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Morphometric Studies Of Testes In Fetal Alcohol Syndrome Of Mice.

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ABSTRACT

An investigation was conducted to determine the effect of maternal alcohol consumption during pregnancy on the morphometery of testes of mice. Two groups of mice comprising of 12 females and 6 males were used. The offspring of group 1 served as control while those of group 2 were exposed to alcohol during gestation period. From 1-6 weeks of age, 10 male offspring were randomly selected from the two groups and sacrificed by severing the spinal cord at the atlanto-occipital joint. Following sacrifice, the testes were prepared for routine histological examination. Morphometric analysis showed reduced seminiferous tubular diameter in the testes exposed to alcohol during pregnancy when compared with the controls. The investigation has therefore demonstrated that maternal alcohol consumption during pregnancy affects the size of seminiferous tubules.

Key Words: Maternal alcohol consumption, fetal alcohol syndrome offspring, testes, morphometry.

Alcohol consumption by mothers during pregnancy can lead to a condition called fetal alcohol syndrome in their offspring (Jones *et al.,* 1973). This syndrome is characterized by retarded growth during fetal and neonatal life (Leichter and Lee 1979; Lee and Leichter, 1980). In experimental animal models, reduced testicular growth was observed (Tatlor, *et al.,* 1981; Onu and Ezeasor, 2001; Onu *et al.,* 2002).

Alcohol has been known to be a neurotoxin (Leonard, 1987). It is also known to have a destructive effect on developing neurons and even on those already formed (Buer Moffet and Altman, 1977). Damage to the central nervous system has emerged as one of the most serious consequences of fetal alcohol syndrome (Streissguth, *et al.,*1986). It has been observed that brain structure and function are affected in fetuses if their dam drinks alcohol during pregnancy (Clarren et al., 1985). Onu and Ezeasor (2001) in their investigation on the effect of maternal alcohol consumption during pregnancy on offspring, observed retardation of growth of testes of the neonates. The authors suggested that that could be an evidence of the disruption of the hypothalmic hypophyseal gonadal axis regulation. This axis plays critical role in the control of reproduction (Grober *et al.,* 1998). Growth and maturation of the testes in the prepubertal mammals are actually dependent on gonadotropin stimulation (Schaubacher, et al., 1982).

In adult males, alcohol is known to disrupt many of the rhythms of neuro endocrine function, probably through its action on the hypothalamus (Greene and Hollander, 1981). The action of alcohol on hypothalamus could lead to disruption of hypothalamic hypophyseal gonadal axis regulation. In chronic studies with male rats, testicular atrophy was observed in addition to lowered plasma testosterone (Van Thiel, *et al.,* 1975). Histological studies of the testes obtained from chronic alcoholics demonstrate a reduction in the cross sectional area of the seminiferous tubles (Bennett, *et al.,* 1970). Since the endocrine mechanism in the fetus is similar to adults (Nathenielsz, 1976) alcohol could disrupt the fetal neuro endocrine function by its action on the hypothalamus, thereby causing disruption of the hypothalamic hypophyseal gonadal axis regulation as in

adults. This disruption could be the cause of retardation of the growth of testes in fetal alcohol syndrome. The retardation of the growth of testes could reflect in the morphometry. This has not been elucidated, hence the investigation.

MATERIALS AND METHODS

Experimental Animals.

Experimental procedures employed in this investigation were similar to that of Lee and Leichter (1980). 24 virgin and 6 immature male mice were used as breeding stock. The mice were randomly selected at the weaning age of 21 days from a colony of locally in-bred mice maintained for research in the animal house of faculty of Veterinary Medicine, University of Nigeria, Nsukka. The females were randomly divided into 2 groups of 12 each. The animals were housed in cages with screened tops and acclimatized for three weeks before the beginning of the investigation. All the animals in the two groups were fed water and commercial diet (Guinea feed produced by Bendel Feed and Flour Mills Nigeria, PLC, Ewu, Delta State) *ad libitum* till six weeks of age. At the commencement of the investigation and the beginning of the 7th week, the mice in group 2 were given 10% ethanol (%v) in drinking water for two weeks and 20% ethanol (%v) for another three weeks. The two groups were then bred overnight by introducing 1 male mice into a cage containing 4 females. Day 1 of pregnancy was established after observing vaginal plug in the vagina the following morning. The alcohol exposed group (2) were given 30% ethanol (%v) till delivery. After delivery, the alcohol for group 2 (gestational alcohol exposed group) was replaced with water. At 1,2,3,4,5 and 6 weeks of age, 10 male offspring were randomly selected from the two groups and sacrificed by decapitation.

Histology And Histomorphometry

After sacrifice, the paired testes were carefully dissected out and fixed in Bouin's fliud. Thereafter the testes were dehydrated in a series of graded ethanol, cleared in xylene and embedded in paraffin. Sections 5um in thickness, were cut from the embedded testes using a rotary microtome and then stained with Haematoxlyin and Eosin. Using a calibrated eyepiece micrometer, the diameter of 20 randomly selected seminiferous tubules of testes was measured in each group.

Statistical Analysis

The results obtained were statistically analyzed. Means and standard errors were calculated for each group. Students 't' test was used to examine whether the difference observed in seminiferous tubular diameter between the control and fetal alcoholic mice were statistically valid.

RESULTS AND DISCUSSION

The results of the parameter measures, which is the diameter of seminiferous tubules are summarized in Table 1. The diameter of the seminiferous tubules of the control mice were significantly greater ($P < 0.05$) than those of mice exposed to alcohol prenatally throughout the period of the investigation.

Table 1: Comparison of the diameter of seminiferous tubules of testes between the controls and those exposed to alcohol prenatally. Mean ± S.E given for each measurement.

Age (weeks)	Control Mice	Prenatal Alcohol Exposed Mice	Probability of Significance	Degrees Of freedom
1	15.38 ± 0.3	9.38 ± 0.4	P < 0.05	38
2	19.50 ± 0.4	14.90 ± 0.4	P < 0.05	38
3	22.03 ± 0.3	18.20 ± 0.3	P < 0.05	38
4	26. 75 ± 0.5	24.73 ± 0.3	P < 0.05	38
5	30.40 ± 0.4	22.33 ± 0.1	P < 0.05	38
6	33. 45 ± 0.4	30.00 ± 0.6	P < 0.05	38

The investigation has demonstrated that maternal alcohol consumption during pregnancy produces decreased mean diameter of seminiferous tubules in the testes of the offspring. This is shown by the fact that from 1 6 weeks of age, the mean diameter of the seminiferous tubules of the control mice were significantly greater than those of the mice exposed to alcohol prenatally. This could be as a result of retardation of growth of testes in fetal alcohol syndrome as observed by Onu and Ezeasor (2001). This reduced mean diameter of seminiferous tubules of the testes is similar to the observation of Bennett, *et al.*, (1970).

The mecahnism by which the alcohol consumed during pregnancy affects the morphometry of testes of the neonates was not investigated. However, alcohol is known to be a neurotoxin (Leanard, 1987) and could destroy the developing neurons of the brain including possibly those of the hypothalamus. Several researchers (Buer-Moffef and Altiman, 1977; Clarren et al, 1985; Streissguth, *et al.*, 1986; Maisto *et al.*, 1999) have highlighted the deleterious effect of alcohol on the brain. Since the endocrime mechanism in the fetuses is similar to adults (Nathanielsz 1976). Alcohol could disrupt the neuro endocrine function of the fetuses by its action on the hypothalamus. This then could lead to the disruption of hypothalamic-hypophyseal-gonadal axis regulation, which plays significant role in reproduction (Grober *et al.*, 1998). Neomeroff *et al.*, (1977) observed smaller testicular weight as a consistent feature in a marked disruption of hypothalamic-hypophyseal- gonadal axis regulation. Gonadotropins stimulation controls the growth and maturation of testes in the prepubertal mammals (Schaubacher, 1982). Therefore, the disruption of hypothalamic hypophyseal gonadal axis regulation could have affected the growth of the testes as observed by Onu *et al.*, (2002) and by extension the seminiferous tubular diameter.

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