (Table I). Further analysis using DMRT showed that the epididymis of control mice weighed significantly greater than those from mice exposed to alcohol during pregnancy at week 3, 4 and 5 ( $p < 0.01$ ) and at week 6 ( $p < 0.05$ ). Similarly, the epididymis of the control mice weighed significantly greater ( $p < 0.01$ ) than those of the mice exposed to alcohol during pregnancy and lactation throughout the duration of the study. Comparison of the epididymis of the two alcohol-exposed groups showed that the weights were similar ( $p > 0.05$ ) at week 3 and 4. However, the epididymis of mice exposed to alcohol during pregnancy only weighed significantly greater ( $p < 0.01$ ) than those from the mice that were exposed to alcohol during pregnancy and lactation at week 5 and 6

**Epididymal growth rates**

There was significant difference ( $p < 0.01$ ) amongst the three groups throughout the period of study (Table II). The results of Duncan's analysis showed that the epididymis of the two alcohol-exposed groups had significantly higher ( $p < 0.01$ ) growth rates when compared with the control while the growth rates of the epididymis of the two alcohol-exposed groups were similar ( $p > 0.05$ ) throughout the duration of the study.

Table I: Comparison of epididymal weights (mg) of control mice and mice exposed to alcohol during pregnancy and during pregnancy and lactation

Age (weeks) at sacrifice	Control (n = 10)	Prenatal alcohol-exposed (n = 10)	Pre-and post-natal alcohol-exposed (n = 10)
3	15.65±1.30 <sup>a</sup>	6.23±0.40 <sup>b</sup>	4.77±0.40 <sup>b</sup>
4	22.35±1.00 <sup>a</sup>	12.14±1.20 <sup>b</sup>	9.53±1.00 <sup>b</sup>
5	33.26±1.60 <sup>a</sup>	18.06±1.00 <sup>b</sup>	10.31±1.40 <sup>c</sup>
6	38.09±1.00 <sup>a</sup>	37.05±1.00 <sup>b</sup>	18.19±1.40 <sup>c</sup>

Values represent means±SEM for each measurement Means with different superscript on the same row are significantly different at  $p < 0.05$

Table II: Comparison of epididymal growth rates of control mice and mice exposed to alcohol during pregnancy and during pregnancy and lactation

Age (weeks) at sacrifice	Control (n = 10)	Prenatal alcohol-exposed (n = 10)	Pre-and post-natal alcohol-exposed (n = 10)
4	1.14±0.01 <sup>a</sup>	1.35±0.03 <sup>b</sup>	1.47±0.40 <sup>b</sup>
5	1.29±0.02 <sup>a</sup>	1.59±0.02 <sup>b</sup>	1.53±0.03 <sup>b</sup>
6	1.34±0.01 <sup>a</sup>	1.98±0.02 <sup>b</sup>	1.89±0.10 <sup>b</sup>

Values represent means±SEM for each measurement Means with different superscript on the same row are significantly different at  $p < 0.05$

## DISCUSSION

This study has demonstrated that maternal alcohol intake in mice during pregnancy and during pregnancy and lactation retarded the growth of the epididymis. This is evident when absolute epididymal weights were used as growth indices. When the two alcohol-exposed groups were compared, it was observed that at 3 and 4 weeks of age, the two groups were similar while those exposed to alcohol during pregnancy alone were bigger than those exposed to alcohol during pregnancy and lactation at 5 and 6 weeks of age. The similarity at 3 weeks of age could be attributable to the effect of alcohol on the two groups while the similarity at 4 weeks of age could be as a result of continued effect of alcohol on those exposed during pregnancy after withdrawal at 3 weeks of age (Zetterman and Sorrel, 1981). The superiority of the epididymal weight of the control over the two alcohol-exposed groups at 5 and 6 weeks of age resulted from the effect of the alcohol on the epididymal growth. This observation is similar to the findings of Taylor *et al.* (1981) and Onu *et al.* (2002a) on testicular growth in fetal alcohol syndrome in rat. The observation also agrees with what was described as lack of catch-up growth in fetal alcohol syndrome in humans (Hanson *et al.*, 1976) and in experimental animals (Lee and Leichter, 1980; Ihemelandu, 1984). However, the results of this study also revealed that the epididymis exposed to alcohol during pregnancy and during pregnancy and lactation attempted to catch-up growth with their control counterparts. This is shown by the superiority of the growth rates of the alcohol-exposed groups over the control. This is an indication of an attempt by the alcohol-exposed groups to catch-up growth with the control. This observation suggests that the intake of alcohol during pregnancy and during pregnancy and lactation did not suppress completely the attempt by the epididymis to catch-up growth with the controls. This is similar to the observation of Nwaogu and Ihemelandu (1999a, b) on muscle and visceral organs of rat respectively.

The superiority of the epididymal weight exposed to alcohol during pregnancy only over those exposed to alcohol during pregnancy and lactation showed that alcohol ingestion during pregnancy and lactation produced adverse cumulative effect on the growth of epididymis.

This observation is similar to that of Nwaogu and Ihemelandu (1999a, b) on muscle and visceral organs of rats respectively and Onu *et al.*, (2000) on testes and accessory sex organs of mice.

The mechanism by which alcohol intake during pregnancy and during pregnancy and lactation retarded the growth of epididymis was not determined in this study. However, Lee and Leichter, (1980) observed that alcohol consumed during pregnancy adversely affects the regulatory mechanism of growth of the pups and the effects persists after birth. It is well established that severe structural damage occurs to the brains of infant exposed to alcohol during pregnancy (Clarren and Smith, 1978). Taylor (1984) also observed that alcohol impairs fetal hormonal system. Alcohol affects the activity of the endocrine system, both the hormone-secreting glands and the activity of the hormones themselves. Since the endocrine mechanism in the fetuses is similar to that of the adults (Nathanielsz, 1976), the alcohol consumed during pregnancy and during pregnancy and lactation could have disrupted the neuroendocrine functions of the fetuses by its action on hypothalamus sequel to destruction of brain structure and function (Clarren *et al.*, 1985). This action on the hypothalamus could lead to disruption of the hypothalamic-pituitary-gonadal axis as suggested by Onu and Ezeasor, (2001) which plays significant role in reproduction (Grober *et al.*, 1998). The hormone from this axis plays a critical role in the growth and activity of the reproductive system (Fugii, 1976).

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