Anti-fertility Activity of Ingestion of Nigerian Bonny Light Crude Oil Contaminated Feed in Male Rat Reproduction: A Possible Hypothalamo-Pituitary Axis Mechanism

[a]Nwafor A, [a]Asiegbu L N, [a]Adienbo MO and [b]Mmom FC

[a]Department of Human Physiology, Faculty of Basic Medical Sciences, College of Health Sciences,
University of Port Harcourt, PMB 5323, Choba, Port Harcourt
[b]Department of Obstetrics and Gynaecology, University of Port Harcourt Teaching Hospital, Port Harcourt.
Correspondence author E-mail: arthur.nwafor@uniport.edu.ng

ABSTRACT

Over the past decades, particularly in the Niger Delta region of Nigeria, oil and gas flared and/or oil-spilled microscopic pathogens are continually introduced into the environment and the immediate or short term impact on humans (as well as wildlife) can hardly be noticed except when damage has been done. This study therefore investigated the potentially dangerous adverse reproductive fertility impact of continuously exposure to short-term low-levels of Nigerian Bonny Light crude oil (BLCO) contaminated feed. Virgin fertile male rats of reproductive age were exposed to 0.3ml, 1.0ml and 2.5ml of BLCO mixed with 20g of rat feed respectively for two weeks. Thereafter, one group were mated with fertile virgin female rats also of reproductive age till parturition. Whereas another group were sacrificed and the testes were removed for histological examination, and hormonal profiles and semen qualities were analysed. Our results showed histologically no detectable differences in the testicular architecture, but dose-response decrease association between the crude oil polluted diet, hormonal and semen parameters, growth rate and pups with low - birth rate compared with control ($p < 0.05$). It is suggestive that the hypothalamus part of the brain may be "turned off" when triggering the pituitary glands to produce sex hormones. More so, the environmental pollutant probably acted in the control of reproduction fertility by interfering with the feedback mechanisms on the hypothalamic-pituitary-gonadal axis, thereby bringing about oxytocin-like type of contractions of the uterus as in the females. The values of the calculated reproduction indices indicated that BLCO adversely affected reproductive fertilities-sexual life quality-sperm quality and their productions as well. The significance of the environmental pollutant induced reproductive fertility hazards is discussed.

***Keywords*:**, Crude oil, feed, infertility, sex hormone, semen quality, low-birth rate, testicular histology.

{**Citation:** Nwafor A, Asiegbu L N, Adienbo MO, Mmom FC. Anti-fertility activity of ingestion of Nigerian bonny light crude oil contaminated feed in male rat reproduction: a

possible hypothalamo-pituitary axis mechanism. American Journal of Research Communication, 2013, 1(7): 210-218} www.usa-journals.com, ISSN: 2325-4076.

INTRODUCTION

The decline in reproductive fertility, a disease of the reproductive system as well as in life span is a public health concern. Although the global rate of infertility has not significantly changed over the past two decades, the exception is the regions in the Sub-Saharan Africa where infertility is greatest primary infertility for instance, dropping from 2.7% in 1990 to 1.9% in 2010, and ranked 1st for global infertility. Nigeria, in particular, has low life expectancy level, at just 46.94 years in 2005-2010, and ranked 211nd (UN 2011, Maya et al 2012, WHO 2013). Whilst it has been acclaimed primarily to resurgent infectious diseases such as malaria, spread of HIV/AIDS epidemic, the research on the impact of prolonged environmental associated exposure to oil and gas flared pathogens in health and disease is limited. Whereas exposure to petrol and diesel (Akintunde et al 2012), and inhalation of environmental air pollutants (Messenger 2010) has been suggested to lower fertility in males. Beside, the poor sperm quality following ingestion of Nigeria bonny light crude oil (BLCO) contaminated feed has been attributed to environmental - toxin induced oxidative damage of the antioxidant systems in the testes/epididymis or through the production / elevation in oxidative stress in sperm cells in males (Farombi et al 2010, Adesanya et al. 2009). The environmental pollutants generate a high concentration of free radicals in blood, which is capable of lowering sperm quality even in the infertile males (Messenger 2010). It is imperative that in the oil rich Niger Delta region of Nigeria, exposure to oil-spill and/or oil and gas flared microscopic pollutants as well as exposure of food consumables to environmental contaminants are common occurrences and account for a more serious environmental exposure to crude oil by humans (as well as wildlife). It is hypothesised that ingestion of feeds exposed to chronic - low - levels of potentially hazardous chemically unrelated substances generated from oil and gas contaminated environment have reproductive modulatory and anti-fertility potentials involving a complex reaction between the higher centres – the brain - the hypothalamus – pituitary- axis and the sex organs

Insofar, there is paucity of literature on the impact of ingestion of oil and gas flared and /or oil-spilled contaminated feed on the reproduction - endocrine physiology system in humans. In this study therefore, the impact of short-term exposure of bonny light crude oil (BLCO) contaminated feed on some reproductive indices in animal model system has been studied. An attempt has been made to elucidate the impact of oil and gas flared and/or oil-spilled pathogens associated

contaminated feed in the environment on the reproductive systems admitting that the scientific data came from experimental animals which the conclusions, if any, may or may not be applied to humans easily; but from which our understanding of the relationship between the impact of environmental associated oil and gas related toxins and reproductive health and disease may emerge.

MATERIALS AND METHODS

Animal model: Thirty two fertile virgin male and twelve fertile female Wistar albino rats of reproductive age weighing between 175g – 235g were maintained separately in the departmental animals' facility and fed on rat's feed (Pfizer feed). The thirty two adult Wistar rats were randomly divided into four groups of eight rats per group, including one control group and three groups of rats treated with vehicle. Treatments were given for 14 days. Rats were weighed before treatment and thereafter. It should be emphasized that animal models are often employed in scientific investigations owing to its convenience, reproducibility, and availability.

Contaminated feed studies- hormonal, semen and histological analysis: Bonny light crude oil (BLCO) employed in the study were obtained from Shell Petroleum Development Company (SPDC) Port Harcourt. Twenty (20g) grams of rat feed contaminated with 0.3ml, or 1ml, or 2.5ml/rat of Bonny light crude oil respectively were fed the test groups while the control groups received uncontaminated feed. Both the treated and control groups were allowed access to water *ad libitum*. Prior to the study, BLCO contaminated feed were administered to virgin male rats after a 24h fasting and subsequently daily for two weeks. Thereafter, five animals each from both the control and test groups were sacrificed for sperm and hormonal analysis as described elsewhere (Egwurugwu and Nwafor 2011,); and the testes were histologically examined after haematoxyline – eosin staining. While in another study, the remaining male animals were mated with fertile virgin females (in the ratio 1 male: 1 female) till parturition for reproduction studies as previously described (Nwafor and kalio 2006). The male fertility index (%) was described by the relationships: - (No. of pregnancies / No. of mating) x 100

Statistical Analysis

The weight data were expressed in mean+ S.D as well as in percentage deference. Statistical analysis were carried out by using the student t-test and ANOVA where applicable with input into SPSS version 17 software Microsoft computer (SPSS, Chicago, Illinois).Statistical significance was considered at $P<0.05$.

RESULTS

Effect of crude oil contaminated feed on reproductive hormone

Ingestion of Nigerian Bonny light crude oil (BLCO) contaminated feed, in-vivo, in a dose-response association statistically significantly caused a gradual decrease ($p < 0.05$) in the reproductive hormones testosterone (82% - 89%), luteinizing hormone (83% - 89%) and follicle stimulating hormone (20% - 40%) compared with control. Fig.1 depicts the relative percentage difference in the hormonal profiles in relation to the control at different concentrations of BLCO.

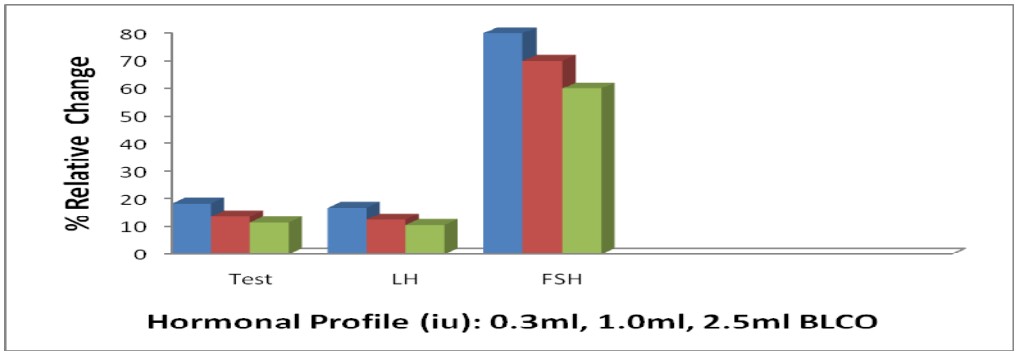


Fig.1 The effect of crude oil contaminated feed on hormonal profiles in male rats.

Effect of crude oil contaminated feed on semen parameters

BLCO statistically significantly ($p < 0.05$) caused a dose-response decrease in sperm motility (42% - 48%), sperm count (41% - 59%), sperm viability (47% - 63%) while the decrease (20%) in their physical attributes (morphology) were marked at a higher dose (2.5ml/day/rat,) (Fig. 2); correspondingly, the debris significantly increased (69% - 77%) in the treated groups in a dose response association compared with control.

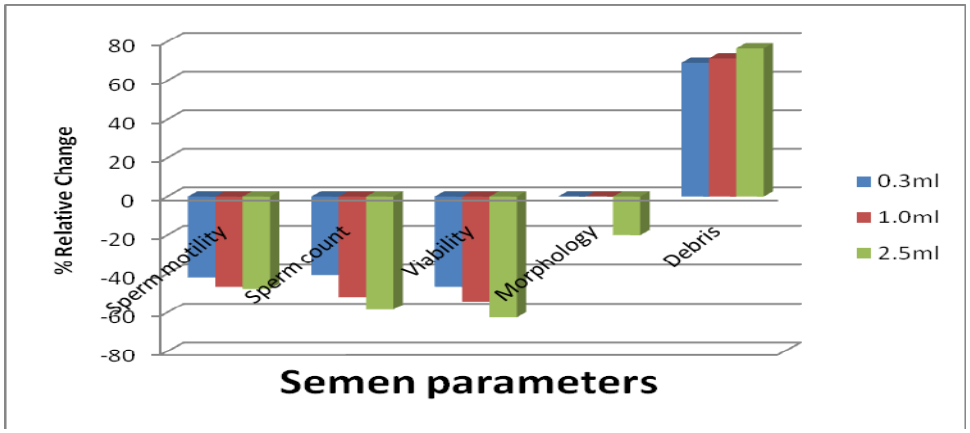


Fig. 2 The effect of crude oil on semen parameters.

Relationship between growth rate, litter birth rate and reproduction index

The relative body weight of the male adult rats fed on crude oil contaminated feed and the weight of litters produced by pregnant rats decreased significantly ($p < 0.05$) by 33% - 35% and 23% - 27%; correspondently, the calculated values of the reproductive indices, - fertility index, also decreased significantly (33.6% - 100%) respectively compared with control with increase in BLCO concentration (Fig.3).

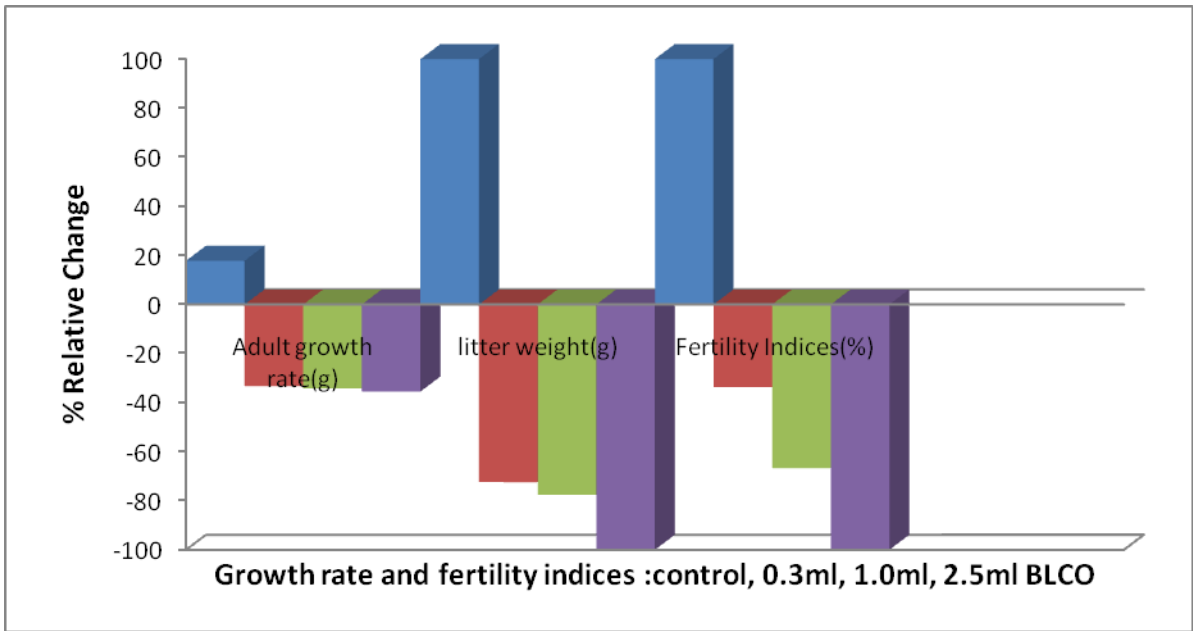
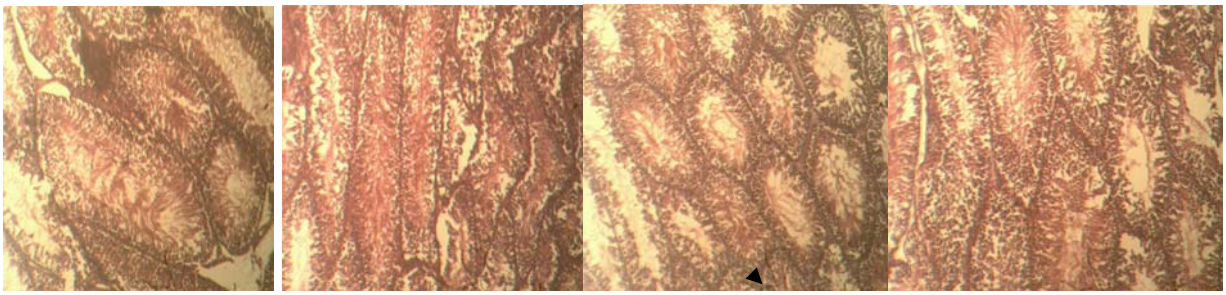


Fig.3 The effect of crude oil on growth rate and reproductive indices.

When compared with the control group, the litters from pregnancies in the female rats mated with males rats fed on 0.3ml and 1.0ml of bonny light crude oil contaminated feed were significantly reduced giving low birth rate of 33.3% and 16.7% respectively. A higher dose of BLCO (2.5ml/rat) decreased female rat's chances of conceiving and carrying pregnancies to term (Fig. 3).

Testicular Histology

Fig. 4(A-D) show testicular photomicrographs of the treated animals compared with control. There were no detectable differences in the architecture of the testicular sections as compared with the control group (A), and the animals fed on contaminated feed with 0.3ml (B), 1.0 ml (C), or with 2.5ml (D) BLCO; all showing normal spermatocytes , maturing spermatogonias, sertoli and interstitial leydig cells respectively.



Group A (control) Group B (0.3ml/rat/day) Group C (1.0ml/rat/rat/day) (Group D (2.5ml/rat/day)

Fig.4 Testicular sections from (A) control, (B) test group (0.3ml/rat/day), (C) test group (1.0ml/rat/day), and (D) test group (2.5/rat/day), showing normal spermatogenic and intestinal leydig cells, H&E x 400.

DISCUSSION

Fertility, rather infertility, is a major clinical problem affecting people medically, psycho-socio-economically, as well as family instability. According to World Health Organization (WHO) infertility affects about 8 to 12 % of the world's population and the cause or causes of infertility can involve one or both partners, with one third being due to the male partner, one third the female and one third involving both the man and woman; and unknown factors 5% of causes of infertility. Besides, WHO estimated that in 2010, 48.5 million couples worldwide were unable to have a child and that 1.9% of women in the age of 20 – 44 who wanted a child were unable to conceive, while 10.5% of those who had already given birth were unable to have another child (Warren-Gash 2013). The derived values for the reproductive indices showed that environmental pollutants of oil and gas origin significantly contributed to adverse reproduction effects by lowering fertility in males and thus complicating pregnancies in females and can account for some of the cases of unknown infertility in many infertile couples where no apparent causes of infertility are found even after thorough medical examination. In fact, male infertility has been associated with the inhalation of air pollutant (Messenger 2010), and exposure to environmental - pollutant - associated lifestyle and endocrine disrupting compounds (Akintunde 2012, Miyamoto et al 2012, Priskorn et al 2012,). Exposure to high level of airborne particulate matter (PM_{10}) has been found to contribute to babies with low birth rate (Messenger, 2010). Our results showed that crude oil pathogens significantly contributed to endocrine disruption of blood hormonal levels in agreement with previous studies (Farombi et al 2010, Otitoju et al 2011,) but those were studies carried out at BLCO concentrations which distorted testicular physiology (Adesanya et al 2009). It is obvious that fertility in men requires normal functioning of the

hypothalamus and pituitary gland (hormone-producing glands in the brain), and the testes. Our results showed normal testicular architecture histologically in the treated groups but dose-response - association between both the crude oil pullulated diet and male sex hormones compared with control, which indicates that the brain's mechanisms are especially important because it's there that the problem begins. The hypothalamus part of the brain following exposure to low-levels of the crude oil pathogens (via the blood system) may be "turned off" when triggering the pituitary glands to produce the most important male sex hormone, testosterone. Accordingly, in humans, the men (younger men) with low testosterone levels (or hypogonadism) may be predisposed to developing erectile dysfunction, and also be at a higher risk of developing heart disease, obesity or increased body fat, type -2 diabetes and high cholesterol level as well as decrease in sexual endurance, or metabolic syndrome and many more. What's more, low testosterone levels may slow or stop sexual maturation - the males (teenagers) could grow up to be inadequate in sexual performance and also in terms of fertility (Mogri et al 2013).

In the present study, BLCO, in-vivo, also significantly contributed to a decline in sperm count, sperm motility, and sperm viability with corresponding increase in abnormal sperm morphology, in a dose - response fashion, compared with control. The actions of the crude oil polluted diet in the male reproductive system and male fertility are of great interest in that the chemicals are hazardous to animal (including human) biology and health, particularly in the uterus and in the developing pups. It is plausible that the environmental pollutants, in-*vivo,* acted in the control of fertility by interfering with the feedback mechanisms on the hypothalamic-pituitary- gonadal axis, thereby bringing about oxytocin-like type of contractions of the uterus as in females or impair reproductive capabilities in males either by hindering the release of semen, reduced sperm quality consequently lowering the survival and development of the offspring controlled by the blood hormonal level. Thus the decrease in the values of the reproduction indices calculated for the reproduction physiology are indications that exposure to Nigerian bonny light crude oil adversely affected reproductive fertilities. However, it is not clear whether or not the sperm DNA was compromised, and needed further studies. The results showed that exposure to BLCO significantly cause low birth rate as well as a decrease in growth rate or poor sperm quality and may probably be associated with environmental - toxin - induced reactive oxygen species (ROS) production or oxidative stress damaged in cells (Farombi et al 2010). A high concentration of free radicals in blood has been suggested to lower sperm quality even in the infertile males

(Messenger 2010). The negative impact of BLCO on growth rate is in consonance with the studies on rabbits (George and Sese, 2010) and rats (Otitoju et al 2011) but differs from the study with mice (Adesanya et al 2009). Researchers have suggested that there are many heavy metals in the oil and gas flared and/or oil spilled in Nigeria which are capable of affecting body functions (Joffa et al 2013, Egwurugwu et al 2013). Unfortunately, wildlife (as well as humans) has not evolved physiological mechanisms or biochemical pathways to detoxify and excrete the environmental associated chemical substances. They are stored and accumulated in the body lipids and fatty tissues, and raise rare disease risk in humans (as well as wildlife) in the annihilated areas both young and old as well as exacerbate communicable and/or non-communicable chronic diseases (Adienbo and Nwafor 2010, Egwurugwu et al 2013). The continually exposure in the sub-Saharan region, particularly Nigeria, to potentially harmful environmental associated oil and gas flared pathogens probably induced endothelial cell injury, activation or dysfunction (Cines et al 1998,) and may be responsible for the pathophysiology of several diseases in these regions. Hitherto, our findings can account for the predicted shortened life span and/ or decreased fertility (UN, 2011) in the developing countries such as Nigeria, particularly in the Niger Delta region where continuous exposure to uncontrollable environmental associated pollutants are a common feature, need be controlled, as man is responsible for its production.

REFERENCES

Adesanya OA, Shittu LAJ, Omonigbehin EA and Tayo AO (2009). Spermatotoxic impact of bonny light crude oil (BLCO) ingestion on adult male Swiss albino mice.Inter.J.Phy.Sci. 4(5); 349-357.

Adienbo OM& Nwafor A, (2010) Effect of prolong exposure to gas flaring on some haematological parameters of humans in the Niger Delta region of Nigeria. J. Appl. Sci. Environ. Mgt 14(1) 13-15

Akintunde OW, Ameen SA and Olaniyan LWB (2012): Commonly used hydrocarbons (petrol and diesel) adversely affects male reproduction. Veterinary Research 5(2): 8 – 12

Cines DB, Pollak ES, Buck CA, Loscalzo J et al (1998) Endothelial cells in Physiology and in the pathophysiology of the vascular disorders. Blood 91(10) 3527-3561

Egwurugwu JN &Nwafor A (2011) Relationship between Body Mass Index, Semen and Sex Hormones in Adult Males Nig J.Phy.26 (1) 29-34.

Egwurugwu JN, Nwafor A, Chinko BC, Olorunfemi O J, Iwuji SC &Nwankpa P(2013). Effects of prolonged exposure to gas flares on the lipid profile of humans in the Niger Delta region, Nigeria. Am. J. Res. Com. 1(5): 115-145

Farombi EO, Adedara IA, Ebokaiwe AP, Teberen R, and Ehwerhemuepha T (2010) Nigerian Bonny light oil disrupts antioxidant systems intestes and sperm of rats. Arch. Environ.Cohtan.Toxicol.59(1): 166-74

George OS and Sese BT (2010): Effects of crude oil contaminated feed on performance and organ weights of growing rabbits in the Niger Delta Area of Nigeria.www.woaj.org/man.

Joffa P K P, Nwafor A & Adienbo MO (2013*)* Correlation between body mass index and peak expiratory flow rate of an indigenous Nigerian population in the Niger Delta Region. Res. J. Rec. Sci. 2(2): 28-32

Mahendra P (2013) Global infertility unchanged; medwireNews Reporter: www.news-medical.net.

Maya NM, Seth RF, Ties B, Sheryl V, Gretchen AS (2012) National, regional, and global trends in infertility prevalence since 1990: A systematic analysis of 277 health surveys. PLoS Medicine,9(12)

Messenger S (2010): Air pollution can harm human production: In Living/ Green food. www.treehugger.com.

Miyamoto T, Tasujimura A, miyagawa Y, Koh E, Namiki M, and Sengoku K (2012). Male infertility and its causes in human. Advances in Urology, 2012: 1155-1162.

Mogri M, Dhindsa S, Quattrin T, Ghanim H and Dandona P(2013)Testosterone concentrations in young pubertal and post-pubertal obese males. Clin. Endocrinol (Oxf). 78(4): 593-599.

Nwafor A & Kalio ID (2006) Physiological effects of *xylopia aethiopica* on pregnancy in albino rats. J.Med & Pharm. Sci. 2(1): 1-4

Otitoju O, Onwurah INE, Otitogu GTO, Ndem JI and Uwah AF (2011). Effect of bonny light crude oil contaminated diet on serum cholesterol and testerone concentration in wistar albino rats. Afri. J. Biotech. 10(53): 10986-10990.

Priskorn L, Holmboe SA, Jørgensen N, Andersson AM, Almstrup K,Toppari J and Skakkebæk NE (2012) Adverse trends in male reproductive health and decreasing fertility rates. Anim. Reprod., 9(4) p.760-771

United Nations, Department of Economic and Social Affairs, Population Division (2011): World Population Prospects: The 2010 Revision, Highlights and Advanced Tables. New York.

Warren-Gash C (2013). Worldwide infertility rates unchanged in 20 years says World Health organisation. Bio News 687

World Health Organization (2013): World development indicators