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Original article

Testicular growth and testosterone levels in male wistar rat offspring exposed to alcohol during pregnancy and/or lactation

J.E. Onu[a,]*, B.O. Oke[b], P.C. Ozegbe[b], J.O. Oyewale[c]

[a] *Department of Veterinary Anatomy, Usmanu Danfodiyo University, Sokoto.*
[b]*Department of Veterinary Anatomy.*
[c]*Physiology, Biochemistry and Pharmacology, University of Ibadan, Nigeria.*

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ABSTRACT

The effect of alcohol on testicular growth and testosterone levels in male rat offspring whose dams were exposed during pregnancy and/or lactation was investigated. Seventy-five adult female Wistar rats and their offspring were used. The female rats were divided into 3 groups of 25 each. The offspring of group 1 served as control, the offspring of group 2 was exposed to 2g/kg body weight of 30% ethanol (v/v) during pregnancy and lactation (APL) while those of group 3 was exposed to the same dose of ethanol during lactation only (AL).. At Day (D)7, D14, D21, D35 and D49 of postnatal life, 5 male offspring were randomly selected from the three groups and sacrificed. After the sacrifice, the two testes were dissected out and their weights determined. At D21, D35 and D49, blood sample for testosterone determination was collected from 5 male offspring. The result of the investigation showed significant reduction ($P<0.05$) in relative weights of the testes and significant increase ($P<0.05$) in testosterone levels in the alcohol-exposed male offspring relative to the controls. The reduced testicular weights and increased testosterone levels which persisted into adulthood may have implications for male fertility in offspring of dams who abuse alcohol during pregnancy and/or lactation.

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*Corresponding author ; Department of Veterinary Anatomy, Usmanu Danfodiyo University, Sokoto.

1. Introduction

Prenatal alcohol exposure has marked effects on the development of hypothalamic-pituitary-gonadal axes in the offspring (Lan et al., 2006). Male rat offspring whose dams were given alcohol during late pregnancy display atypical sexual behavior in adulthood (Ingeborg et al., 2003). In man, the brain growth spurt which is a period during which the brain is particularly vulnerable to environmental influences begins at about mid-pregnancy and peaks around birth (Dobbing and Sands, 1973). In laboratory animals such as rats, the brain growth spurt occurs primarily during the first 2 weeks after birth (Dobbing and Sands, 1979). The period of maximal vulnerability in the rat is thought to coincide with the postnatal period. Alcohol may act directly on the developing foetal glands and target organs for hormone production (Jeff and Carey, 2005). Neonatal alcohol exposure has been shown to cause cerebellar defects (Dobbing and Sands, 1979). If neonatal alcohol exposure can affect the development of cerebellum, it is likely that other parts of the brain like the hypothalamus can be affected. This could also lead to marked effects on the development of hypothalamic-pituitary-gonadal axis like prenatal alcohol exposure. This axis plays a 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). It is possible that the marked effect on the hypothalamic-pituitary-gonadal axis could have effect on testicular weight and testosterone synthesis and release in rats whose dams were exposed to alcohol during pregnancy and/or lactation. This study attempts to investigate these parameters,the report of which appears to be unrecorded in the literature.

2. Materials and methods

2.1. Experimental animals

Seventy five female Wistar rats aged between 4 and 5 weeks and 21 fertile adult male rats were used in this investigation. Both the females and males were obtained from the Laboratory Animal Unit, Faculty of Veterinary Medicine, University of Ibadan, Nigeria. All the animals were fed water and commercial diet (Guinea Feed, Bendel Feed and Flour Mills, Nigeria Plc.) ad libitum throughout the duration of the study. After acclimatization for five weeks, the 75 female rats were into 3 groups of 25 each. Group 1 served as control (C), group 2 was exposed to alcohol during pregnancy and lactation (APL) while group 3 was exposed to alcohol during lactation only (AL).

2.2. Breeding of experimental rats

At the commencement of the study, the female rats weighing 145-170g were bred overnight by introducing 1 male rat into a cage housing 5 female rats. Day 1 of pregnancy was presumed after observing copulation plug the following morning.

2.3. Alcohol administration

Following pregnancy detection, 2g/kg body weight of 30% ethanol (v/v) was given to the pregnant rats in APL per os the rats in APL. Theses continued throughout pregnancy and lactation. After delivery, the lactating rats in AL were given the same quantity of ethanol per os and this lasted throughout lactation.

2.4. Sample collection

At D7, D14, D21, D35 and D49 of postnatal life, 5 female offspring were randomly selected from the three groups, weighed and sacrificed and the two testes removed and weighed At D21, D35 and D49 of postnatal life, blood samples were also collected from 5 male Wistar rat offspring from each of the 3 subgroups (Control, APL and AL) after anaesthesia achieved by intramuscular injection of a combination of Ketamine and Diazepam at a dose of 75mg/kg and 10mg/kg body weight respectively. The blood was transferred into heparinized sample tubes. The collection was done between 8 and 10 am to rule out any diurnal variation in hormone levels (Wilcoxon and Redei, 2004). The blood in heparinized sample tubes was spun in a centrifuge at 3000 rpm for 5-10 minutes. The blood in the heparinized sample bottles was spun in a centrifuge at 3000 rpm for 20 minutes. The plasma component was withdrawn into a clean, dry glass vial and stored at – 20oC till the testosterone assay was done.

2.5. Quantitative measurement

Following removal, the testicular weights were determined with Metler's Analytical Balance (MICROWA SWISS, 5540) and expressed as relative weights according to Riser and Shirer (1967).

2.6. Enzyme – linked immunoassay

The kit used for the assay of testosterone was from immunometrics (UK) Ltd., 280 Munster Road, London, SW6 6BQ. Serum testosterone was measured immunometrically according to the manufacturer's instructions. Testosterone enzyme-linked immunoassay is a direct assay of a limited or competitive type. Sodium azide was used to displace testosterone from binding proteins thereby making it available for antibody binding. The testosterone in the sample equilibrated with a fixed amount of alkaline phosphatase labeled testosterone in binding to a limited amount of fluorescein labeled polyclonal anti-testosterone antibody. Then an anti-fluorescein serum bound to magnetic particles was used to separate the testosterone/testosterone labeled-antibody complex from unbound components by magnetic sedimentation and wash step. The magnetic particles were incubated with enzyme substrate solution for a fixed time and the reaction ended by an addition of stop buffer. The amount of colour produced by the alkaline phosphatase from yellow to pink was inversely proportional to the amount of testosterone present in the sample. The testosterone concentration of test sample was interpolated from a calibration curve.

2.7. Statistical analysis

The data generated were subjected to statistical analysis. Means and standard errors of means were calculated for each group. Analysis of variance (ANOVA), was used to examine whether there were differences among the three groups in the parameters measured. Duncan's Multiple Range Test (DNMRT) was used to determine which groups differed (Duncan, 1955). For the statistical tests, $P<0.05$ was statistically significant.

In October 2011, 20 specimens (length, 12.9 ± 0.3 cm) of A. cygnea were collected from the Tajan River estuary (36°48′46" N, 53°6′57" E) (Mazandaran Province, Iran). The valves of specimens were opened by cutting anterior and posterior abductor mussels using a scalpel. Samples of ≈0.5 cm3 from mantle and foot were removed and fixed into Bouin's solution (saturated picric acid, 75 parts, 40% formaldehyde, 25 parts and glacial acetic acid, 5 parts) for 48 h and then stored in 70% ethanol. The histological sections (5-7 μm thickness) were prepared based on Hewitson and Darby (2010) and stained using haematoxylin and eosin. Stained tissues were observed under a light microscope (Leica MS5).

3. Results

3.1. Relative weights of the testes

At D7, D14, D21, D35 and D49 of postnatal life, the relative weights of the testes in APL were significantly reduced ($P<0.05$) relative to the control (Table 1). However, in AL, the relative weights of the testes were significantly reduced ($p<0.05$) at D7, D14, D21 and D35 relative to the control while at D49, the relative weights were similar ($p>0.05$) to the control. Further comparison showed that the relative weights in APL and AL were

similar ($p>0.05$) at D7 while at D49, the relative weights in APL were significantly reduced ($p<0.05$) when compared with the AL.

3.2. Testosterone levels

There was a significant increase ($P<0.05$) in plasma testosterone levels in APL at D35 and D49 as against the control while the levels in AL were significantly increased ($p<0.05$) at D21, D35 and D49 relative to the control (Fig. 1).

Table 1
Comparison of the relative weights (%) of testes of male rat offspring whose dams were exposed to alcohol during pregnancy and/or lactation.

Age at sacrifice (Days)	Control group	APL	AL
7	0.14±0.01a	0.075±0.02b	0.10±0.10b
14	0.20±0.002a	0.11±0.01c	0.13±0.01b
21	0.27±0.01a	0.17±0.002c	0.21±0.001b
35	0.49±0.04a	0.26±6.70c	0.35±0.04b
49	0.41±0.01a	0.22±0.03b	0.37±0.01a

Means with different superscripts on the same row are significantly different at ($P<0.05$) while means with common superscripts on the same row are similar at ($P>0.05$).
APL, Male offspring of dams exposed to alcohol during pregnancy and lactation
AL, Male offspring of dams exposed to alcohol lactation only

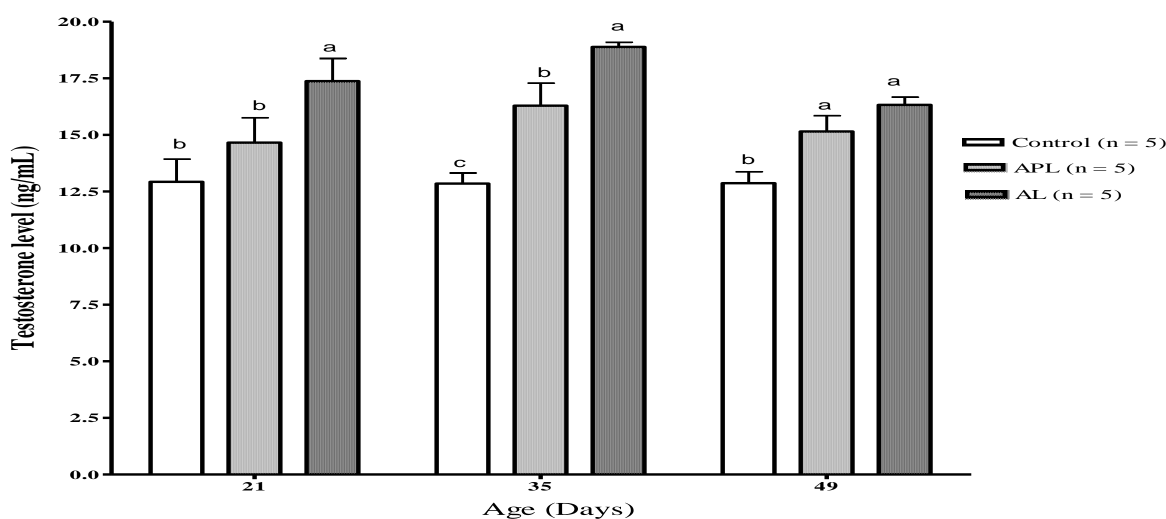

Fig.1: Plasma testosterone levels (mean ± SEM) in APL or AL or control.

Bars with different alphabets (a,b,c) are significantly different at $p<0.05$ using DMRT

APL: Male rat offspring whose dams were exposed to alcohol from pregnancy to lactation

AL: Male rat offspring whose dams were exposed to alcohol during lactation

n: number of animals

4. Discussion

This investigation has shown that maternal alcohol consumption in rats during pregnancy and/or lactation produced retardation of the growth of the testes. This is evident when the relative weights of the testes were

used as growth indices. The use of relative weights is necessary in the comparison of organ weights since organ weights are dependent upon the body weights. The difference in relative weights, therefore confirmed that there was true significant difference between the weights of testes of the control and the alcohol-exposed groups. This observation is similar to that of Murillo-Fuentes et al., (2001) on liver, kidney and spleen of offspring of rats exposed to alcohol during pregnancy and/or lactation. He also observed significant reduction in milk consumption by the offspring. However, milk consumption by the offspring was not determined in this investigation. The 30% and 20% ethanol used in this investigation and that of Murillo-Fuentes et al., (2001) respectively, which produced retardation of growth of organs implies that even at a lower dose levels, alcohol can be detrimental to the growth of foetal and neonatal organs (Anuppa, 1989). The similarity in relative weights of the testes of the control and the lactational alcohol-exposed group at the D49 is an indication of an attempt to catch-up growth with the controls. This implies that the effect of lactctional alcohol exposure on the growth of the testes is not as severe as exposure during pregnancy and lactation in which there was no attempt to catch-up growth (Onu et al., 2002).

The mechanism of the decrease in testicular weights was not known from this investigation. However, alcohol is a known neurotoxin (Leonard, 1987) and could destroy the developing neurons of the brain including possibly those of the hypothalamus. Passage of alcohol from mother to foetus occurs via the placenta to a point where foetal and maternal concentrations are almost equal (Anuppa, 1989) and alcohol has direct toxic effect on foetal organ development (Fisher et al., 1981). Alcohol administered to lactating rats is transferred to neonates through the milk (Mennella, 2001). The lactational period coincides with the brain growth spurt which is the period of maximal vulnerability to environmental insults.

Neonates have limited capacity to metabolize alcohol which in turn accumulates in the tissues. This limited capacity may be due to lower activity of alcohol dehydrogenase and cytochrome P-450 system found in the liver which is involved in alcohol metabolism (Mennella, 2001). This accumulated alcohol could be destructive to the tissues of the foetus and neonates leading to poor growth. In this investigation, alcohol was administered to the dams during pregnancy and/or lactation. It is possible therefore that the alcohol may have affected the development of the brain including the hypothalamus. This action on hypothalamus could lead to disruption of the hypothalamic-pituitary-gonadal axis regulation as suggested by Onu and Ezeasor, (2001). The hypothalamus has neurosecretory neurons which synthesize and release hypothalamic releasing factors that act on rostral pituitary gland to release gonadotropins which control the maturation and regulation of the action of the gonads including growth (Grober et al., 1998).

The results of this investigation demonstrated increases in the testosterone levels in male rat offspring whose dams were exposed to alcohol during pregnancy and/or lactation. This is at variance with the observation of Ward, (2002) who observed attenuated testosterone increase in neonatal rats exposed to alcohol during foetal life. However, the rats in this investigation were exposed to alcohol during pregnancy and/or lactation.

The cause of increased testosterone levels in male rat offspring whose dams were exposed to alcohol in this study was not immediately known. However, it is a well established phenomenon that chronic alcohol exposure to adult rats reduces blood levels of testosterone (Adams et al., 1997). However, alcohol markedly elevates (by 200-300%) plasma testosterone levels in prebuscent rats (Little et al., 1992) and adolescent hamsters (Ferris., 1998). One possible explanation could be due to differences in adrenal/testicular contribution to circulating testosterone in immature male rats with testicular contribution being minimal. Little et al., (1992) studied the effects of alcohol on the pituitary-gonadal axis in sexually immature rats and reported increased testosterone levels. Since the testicular contribution is minimal, the stimulation of adrenal cortex could have led to increased testosterone levels as observed in this study. However, the adrenal androgens; dehydroepiandrosterone sulphate and dehydroepiandrosterone are not directly associated with the development of reproductive organs and sexual maturity (Watanabe and Oonuki, 1999) but are converted to testosterone by aromatization (Haning et al., 1991). Thus this increased plasma levels of testosterone observed in the male rat offspring of alcohol-exposed dams in this investigation did not improve spermatogenesis perhaps because of the suppressive effect of alcohol on the development of spermatogenic cells.

References

Adams, M.L., Meyer, E.R., Cicero, T.G., 1997. Interaction between alcohol- and opioid- induced suppression of rat testicular steroidogenesis in vivo. Alcohol. Clin. Exper. Res., 21, 684-690.

Anuppa, C., 1989. Fetal Alcohol Syndrom. Health Science and Technology Gallery. www.digitalism.org

Dobbing, J., Sands, J., 1973. Quantitative growth and development of human brain. Archives of Disease in childhood., 48, 757 – 767.

Dobbing, J., Sands, J., 1979. Comparative aspects of the brain growth spurt. Early Human Dev., 3(1), 79 – 83.

Duncan, D.B. (1955), Multiple Range and Multiple Fisher's tests. Biometrics. II., 1 – 42.

Ferris, C.F., Shitiegman, K., King, J.A., 1998. Voluntary ethanol consumption in male adolescent hamsters increases testosterone and aggression. Physiol. Behav., 63, 739-744.

Fisher, S.E., Barnicle, M.A., Steis B., Holzman, I., Van Thiel, D.H., 1981. Effect of acute ethanol exposure upon in vivo leucine uptake and protein synthesis in the fetal rat. Pediatr. Res., 15, 335-339.

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

Hafez, E.S.E., 1970. Reproduction and Breeding Techniques for Laboratory Animals. Philadelphia. Lea and Febiger., 5 – 17.

Haning, R.V., Carlson, I.H., Flood, C.A., Hackett, R.J., Longcope, C., 1991. Metabolism of dehydroepiandroterone sulfate (DS) in normal women and women with high DS concentration. J. Clin. Endocrinol. Metabol., 73, 1210-1215.

Ingeborg, L.W., Ward, O.B., Affuso, J.D., Long III, W.D., French, J.A., Hendricks, S.E., 2003. Fetal testosterone surge, Specific modulations induced in male rats by maternal stress and/or alcohol consumption. Horm. Behav., 43, 531-539.

Jeff, S., Carey, L.C., 2005. Programming effects of moderate binge alcohol consumption. Early life Origins of Health and Disease. Marelyn, Wintour- Coghlan. E. Owens., J. 1-8. (eds).

Lan, N., Yamashita, F., Halpert, A.G., Ellis, L., Yu, W.K., Viau, V., Weinberg, J., 2006. Prenatal ethanol exposure alters the effects of gonadectomy on hypothalamic- pituitary-adrenal activity in male rats. J. Neuroendocrinol., 13,672-684.

Leonard, E.P., 1987. Ethanol as a neurotoxin. Biochem. Pharmacol., 36(23), 2055-2059.

Little, P.J., Adams, M.L., Cicero, T.J., 1992. Effects of alcohol on hypothalamic-pituitary- gonadal axis in the developing male rats. J. Pharmacol. Exper. Therapautics., 263, 1056-1061.

Maier, S.E., West, J.R. 2004. Patterns of alcohol-related birth defects. National Institute on alcohol Abuse and Alcoholism. Bethesda., Maryland hppt,//www.niaaa.nih.gov

Mennela, J., 2001. Alcohol's effect on lactation. http,//www.niaaa.nih.gov.

Ward, I.L., Ward, O.B., Affuso, J.D., Long III, W.D., French, J.A., Hendricks, S.E., 2003. Fetal testosterone surge, Specific modulations induced in male rats by maternal stress and/or alcohol consumption. Horm. Behav., 43,531-539.

Murillo-Fuentes, L., Artillo, R., Carreras, O., Murillo, L., 2001. Effects of maternal chronic alcohol administration in the rat; lactation performance and pup's growth. Eur. J. Nutr., 40(4), 147-54.

Onu, J.E., Ezeasor, D.N., 2001. Retardation of testicular growth in mice in fetal alcohol syndrome, Suspected evidence of disruption of hypothalamic-hypophyseal- gonadal axis regulation. Niger. Veter. J., 22(2),35-51.

Onu, J.E., Ezeasor, D.N., Ihemelandu, E.C., 2002. Morphological analysis of mouse testes following gestational and lactational alcohol exposure. Niger. Veter. J., 23(2), 15-22.

Riser, W.H., Shirer, J.F., 1967. Correlation between canine hip dysplasia and pelvic muscle mass. A study of 95 dogs. J. Amer. Veter. Res., 28, 767-777

Schaubacher, B.D., D'Oechio, M.J., Kinder, J.E., 1982. Initiation of spermatogenesis and testicular growth in oestradiol 19β-implanted bull calves with pulsatile infusion of LHRH. J. Endocrinol., 93, 183-192.

Ward, I.L., Ward, O.B., Affuso, J.D., Long III, W.D., French, J.A., Hendricks, S.E., 2003. Fetal testosterone surge, Specific modulations induced in male rats by maternal stress and/or alcohol consumption. Horm. Behav., 43,531-539.

Watanabe, N., Oonuki, Y., 1999. Inhalation of diesel engine exhaust affects spermatogenesis in growing male rats. Env. Health Perspective., 107(7), 539-544.