MORPHOLOGICAL ANALYSIS OF MOUSE TESTES FOLLOWING GESTATIONAL AND LACTATIONAL ALCOHOL EXPOSURE

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SUMMARY

The effect of maternal alcohol consumption during gestation and lactation on the morphology of the testes of offspring was studied using 180 male mice. The 180 mice were offspring of a breeding stock comprising 36 female and 18 male mice. The 36 female mice were divided into 3 groups of 12 each. The offspring of group 1 served as control while those of groups 2 and 3 were exposed to 30% ethanol (V/V) prenatally and pre- and postnatai respectively. At 1, 2, 3, 4, 5 and 6 weeks of age, 10 male offspring were randomly selected from groups 1, 2 and 3 and sacrificed. Following sacrifice, the testes were carefully dissected out. Determination of the weights of the testes showed that the testis of controls weighed significantly heavier than those of the alcohol exposed groups. Histologically, seminiferous tubular diameter of controls was larger than those of the alcohol exposed groups. There was also delay in the development of spermatogenic cells in the testes of those exposed to alcohol.

KEY WORDS: Maternal alcohol consumption, Morphology, Offspring, Testes.

INTRODUCTION

Excessive ingestion of alcohol by either pregnant women or experimental animals, is known to induce a characteristic pattern of developmental defects known as the fetal alcohol syndrome (FAS) (Abel, 1982). This syndrome is characterized by specific facial features such as short palpebral fissures, epicanthal folds, short nose, indistinct philtrum, thin lip and flat midface, pre- and postnatal growth retardation, microcephaly and mental retardation (Sampson et al., 1997). Damage to the central nervous system (CNS), has recently been identified as one of the most serious consequences of fetal alcohol syndrome (Streissguth et al., 1986). Human and non-human primate studies on brain structure and function now strongly suggest that maternal alcohol consumption during pregnancy can affect fetal brain structure and function (Claren et al., 1985). The hypothalamic pituitary-gonadal axis regulation could be disrupted resulting in abnormal endocrine environment which affects the development of the reproductive system (Pineda and Faulkner, 1980). There have been documented reports on the effects of
maternal alcohol consumption on the growth and development of some parts of the body in humans and experimental animals (Clarren et al., 1985; Streissguth et al., 1986; Nwoagui; and Ihemelandu, 1999a and b, Onu and Ezeasor, 2001; Onu et al., 2002a and b). The effect on the morphology of the testes has not been studied, hence the study.

MATERIALS AND METHODS

Experimental animals
The method employed in producing experimental fetal alcohol syndrome in this study was similar to that of Lee and Leichter, (1980). Thirty six (36) female and eighteen (18) male mice were used in this study as breeding stock at the weaning age of 21 days from a colony of locally inbred mice maintained for research in the Animal House of the Faculty of Veterinary Medicine, University of Nigeria, Nsukka. The female mice were randomly divided into 3 groups of 12 each. Both the males and the females were housed in cages with screened tops and were acclimatized for three weeks before the commencement of the study. Feed and water were provided to the groups ad libitum. The offspring of group 1 served as control while the offspring of group 2 were exposed to alcohol during gestation only, those of group 3 were exposed during both gestation and lactation.

At seven weeks of age when the female were sexually matured (Hafez, 1970), the mice in group 2 and 3 were given 10% ethanol (v/v) in drinking water for 2 weeks. The three groups were then bred overnight by introducing 1 male mouse into a cage containing 4 females. Day 1 of pregnancy was established when plug was observed in the vagina in the following morning. Following diagnosis of pregnancy, the quantity of alcohol for the experimental groups was raised to 30%. After delivery, only group 3 (gestational and lactational exposure) continued to receive 30% ethanol (v/v) till weaning their offspring at 21 days. A total of 180 male offspring were used for this study. At 1, 2, 3, 4, 5, and 6 weeks of age, 10 male offspring were randomly selected from each of group 1, 2 and 3 and sacrificed.

Quantitative measurement
Following sacrifice the two testes of the animals were carefully dissected out and weighed.

Histology and histomorphometry
After determination of their weights, the testes were fixed in Bouin’s fluid for 24 hours. Thereafter, routine method Humason (1979) was used to prepare the testes for histological examination. Using a calibrated eyepiece micrometer, the diameter of 20 randomly selected seminiferous tubules of the testes was measured in micrometer (μm) in each group. The characteristic cell populations of the testes were noted and described.

Statistical analysis
Means and standard errors were calculated for each group. Analysis of variance (ANOVA) using F-ratio was used to examine whether the differences observed in the parameters measured were statistically significant. Following the observations that there were significant differences between groups, Duncan’s New multiple Range Test (DNMRT) (Duncan, 1955) was used to determine which groups differed.
RESULTS

Testicular weights
Comparisons made using F. ratio showed that the mean testicular weights of the mice in the three groups differed significantly (P<0.01) at 1, 2, 3, 4, 5 and 6 weeks of age (Table I). Comparison using DNMRT following F-ratio analysis showed that the testes of the control mice were significantly heavier (P<0.01) at 1, 2, 3, 4, 5 and 6 weeks of age than those exposed to alcohol during gestation only. But on the contrary the testes of mice exposed to alcohol both during gestation and lactation weighed significantly less (p<0.01) than those of the control at 1, 2, 3, 4, 5 and 6 weeks of age. Furthermore, it was observed that the testicular weights of mice exposed to alcohol during gestation only were similar to those exposed to alcohol both during gestation and lactation at 1 and 4 (p>0.05) weeks of age. However, the testicular weights of the former were significantly heavier than those of the latter at 2 and 5 (p<0.01) and 3 and 6 (p<0.05) weeks of age.

Histomorphometry
Seminal tubular diameter
When comparison were made using F. ratio, the mean diameter of the seminiferous tubules of the mice in the three groups differed significantly (p<0.01) at 1, 2, 3, 4, 5 and 6 weeks of age (Table II). Comparison using DNMRT, following F-ratio analysis showed that the diameter of the seminiferous tubules of the control mice were larger at 1, 2, 3, 4, 5 and 6 (p<0.05) and 4 (p<0.05) weeks of age than those of mice exposed to alcohol during gestation only. The diameter of the seminiferous tubules of mice exposed to alcohol both during gestation and lactation were smaller at 1, 2, 3, 4, 5 and 6 (p<0.01) and 4 (p<0.05) weeks of age than those of the controls. On the other hand, the diameter of the seminiferous tubules of mice exposed to alcohol during gestation only were larger at 2, 3, 5, 6 (p<0.01) and 4 (p<0.05) weeks of age than those of mice exposed to alcohol both during gestation and lactation. The diameter of the seminiferous tubules of the latter were bigger (p<0.01) at 1 week of age.

TABLE I: Comparison of testicular weights (mg) between control, gestational and both gestational and lactational alcohol-exposed mice, using F-ratio

<table>
<thead>
<tr>
<th>Age (weeks)</th>
<th>Control</th>
<th>Gestational alcohol exposed mice</th>
<th>Gestational and lactational alcohol-exposed mice</th>
<th>F-ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>20.00 ± 0.4</td>
<td>11.10 ± 0.6</td>
<td>11.40 ± 1.03</td>
<td>49.40*</td>
</tr>
<tr>
<td>2</td>
<td>28.40 ± 0.3</td>
<td>17.36 ± 1.03</td>
<td>12.60 ± 1.03</td>
<td>87.94*</td>
</tr>
<tr>
<td>3</td>
<td>65.83 ± 2.7</td>
<td>29.09 ± 1.2</td>
<td>23.38 ± 1.6</td>
<td>137.51*</td>
</tr>
<tr>
<td>4</td>
<td>106.75 ± 3.0</td>
<td>43.59 ± 5.9</td>
<td>30.85 ± 2.3</td>
<td>102.03*</td>
</tr>
<tr>
<td>5</td>
<td>134.12 ± 2.5</td>
<td>91.24 ± 5.5</td>
<td>35.29 ± 2.7</td>
<td>172.92*</td>
</tr>
<tr>
<td>6</td>
<td>166.88 ± 3.6</td>
<td>116.85 ± 4.6</td>
<td>103.10 ± 2.6</td>
<td>77.04*</td>
</tr>
</tbody>
</table>

Degrees of freedom for all ages =27  *p<0.01
TABLE II: Comparison of seminiferous tubular diameter (μm) between control gestation and both gestational and lactational alcohol-exposed mice testes using F-ratio.

<table>
<thead>
<tr>
<th>Age (weeks)</th>
<th>Control</th>
<th>Gestational alcohol exposed mice</th>
<th>Gestational and lactational alcohol-exposed mice</th>
<th>F-ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>15.38 ± 0.3</td>
<td>9.83 ± 0.4</td>
<td>11.98 ± 0.3</td>
<td>67.00*</td>
</tr>
<tr>
<td>2</td>
<td>19.50 ± 0.4</td>
<td>14.90 ± 0.4</td>
<td>13.03 ± 0.4</td>
<td>67.00*</td>
</tr>
<tr>
<td>3</td>
<td>22.03 ± 0.3</td>
<td>18.20 ± 0.3</td>
<td>13.03 ± 0.4</td>
<td>97.24*</td>
</tr>
<tr>
<td>4</td>
<td>26.75 ± 0.5</td>
<td>24.73 ± 0.3</td>
<td>17.53 ± 0.4</td>
<td>80.30*</td>
</tr>
<tr>
<td>5</td>
<td>30.40 ± 0.4</td>
<td>22.33 ± 1.0</td>
<td>19.90 ± 0.4</td>
<td>99.00*</td>
</tr>
<tr>
<td>6</td>
<td>33.45 ± 0.4</td>
<td>30.00 ± 0.6</td>
<td>25.63 ± 0.5</td>
<td>62.20*</td>
</tr>
</tbody>
</table>

Degrees of freedom for all ages = 57 *p<0.01

Histology
At three weeks of age, the seminiferous tubules of testes from the control group contained primary spermatocytes in diplotene stage and round spermatids. Leydig cells were present in the interstitial stroma separating the tubules and there was evidence of canalization (Fig. 1).

In the testes of gestational alcohol-exposed group some profiles of the seminiferous cords contained primary spermatocytes at zygotene and pachytene stages. Leydig cells and fibroblasts were present in the interstitium and there was no evidence of canalization of the cords (Fig. 2).

Fig. 1: Light micro-graph showing developmental changes in seminiferous tubules of 3-week old control mice. X 600

D=Diplotene primary spermatocytes;
RS=Round spermatids; C=Canalized lumen;
L=Leydig Cells

Fig. 2: Light micro-graph showing developmental changes in seminiferous tubules of 3-week old gestational alcohol-exposed mice. X 600

Z=Zygotene primary spermatocytes;
P=Pachytene primary spermatocytes;
LF=Leydig Cells and fibroblasts
At six weeks of age, the seminiferous tubules of control testes contained a full spectrum of spermatogenesis and has expanded greatly. Leydig cells were present in the interstitium and Regaud body in the lumen of the tubules indicated that spermatozoa has been produced (Fig. 3).

Fig. 3: Light micro-graph showing developmental changes in seminiferous tubules of 6-week old control mice. X 600

E=Elongated spermatids; RS=Round spermatids; R=Regaud body; L=Leydig Cells

In the testes of gestational and lactational alcohol-exposed mice, some profiles of the seminiferous cords contained primary spermatocytes and few round spermatids (Fig. 4).

Fig. 4: Light micro-graph showing developmental changes in seminiferous tubules of 6-week old gestational and lactational alcohol-exposed mice. X 600

R=Round spermatids
P=Pachytene primary spermatocytes;

**DISCUSSION**

This study has demonstrated that maternal alcohol consumption in mice during pregnancy and lactation produced retardation of growth and lack of “catch-up growth” postnatally in testes. This is illustrated by the fact that from 1-6 weeks of age, the weights and seminiferous tubular diameter of the testes of the control group were significantly greater than those exposed to alcohol both during pregnancy and lactation. This is similar to what was described as lack of “catch-up growth” in fetal alcohol syndrome in humans (Hanson et al., 1976) and experimental animals (Lee and Leichter, 1980).

Comparison of the testicular weights of the two alcohol-exposed groups showed
that the groups exposed to alcohol during pregnancy only were significantly greater than those exposed to alcohol during pregnancy and lactation at 2, 3, 5 and 6 weeks of age. Comparison of the seminiferous tubular diameters of the two alcohol-exposed groups indicated that the group exposed to alcohol during pregnancy only were significantly greater than those exposed to alcohol during pregnancy and lactation at 2, 3, 4, 5 and 6 weeks of age. These observations were indications that maternal alcohol consumption during lactation following gestational exposure had cumulative adverse effect on the growth of the testes. This is similar to the observation of Ihemelandu (1984) and Nwaogu and Ihemelandu (1999) on muscle growth. These authors observed decreased muscle growth in rats exposed to alcohol pre-and postnatally when compared with those exposed to alcohol prenatally only. The testicular weights of the two alcohol-exposed groups were similar at 1 and 4 weeks of age while their seminiferous tubular diameter were similar at 1 week of age. The reason for this similarity is not clear. However, the similarity of testicular weights at 1 week of age could be an attempt of the testes exposed to alcohol both during pregnancy and lactation to “catch-up growth” with those exposed to alcohol during pregnancy only. This is further evidenced by the greater seminiferous tubular diameter of those exposed to alcohol both during pregnancy and lactation.

The study has also demonstrated histologically that maternal alcohol consumption in mice during pregnancy and lactation produced retardation of growth of the testes using the development of spermatogenic cells as growth index. The seminiferous tubules of the control testes showed full spectrum, of spermatogenesis. In addition, there was the presence of Regnald body in the lumen of the seminiferous tubules indicating production of spermatozoa. On the other hand, the seminiferous tubules of the alcohol exposed testes contained primary spermatocytes and few spermatids.

The mechanism by which alcohol retarded the growth of testes was not investigated in this study. However, since alcohol crosses the placenta (Mann et al., 1975) and reaches the amniotic fluid, kidney, lungs, thymus, heart and brain (Aka and Gammaniel, 1997), it is capable of affecting the developing fetus and considerable evidence in the literature suggests that this is the case (Abel, 1982; Sokol et al., 1986). Alcohol is known to be neurotoxic (Leonard, 1987) and could destroy the developing neurons of the brain, and possibly those of the hypothalamus. This could then lead to disruption of the hypothalamic-hypophyseal-gonadal axis and subsequent impairment of gonadotropin stimulation and eventual retardation of the growth of the testes. However the hormonal aspect needs to be investigated and the investigation is currently going on.

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