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Research Article

Effects of Exposure to Effluent Contaminated River Water on Boar Reproduction

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ABSTRACT

This study was designed to evaluate the effects of exposure of boars to wastewater/effluent contaminated water on testicular function. Boars reared on contaminated water were purchased, and their testicular tissue was processed for histopathological observations. The lesions observed included; vacuolated seminiferous epithelium, sloughing of germ cells and depletion of the seminiferous epithelium. The findings indicate that exposure of boars to mixtures of environmental toxicants can result in perturbation of spermatogenesis with consequences on fertility.

Key words: River contamination, Endocrine disruptors, Testicular histopathology

INTRODUCTION

Endocrine disrupting chemicals have been detected in urban draining rivers globally (Huang and Sedlak, 2001; Kolpin *et al.,* 2002) and have been shown to have adverse effects the reproductive system of animals (Paul *et al*., 2005) through disturbance of estrogen and or androgen mediated processes (Bello *et al.,* 2014). Known endocrine disrupting chemicals include: plasticizers, surfactants, pesticides and pharmaceutical wastes, the sources of these compounds have been identified to be indiscriminate discharge from industries, households and farmlands (Stevens *et al.,* 2003). Pollution of surface water due to inadequate municipal sewage treatment and runoff from informal settlements close to the river has been demonstrated in various regions (Sibanda *et al.,* 2015). In Kenya, studies on urban draining rivers revealed heavy contamination by microbial organisms (Musyoki *et al.,* 2013), loaded with heavy metals (Ndeda and Manohar, 2014) and pesticides (Wandiga 2001). These contaminants emanate from informal settlements, surface run-off from the city, urban farmlands and light industries like garages along the banks of the rivers.

Urban rivers and wastewater effluents are increasingly becoming a reliable water source for urban agriculture due to water scarcity. Thirty percent (30%) of Nairobi residents raise livestock and cultivate food crops along the river banks, on vacant plots, marginal land and backyards (Hussain *et al.,* 2001). The majority use untreated or partially treated sewage to irrigate crops and fodder (Karanja *et al.,* 2010).

In pig farming areas, pigs are seen roaming in search of food in drainage canals and water ways, thus exposed to a cocktail of water contaminants. Whereas, it has been shown that effluent contaminated water has adverse effects on the reproduction of aquatic organism (Shalaby and Migeed, 2012), little is documented about endocrine disruption compounds in relation the fertility of domestic animals especially the pig. With over 80% genomic similarity with man, the pig serves as the best animal model to use for studies that would shed an understanding on environmental endocrine toxicity as those taken up within the carbon chain in wastewater effluents in cities like Nairobi. The domestic pig is in this case used to demonstrate these effects and by extension postulate the possible dangers to man through consumption of these animals or crop carbon chain, especially in pork and vegetables like kales planted using contaminated water.

This study aimed at investigating the possible reproductive effects of exposure to mixtures of contaminants in wastewater on the boar (*Sus scrofa domestica*) by examining their testicular histopathology. This study has a direct human relevance too, based on the observa-tion by Svechnikov*et al.,* (2014) that residence location in the vicinity of hazardous waste disposal site induces reproductive abnormalities in boys of the resident families.

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MATERIALS AND METHODS

Study area

The study was carried out in the informal settlements in Nairobi County, Kenya. The sites selected were Kibera, Mathare, and Dandora. These locations were selected for three main reasons; (1) proximity to the contaminated Nairobi river, (2) the physical appearance of the flowing water and (3) the high concentration of small holder pig farms with the animals scavenging along the riparian of the river.

Animal selection and sample collection

Five (5) adult boars reared since birth along the river riparian were selected as the experiment group and a control group of five (5) boars reared in non-contaminated environment were also selected. Boars of both groups were castrated under general anaesthesia using Xylazine 4.4mg/kg, Telasol 4.4mg/kg and Ketamine 2.2mg/kg. The testes were cleaned, epididymis trimmed off and the testis parenchyma cut into $1mm^3$ blocks for histopathology.

Tissue processing and examination

From each half of the testis, $1mm^3$ sections were made from the area between the mediastinum testis and the tunica albuginea. These sections were fixed in freshly prepared 10% neutral buffered formalin for 24 hours. The sections were then processed by dehydration in alcohol, clearing with xylene and embedding in paraffin. Five µm sections were obtained, stained and observed under a light microscope at magnification of ×40.

RESULTS

The figures 1 to 3 showed the histological appearance of testicular sections from boars accessing wastewater in selected slum areas of Nairobi. There was evidence of vacuolated seminiferous epithelium, sloughing off of germ cells and depletion of the seminiferous epithelium. The effect varied with some tubules showed complete spermatogenesis. The interstitial tissue appeared unaffected. The seminiferous tubules in control animals showed normal spermatogenesis as seen in figure 4.

DISCUSSION

The main lesions observed in this study were vacuolation within seminiferous epithelium, sloughing of germ cells and partial depletion of the seminiferous epithelium. The findings are indicative of environmental endocrine toxicity pointing towards effects of estrogens on the testicular tissue but further analyses are needed to identify the toxicants. The findings are however a major indication of infertility due to disrupts of testicular function.

The chemical toxicants are expected with increasing human population and industrialization that lead to an increase in the manufacture, use and discharge of a wide variety of chemicals into the environment (Knez, 2013). These chemicals end up contaminating surface water from household and industrial discharge, runoffs from agricultural fields, indiscriminate discharge or release from wastewater treatment plants. There is mounting evidence that exposure to such environmental contaminants affect animal and human reproduction (Bustos-Obregón and Hartley, 2008; Kipyegon *et al*., 2016).

The toxicants in water would serve as environmental contaminants to animals mainly through ingestion of contaminated water or feed (Norstrom, 2002). Although it has been argued that the exposure rates of such contaminants are low and the reproduction of the majority of ruminants appears unaltered by such low levels of exposure (Rhind *et al*., 2010). Certain production systems expose animals to a higher concentration of environmental contaminants already shown to have reproductive side effects (Norstrom, 2002). This is the first report showing effects of continuous exposure to effluent contaminated water on boar testicular function, but *in-vitro* effects of nonylphenol, phthalate and bisphenol A on the pig have been shown (Yuh *et al*., 2013).

Histopathological evaluation of the testis as a measure of toxicity on fertility has been used by previous authors in various animals (Leino *et al.*, 2005; Svechnikov *et al.*, 2014). In the current study, the histopathological changes observed in the Seminiferous epithelium varied from mild to severe; while some tubules showed developing sperm (Figure 3), others exhibited disorganization of seminiferous epithelium, lacking the characteristic basal to luminal maturation of germ cells (Figure 1). This disruption has been reported to cause a decrease in spermatogenesis in fish living in contaminated lake (Shalaby and Migeed, 2012). Similar findings have also been reported in rats exposed to cadmium (Adamkovicova *et al.*, 2014) and were speculated to cause a reduction in reproductive success.

Exposure to exogenous chemicals is reported to affect/damage the ectoplasmic specialisation between the adjacent Sertoli cells and Sertoli germ cell interaction (Anahara *et al.*, 2006) leading to sloughing of germ cells into the lumen. Similar findings have been widely reported as an effect of EDC exposure (Bello *et al.*, 2014; Adamkovicova *et al.*, 2014). In this study, the effect is evidenced by a reduction in thickness of the seminiferous epithelium (Figure 1). The loss of germ cells through sloughing into the lumen further lead to some tubules with almost complete absence of spermatozoa; a consequence of testicular toxicity. This leads to infertility due to a decrease in the number of spermatogonia produced. The infertility however is not manifested when the affected boars are used for mating because the seminiferous tubules are not affected uniformly as some tubules were observed to exhibit normal spermatogenesis.

The findings of this study indicate that exposure to contaminants in urban wastewater can result in testicular disruption seen as sloughing of germ cells and vacuolization of Sertoli cells. It is speculated in this study that wastewater contains sufficient levels and mixtures of toxicants capable of affecting the junctions between the sertoli cells and germ cells. The histopathological findings of the testis are not uniform in all the animals examined, there were variation in the type and severity of the lesions; a finding similar to that seen in sheep exposed to sewage sludge by Paul *et al* (2005). This is thought to be due to uncontrolled factors; for example boars sampled from informal settlements with rivers receiving predominantly industrial discharge had more severe histopathological changes than those sampled from rivers receiving predominantly household discharges.

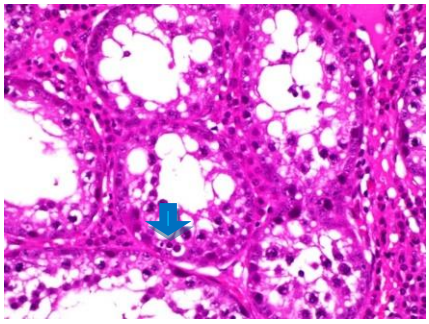

Fig. 1: Photomicrograph of the testis of a boar accessing contaminated river water. There is a disruption of the seminiferous epithelium due to desquamation, vacuolation is also evident (arrow) with reduction in the thickness of the seminiferous epithelium (transverse section ×400 H&E stain).

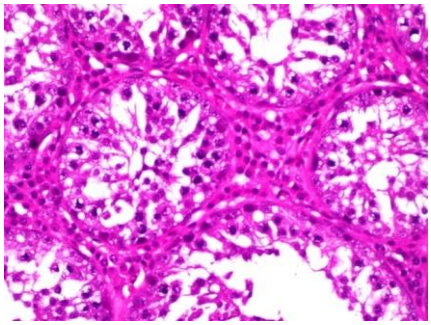

Fig. 2: Photomicrograph of the testis of a boar accessing contaminated river water. Desquamation is not affecting all seminiferous tubules uniformly; some tubules are more affected than others (transverse section ×400 H&E stain).

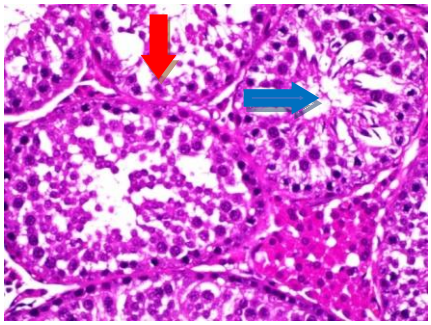

Fig. 3: Photomicrograph of the testis of a boar accessing contaminated river water. The less affected tubules were able to proceed on with spermatogenesis. Notice intraepithelial empty spaces in one tubule (red arrow) and elongated spermatids in another (Blue arrow) (transverse section × 400 H&E stain).

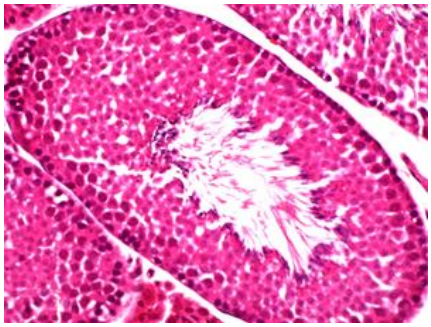

Fig. 4: Normal spermatogenesis (transverse section ×400 H&E stain).

In conclusion, our results indicate that prolonged exposure of pigs to wastewater results in testicular disruption, as an indicator of exposure to a mixture of environmental chemicals, some of which may or may not be EDCs as such. The results of this study add to the existing knowledge of the potential of environmental contaminants exposure to disrupt/affect reproductive function. However, like Liney *et al.* (2006) concluded, this study cannot say which chemical is responsible for the testicular effects reported. The concentration of chemicals to which the animal was exposed to and the specific mixture of chemicals involved was not determined (Paul *et al.,* 2005). Strong associations are drawn between the effects seen and certain chemicals present in effluents, based on findings in the published literature (Bello *et al*., 2014). The findings are however a major indication of infertility due to disrupts of testicular function.

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