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Histological Evidence of Retardation of Spermatogenesis in Mice in Experimentally Induced Fetal Alcohol Syndrome

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Abstract: The effect of maternal alcohol consumption during pregnancy and lactation on the spermatogenesis in male offspring was studied in neonatal mice. Three groups of mice comprising 12 female and 3 male mice group were used. 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 postnatally, respectively. At 6 weeks of age 10 male offspring were randomly selected from the three groups and sacrificed. Following sacrifice, the testes and cauda epididymis were carefully dissected out, fixed in Bouin's fluid and prepared for routine histological examination. Examination of the seminiferous tubules showed delay in the development of spermatogenic cells as evidenced by few to no spermatozoa in the cauda epididymis of the alcohol-exposed groups.

Key words: Fetal alcohol syndrome, spermatogenesis, neonates, testes, epididymis

INTRODUCTION

Alcohol ingestion by mothers during pregnancy can result to fetal alcohol syndrome (Jones and Smith, 1973). In experimental animal models, the syndrome is characterized by retardation of growth during fetal and neonatal life (Abel and Greizerstein, 1979; Lee and Leichter, 1980). Damage to the central nervous system (CNS) of the offspring has emerged as one of the most serious consequences of maternal alcohol consumption (Streissguth *et al*., 1986).

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). Since fetal brain structure and function could be affected, the hypothalamic-pituitary-testicular axis regulation could be disrupted thereby leading to impairment in the synthesis and release of gonadotropins. The gonadotropins maintain the growth and maturation of testes in prepubertal mammals as well as the maturation and action of gonads (Grober *et al*., 1998).

The effect of maternal alcohol consumption on the growth and development of some parts of the body in both human and experimental animals have been documented (Lee and Leichter, 1980 ; Clarren *et al*., 1985; Ihemelandu, 1984; Nwaogu and Ihemelandu, 1999a,b; Onu and Ezeasor, 2001; Onu *et al*., 2002a,b,c,d). The possible effect of alcohol consumed during pregnancy and lactation on spermatogenesis in the offspring has not been studied, hence this study.

MATERIALS AND METHODS

Experimental animals: The method employed in producing experimental fetal alcohol syndrome in this study is similar to that of Lee and Leichter (1980). 36 virgin female and 9 immature male mice were used in this study. They 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 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 three female groups and the immature males were housed separately in cages with screened tops and were acclimatized for 3 weeks before the commencement of the study.

At the beginning of the 7th week of age, the mice in groups 2 and 3 were given 10% ethanol ($^{V}/_{V}$) in drinking water for 2 weeks and 20% ethanol ($^{V}/_{V}$) for another 3 weeks. The three groups were then bred overnight by introducing one male mouse into a cage containing 4 females. Day 1 of pregnancy was presumed after observation of vaginal plug the following morning. Following diagnosis of pregnancy, the alcohol-exposed groups, 2 and 3 received 30% ethanol ($^{V}/_{V}$) till delivery. After delivery, the alcohol for group 2 (prenatal alcohol-

exposed) was replaced with water while group 3 (pre-and postnatal alcohol-exposed) continued to receive 30% ethanol ($^{V}/_{V}$) till weaning their offspring at 21 days of age. At the sixth week of age which is the age of sexual maturity in male mice (Hafez, 1970) 10 male offspring were randomly selected from groups 1, 2 and 3 and sacrificed.

Histology: After sacrifice, the paired testes and cauda epididymis were dissected out and fixed in Bouin's fluid. Thereafter, the tissues were processed in the normal paraffin procedure (Humason, 1979). The epithelium of the seminiferous tubules of the testes and the content of the lumina of the cauda epididymis were examined microscopically and described.

RESULTS

At the sixth week of age, the control testes contained a full spectrum of spermatogenesis. Regnaud body in the lumina of the tubules indicated that spermatozoa had been produced (Fig. 1). Profiles of seminiferous tubules of the prenatal alcohol-exposed testes had lumina but did not contain full spectrum of spermatogenesis. However, some profiles had few round and elongated spermatids (Fig. 2). In the pre-and postnatal alcohol-exposed testes, profiles of seminiferous tubules contained both early and late stages of primary spermatocytes apart from few round spermatids. There was no Regnaud body in the lumina of tubules of the two-alcohol-exposed groups. The lumina of cauda epididymis of the control mice were filled with spermatozoa (Fig. 3) while there was non in the lumina of cauda epididymis of the pre-and postnatal alcohol-exposed testes (Fig. 4).

DISCUSSION

The results of this study have demonstrated that maternal consumption of alcohol during pregnancy and lactation produces retardation of spermatogenesis in the male offspring. This is evidenced by the lack of full spectrum of spermatogenesis in the testes of the two alcohol-exposed groups. This is further highlighted by the sparsity of spermatozoa and none at al in the lumina of cauda epididymis of prenatal and pre-and postnatal alcohol-exposed groups respectively. This could be as a result of retardation of testicular growth in fetal alcohol syndrome as observed by Onu and Ezeasor (2001) and Onu *et al.* (2002a,b,c).

The mechanism by which alcohol consumed during pregnancy and lactation retarded spermatogenesis in the offspring was not established from the result of this study. However, alcohol being a neurotoxin (Leonard, 1989) could destroy the developing neurons including

Fig. 1

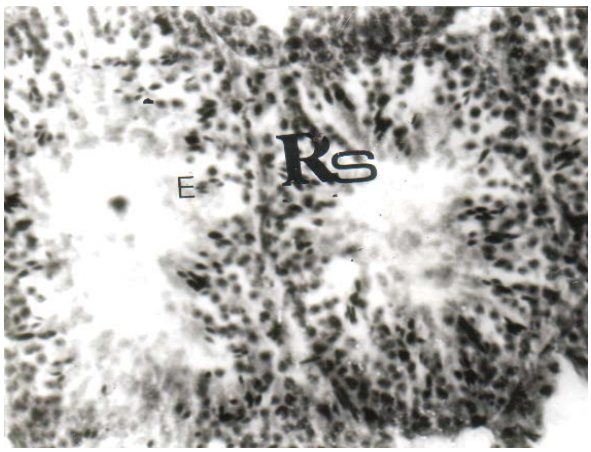


Fig. 2

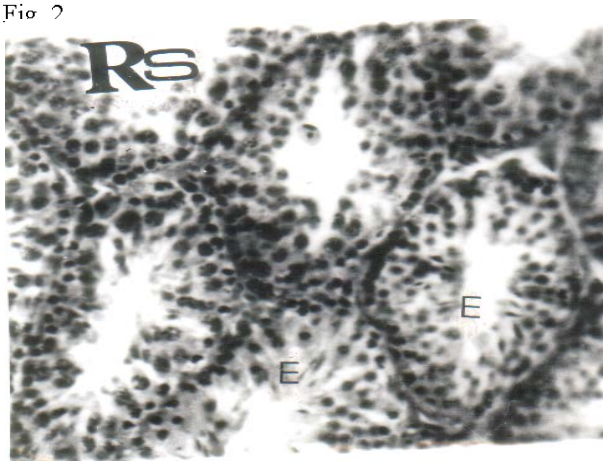


Fig. 1 and 2: Light micrographs showing cross sections of 6-week old testes of mice x 600
Fig. 1: Control,
Fig. 2: Prenatal alcohol – exposed
Rs = round spermatid E = elongated spermatid

possibly those of the hypothalamus. This could lead to disruption of hypothalamic-pituitary-testicular axis regulation and subsequent impairment of gonadotropin release. Taylor (1984) observed that alcohol impairs the fetal hormonal system. Courot *et al.* (1970) observed that spermatogenic division are under endocrine control. Leydig cells produce testosterone, which act on Sertoli cells to drive spermatogenesis. Although the precise mechanism by which this occurs is actually unknown, the requirement for testosterone is absolute (Sharpe, 1984). Steinberger *et al.* (1973) postulated that follicicle stimulating hormone (FSH) is essential for the later stages of spermatogenesis because in the absence of the hormone, maturation of seminiferous epithelium do not progress beyond the spermatid stage. In this study, the

Fig. 3:

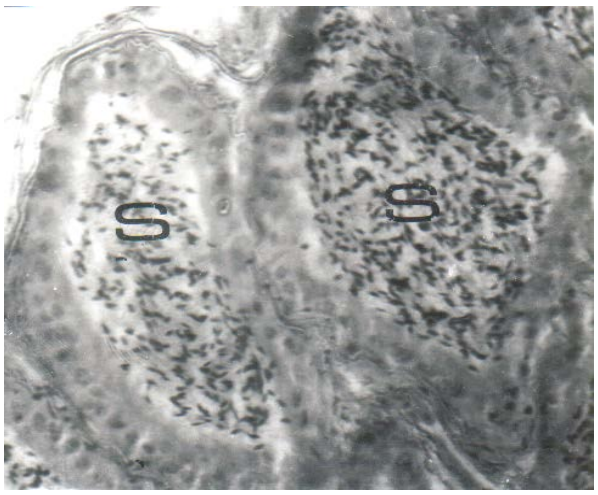
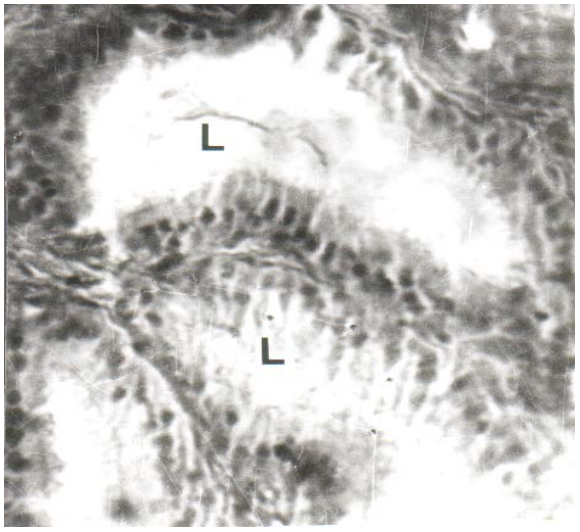


Fig. 4:



Figs. 3 and 4: Light micrographs showing cross sections of 6-week old cauda epididymis x 600
S = spermatozoa in the lumina of the cauda epididymis of the control mice
L = lumina of cauda epididymis of the pre-and postnatal alcohol-exposed mice without spermatozoa

seminiferous epithelium did not progress beyond the spermatid stage in the two alcohol-exposed groups. Therefore, the retardation of spermatogenesis in this study could be due to impairment of fetal hormonal system Taylor (1984), consequent upon disruption of the hypothalamic-pituitary-testicular axis regulation.

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REFERENCES

Abel, E.L. and H.P. Greizerstein, 1979. Ethanol-induced prenatal growth deficiency changes in fetal body composition. J. Pharmacol. Exp. Ther., 211: 660-671.

Clarren, S.K., D.W. Bowden and S. Astley, 1985. The brain in fetal alcohol syndrome. Hlth. Res. World, 10: 20-23.

Courot, M., M.T. Hochereau-de Reviers and R. Ortavant, 1970. Spermatogenesis. In: The Testes: Development, Anatomy and Physiology. Johnson, A.D., W.R. Gomez and N.L. Vandemark (Eds.) Academic Press. New York, pp: 339-342.

Grober, M.S., G.M. Winterstein, A.A. Ghazanfar and V.P. Eroschenko, 1998. The effect of oestradiol on gonadotrophin releasing hormone neurons in the developing mouse brain. Gen. Comp. Endocrinol., 112: 356-363.

Hafez, E.S.E., 1970. Reproduction and Breeding Techniques for Laboratory Animals. Lea and Febiger, Philadelphia, pp: 299-315.

Humason, G.L., 1979. Animal Tissue Technique 4th Edition. W.H. Freeman and Company, San Francisco.

Ihemelandu, E.C., 1984. Effect of maternal alcohol consumption on pre-and postnatal muscle development in mice. Growth, 48: 35-43.

Jones, K.L. and D.W. Smith, 1973. Recognition of fetal alcohol syndrome in early infancy. Lancet., 1: 999-1001.

Lee, M. and J. Leichter, 1980. Effect of litter size on the physical growth and maturation of the offspring of rats given alcohol during gestation. Growth, 44: 327-355.

Leonard, E.B., 1989. Ethanol as a neurotoxin. Biochem. Pharmacol., 36: 2005-2059.

Nwaogu, I.C. and E.C. Ihemelandu, 1999a. Effects of maternal alcohol consumption on the allometric growth of muscles in fetal and neonatal rats. Cells Tis Org., 164: 167-173.

Nwaogu, I.C. and E.C. Ihemelandu, 1999b. Allometric growth of visceral organs in fetal alcohol syndrome in rats. Nig. Vet. J., 20: 39-49.

Onu, J.E. and D.N. Ezeasor, 2001. Retardation of testicular growth in mice in fetal alcohol syndrome: Suspected evidence of disruption of hypothalamic-hypophyscal-gonadal axis regulation, Nig. Vet. J., 22: 35-41.

Onu, J.E., E.C. Ihemelandu and D.N. Ezeasor, 2002a. Testicular growth in fetal alcohol of mice. Sahel Med. J., 5: 47-49.

Onu, J.E., E.C. Ihemelandu and D.N. Ezeasor, 2002b. The effect of maternal alcohol consumption on the grwoth rate of the body and testes in neonatal mice. Nig. J. Exp. Applied Biol., 3: 159-163.

Onu, J.E., E.C. Ihemelandu and D.N. Ezeasor, 2002c. Morphometric studies of testes in fetal alcohol syndrome of mice. J. Exp. Clin. Anat., 2: 33-36.

Onu, J.E., E.C. Ihemelandu and D.N. Ezeasor, 2002d. Morphological analysis of mouse testes following gestational and lactational alcohol exposure. Nig. Vet. J., 23: 15-22.

Sharpe, R.M., 1984. Intratesticular factors controlling testicular function. Biol. Reprod., 30: 29-49.

Steinberger, E., A. Root, M. Ficher and K.D. Smith, 1973. The role of androgen in the initiation of spermatogenesis in man. J. Clin. Endocrinol. Met., 37: 746-751.

Streissguth, A.P., S. Landesman-Dwyer, J.C. Martin and D.W. Smith, 1986. Teratogenic effects of alcohol in humans and laboratory animals. Science. New York, 209: 353-361.

Taylor, H., 1984. Minimal brain dysfunction in perspective. In: H.E. Tarter and G. Goldstein (Eds.) Advanced Clinical Neuropsychology. Plenum Press. New York, pp: 207-229.