CIBTech Journal of Pharmaceutical Sciences ISSN: 2319–3891 (Online)
An Open Access, Online International Journal Available at http://www.cibtech.org/cjps.htm
2014 Vol.3 (2) April-June, pp.15-23/Solomon et al.
Research Article

HISTOMORPHOLOGICAL EFFECT OF DUMPSITE WASTE FORAGE (*CALOPOGONIUM MUCUNOIDES*) ON THE TESTES AND OVARIES OF RABBIT (*ORYCTOLAGUS CUNICULUS*)

I.P. Solomon[1], *Oyebadejo S.A[2] and Akpan E.E.[1]
[1]*Department of Animal Sciences, Faculty of Agriculture, University of Uyo, Akwa- Ibom, Nigeria*
[2]*Department of Human Anatomy, Faculty of Basic Medical Sciences, University of Uyo, Akwa- Ibom, Nigeria*
**Author for Correspondence*

ABSTRACT
Histomorphological effect of the feeding of dumpsite forage (Calapo -*Calopogonium mucunoides*) on the reproductive profile of rabbits (*Oryctolagus cuniculus*) was investigated. 30 rabbits; 20 females and 10 males were obtained and distributed randomly into two treatment groups of 10 females and 5 males. The forage, specifically Calapo (*Calopogonium mucunoides)* was fed to the two groups' *ad-libitum* with the non-dumpsite fed group serving as the control. After a period of 20 weeks, the rabbits were sacrificed, organs (ovaries and testes) were removed and fixed immediately in Bouin's fluid transported to the Laboratory for Histopathological analysis. Fixed tissues were cut at 2-3mm in thickness, processed to paraffin wax, cut at 5 microns, stained using Heamtoxylin and Eosin technique and observed histopathologically under light digital microscope.
The results showed cellular hypertrophy in ovary of the dumpsite compared to the non dumped site which showed normal cellular pattern of the ovaries. For the testes, it showed moderate cellular abnormality, with area of hypertrophy from the dumped site compare to non dumped site showing normal cellular pattern of spermatogenic lining cell, leydig cell and good structural networking within normal constituency.
In conclusion, feeding of dumpsite forages to rabbits could lead to germinal cell abnormalities and cyto-architectural alterations and deleterious effect on reproductive morphology of rabbits

Keywords: *Calapo (Calopogonium Mucunoides), Rabbit (Oryctolagus Cuniculus), Testes, Ovary, Histopathology*

INTRODUCTION
Dumpsites are traditional methods of waste disposal to landfill method of waste management. Dumpsites are often established is disused quarries, mining or excavated pits away from residential areas (Abdusalam, 2009). Poor management of dumpsites could create a number adverse environmental impacts, one of these impact is due to location of dumping site in suitable areas. Locating a dumping site in a suitable area is a very time consuming process.
Soil is a vital resource for sustaining basic human needs, a quality food supply and a livable environment (Wild, 1995). It serves as a sink and recycling factory for both liquid and solid waste. Municipal solid waste has been found to contain appreciable quantity of heavy metals such as copper (Cu), Nickel (Ni), Iron (Fe), Lead (Pb), Arsenic (As) all which may end up contaminating the soil and even get to the forages growing on that soil (Alloway and Aryes, 1997).
Reproduction inefficiency is the most constraint to efficient rabbit production in the tropical (Gdadamosi and Egbunike, 1999). The efficiency of sperm production, libido and quality of sperm tend to remain uniform throughout the reproductive life of an animal but may be significantly attend by age, nutrition, environment, health status, drugs and chemicals (Togun and Egbunike, 2006). Among these factors mutation, drug and hormones are the most prominent. Sexual nutrition is known to be delayed by a poor nutrition requirement during growths (Omole, 1982). It also affects age at puberty and stimulation of hypothalamus indirectly produce interstitial cells stimulating hormone that acts in the testicular tissue (Cogan *et al*., 2004).

Calapo (*Calopogonium mucunoides* Desv.) is a vigorous, hairy annual or short–lived perennial trailing legume. It can reach several meters in length and form a dense, tangled mass of foliage, 30-50 cm deep. The root system is dense and shallow, at most 50 cm deep. The stems are succulent, covered with long, brown hairs. They are creeping in the lower parts, sometimes rooting at the nodes that come in contact with the soil. The upper part of the stem is twining. The leaves are up to 16 cm long and trifoliate. The hairy leaflets are 4-10 cm long x 2-5 cm broad, ovate to elliptical. The inflorescence is a slender hairy raceme that may be up to 20 cm long and bears 2 to 12 blue or purple small flowers. The Fruits are 3-8 seeded hairy pods, 2-4 cm long (FAO, 2011; Cook *et al*., 2005; Chin *et al*., 1997).

Calapo is mainly used as cover crop, alone or in mixture with other legumes, especially in rubber, oil palm or in young forest plantations. Calapo is used for green manure though its value for this use still needs confirmation. Calapo is a pioneer species: it provides soil protection against erosion, reduces soil temperature, improves soil fertility and controls weeds (Cook *et al*., 2005; Chin *et al*., 1997). Although not widely used, calapo is the most popular legume amongst Brazilian farmers and is the legume seed produced in greatest volume in Brazil (Pizarro, 2001).

Calapogonium mucunoides can be grazed and made into hay or silage. Animals especially cattle graze it during the latter part of the dry season (Cook *et al*., 2005). Its good persistence under grazing might be a way to improve overall pasture quality through enhanced soil fertility, subsequent higher pasture growth rate and weed control (Chin *et al*., 1997). One commercial cultivar, derived from plantation agriculture, has been developed in Brazil (Cook *et al*., 2005).

Animals are being fed by human with forages grown on dumpsite which may contain heavy metals like cadmium, lead, mercury and arsenic etc. that can have adverse effect on the reproductive life of the animal. The efficiency of reproduction tends to remain uniform throughout the reproductive life of an animal but may be significantly altered by such factors as bio-climate, chemicals, hormones, drugs and nutrition (Togun and Egbenike, 2006).

Animal studies have shown that accumulation of heavy metals in the reproductive organs of animals like ovary and testes have resulted in infertility in females and reduced libido in males (Herbert *et al*., 2005). Making this study to be important because it will ascertain the effect of the heavy metal on the ovary and testes of the animal and effects on the hormones e.g estrogen hormones, leuteinizing hormones, follicle stimulating hormones and prolactin hormones.

The reported cases of infertility in females and reduced libido in buck rabbits fed dumpsite waste forage are sources of concern (Herbert, 1997, 1998, Herbert *et al*., 2005; Joshi, 2007). The needed improvement in reproductive performance of rabbit may be achieved by investigating the effect of heavy metals in dumpsite waste forages.

The farmer's ability to breed his animals successfully has a high dependence on the fertility status of the animals used. The relevance of sexual behavior in farm animals can be accessed using various parameters. Umesiobi *et al*., (2000) reported that parameters for include: conception rate, little size, milk production and fecundity.

The reproductive performance of the male could be measured using the sperm concentration, sperm motility, level dead spermatozoa and proportion of morphologically deformed sperm (Herbert, 1992. Umesiobi *et al*, 1998; Umesiobi *et al*, 1999; Ogbilewu *et al*; 2007).

Heavy metals are natural components of the Earth's crust. They cannot be degraded or destroyed. To a small extent they enter the body through food, drinking water and inhalation of contaminated air. Heavy metals are considerably environmental concern due to their toxicity and accumulate behavior (Purves, 1985). Their uptake by plants from the soil is largely specified. Although all trace elements are natural constituent of the soil, the dumping of waste on soil has been found to increase their heavy metal profile (Clarkson *et al*., 1983, Adeniyi *et al*., 1993 and Adeniyi, 1996). The effect of this is that their concentration may reach toxic levels, resulting in increased health risk to animals especially if they are fed with forage on that soil.

Studies on heavy metals in ecosystem have shown an indication of a silent epidemic of environmental metal poisoning of ever increasing metals in sub-tropical soils (Nriagu, 1988; Shuman, 1999). With

increasing pressure on agricultural and proliferation of urban and peri-urban farming, waste dumpsite are becoming attractive because of their rich deposit of organic matter and plant nutrients.

Although the nutrient content of waste makes them attractive as fertilizer, land application of many industrial waste and sewage is constrained by the pressure of heavy metals, hazardous organic chemical, salts and extreme pH (Cameron *et al*., 1997). Animals studies have shown the reproductive toxicity of a number of heavy metals, but in this study only four of them will of interest which include; Lead (Pb), Mercury (Hg), Arsenic (Ar) and Cadmium (Cd).

Rabbit as a laboratory animal are very important in research institutes. The current number of rabbits used for research is about 6000 annually world over (Anonymous, 2006). The fur is used as raw material for production of wool, rug and pillows. Rabbits are used as pets in homes and their feaces serves as biologically enhanced fertilizer. More recently, they have been used as source of meat. When feeding and management are of a high standard, the rabbit is one of the most efficient animal in the world at converting food for meat (Fielding, 1991).

The testis produces two highly organized and intricate function, Spermatogenesis which is the process that takes place within seminiferous tubules of the testis with the support of the sertoli cells, leading to the formation of mature spermatozoa from undifferentiated germ cells (Hess, 2008) and steriodiogenesis which is the biological process by which steroids are generated from cholesterol into steroids. The interstitial compartment which comprises of leydig cells is the site of steriodiogenesis in the testis (Osinowo, 2006), testes are round in nature, surrounded by a thick collagenous fiber known as tunica albuginea. In the posterior part of the testis, the connective tissue of the albuginea expands into a thick mass that project into the substance of the testis. The projection is known as mediastinum testis. From the mediastinum testis, there is a septa arising and entering into 250 compartment called testicular lobules and each of the lobules contain coiled structure known as seminiferous tubule (Singh, 2004). The tunica vaginalis is the outermost covering of the testis. The testis develop from the abdominal wall as it descends, it drags along the peritoneal sac which forms a sac known as tunica vaginalis. The tunica vaginalis has an an outer parietal layer and inner visceral layer (Singh, 2004). The inner part of the seminiferous tubule has so many cells known as the myoid cells. The spermatogenic lineage cells are that precusors of spermatozoa. The spermatogenic cells of the testis support and nourish the sperm and also play a role in phagocytosis (Singh, 2004).

Umesiobi (2002) and Fielding (1991) reported that the female reproductive organ in rabbit consists of the ovary (2), fallopian tube (2), uteri (2), cervix, vagina and teats (8-10). The ovary produces eggs and hormones. Each ovum is generated in a recently formed follicle within the ovary. Some tiny follicles develop and ultimately attain maximum size in diameter after having migrated from deep in the ovary to the surface of the ovary. The ovary varies in shape depending on the animal. In cattle/sheep the ovary is almond shaped, in swine it resembles a cluster of grapes, and the ovary of horse and rabbit are bean shaped. In the ovary, the hilius is the point where blood and the nervous system enter the ovary. The nerves that supply the ovary also passes through the mesovarium (Kwhite, 2005). The ovary is the female Gonad homologous to the male Testes. It is usually a paired organ in domestic species, but in the bird only the left Ovary is present. The structures found within the ovary are undergoing constant changes throughout the oestrus cycle from the Follicles containing Oocytes, to the formation of Corpus Haemorrhagicum, Corpus Luteum, and finally Corpus Albicans. Ovaries are ellipsoidal in shape with an irregular surface due to the projection of dominant follicles and corpora lutea. These irregularities are absent in the mare due to the cortex and medulla being reversed with ovulation only occurring from the ovulation fossa. They are greatest in Polytocious animals such as the sow due to many dominant follicles, and so corpora lutea, developing at once.

No recent studies have been conducted to elucidate possible cyto-architectural alterations and possible deleterious effect of heavy metals in forages from dumpsites on testes and ovaries of rabbits, considering the rate of consumption of rabbits as one of the major source of meat with very high protein contents, this study therefore explores the major cellular effect, keeping in view histopathological alterations in dumpsite forage fed and non – dumpsite forage fed rabbits.

MATERIALS AND METHODS

Drugs and Chemicals

Sodium chloride, formaldehyde, sodium trioxocarbonade V, sodium bicarbornate, xylene, paraffin wax, 70% alcohol, 90% alcohol, absolute alcohol, distilled water, hutches, concentrate feed, latex hand glove, weighing scale, graduated vials, measuring tape, they were all procured from BDH Chemicals, England. All other chemicals were of analytical grade.

Experimental Animal and Management

The animals were sourced from the University of Uyo Teaching and Research Farm, Use-Offot, Akwa Ibom, Nigeria. A 2- week experimental period was used to get the animals (rabbits) acclimatized with the experimental procedures. The experiment lasted 20 weeks (June, 2013 to November, 2013). The animals used in this study were 4 bucks and 20 does crossbred rabbits aged 6-7 months. The males weighed between 1350g and 1650g, while the females weighed between 1400g and 1800g.

Four bucks and twenty does respectively were divided into two groups of 12 animals each. When placing the animals into groups care was taken in order to balance the groups such that there were no significant differences between them on the basis of age and weight and the animals were identified individually with the aid of a permanent marker on their ears**.** The groups were randomly assigned to two (2) treatment diets: dumpsite fed animals and non-dumpsite fed animals.

The experimental animals were housed in a wooden hutch with a wire mesh floor and in-built waste trays. The management techniques employed for all the experimental animals included regular cleaning of the hutch, feeding and watering of the experimental animals on a daily basis. The experimental animals were managed well.

Drinkers and feeders were made of plastics and concrete with narrow but blunt mouth to discourage fed wastage and injuries. Forage (experimental diets) and clean water was also supplied *ad libitum*. Permission and approval for animal studies were obtained from the College of Health Sciences Animal Ethnics Committee, University of Uyo.

Experimental Animal Health

The rabbits acquired were treated against internal and external parasites by subcutaneous injection of ivomec (0.2 ml per rabbit) and a broad spectrum antibiotic (Oxytetracyclin L. A.) was also administered at the rate of 0.2 ml per rabbit. Sulphur powder was given occurrences of mange and neomycin was given for diarrhea at the rate of 10g per four (4) liters of drinking water.

Experimental Designing and Feeding of Experimental Diets

Two treatments being the waste dumpsite fed and the non-dumpsite fed. Forages were obtained from two sites, one being the waste dumpsite within Uyo metropolis and the other being a land, which is the non-waste dumpsite. Forage used was *Calapogonium mucunoides* due to its palatability to the animals. The forages were supplied daily to the animals and fed *ad-libitum*. Alongside with the forage, the concentrate of pelleted poultry grower's mash meal (20% CP and 2700Kcal/kg) were fed routinely to facilitate the growth of the animals.

Table 1: Showing the experimental designing of the study

Group	Female	Male	Treatment (site)	Duration
1(NDS)	10	5	Non-Dumpsite Forage	20 weeks
2(DS)	10	5	Dumpsite Forage	20 weeks

Sample Collection for Histopathological Analysis

At the end of the stipulated 20 weeks feeds were withdrawn, the rats were subjected to a 12 hours fast but had access to water. Sacrificed using chloroform vapour.

Testes and ovaries were harvested out from male and female rabbits respectively, harvested organs were carefully dissected out, trimmed of all fat and connective tissue blotted dry to remove any blood. The tissues were immediately in Neutral Buffered Saline and then transferred to a graded series of ethanol. On day 1, they were placed in 70% alcohol for 7 hours, then transferred to 90% alcohol and left in the latter overnight. On day 2, the tissues were passed through three changes of absolute alcohol for an hour each then cleared in xylene. Once cleared, the tissues were infiltrated in molten paraffin wax in the oven at 58°C. Three changes of molten paraffin wax at one-hour intervals were made, after which the tissues were embedded in wax and blocked out. Prior to embedding, it was ensured that the mounted sections to be cut by the rotary microtome were orientated perpendicularly to the long axis of the kidney, liver and pancreas. The sections were designated "vertical sections". Serial sections of 5 µm in thickness were obtained from a solid block of tissue, fixed on clean albuminized slides to prevent sections coming off the slides and later stained with Haematoxylin and Eosin staining techniques, after which they were passed through ascending grade of alcohol, cleared in xylene and mount in DPX mountant, allowed to dry at room temperature and observed Histopathologically under digital light microscope.

Photomicrography

Records of the Histological and histochemical results were obtained by photomicrography using digital photomicrographic microscope at the Gross Anatomy Research Laboratory, Department of Human Anatomy, College of Health Sciences, University of Uyo, Uyo, Akwa- Ibom, Nigeria as illustrated in Plate.1 to 4.

RESULTS

Histopathology of Testes of Rabbits Fed Dumpsite Forage and Non – Dumpsite Forage

Dumpsite (DS) - Plate A(X100) and B(X400) of Testes from the Dumpsite revealed cytp-architectural alterations, area of cellular abnormality, nuclear fragmentation, and focal area of degeneration, cytoplasmic pigmentation and hypertrophy.

Non - Dumpsite *– (NDS)* - Plate C(X100) and D(X400) of Testes from the NDS revealed normal cellular architecture of seminiferous tubules with distinct area of interstitium containing leydig cell, the tubules enclosed the spermatogenic lining cells towards the luminal semen. There is no evidence of cellular abnormality seen.

Dumpsite Fed Forage Ovaries

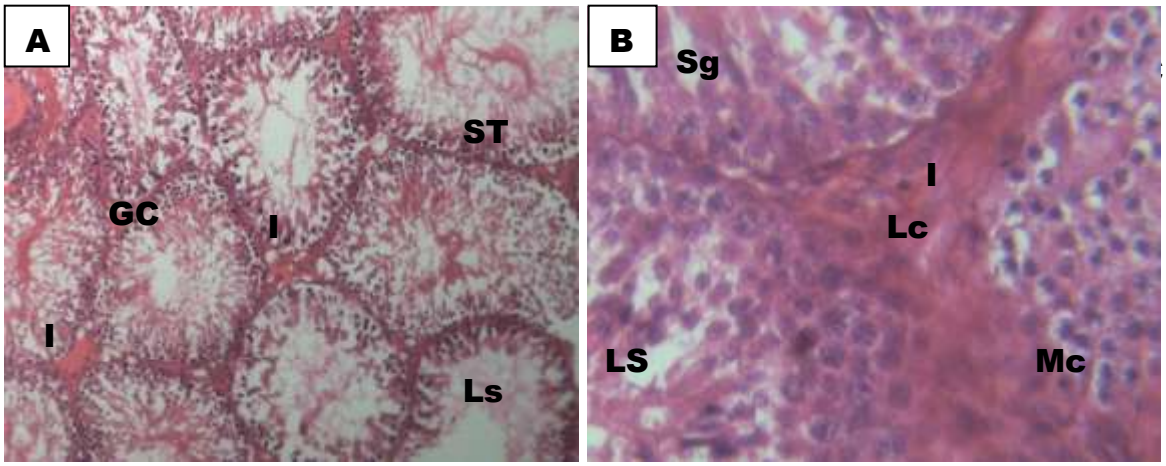

PLATE 1 *- Testes from Dumpsites at Magnification A(**X100**) and B (**X400**) stained with H and E technique*
Keys: Sglc *–Spermatogenic lining cells,* ***I*** *– Interstitium,* ***Ls****- luminal semen,* ***Sz****-Spermatozytes,* ***Mc*** *– Myoid cells,* ***Sp*** *–spermatocytes,* ***ST*** *– Seminiferous tubules.*

Non –dumpsite fed forage Ovaries

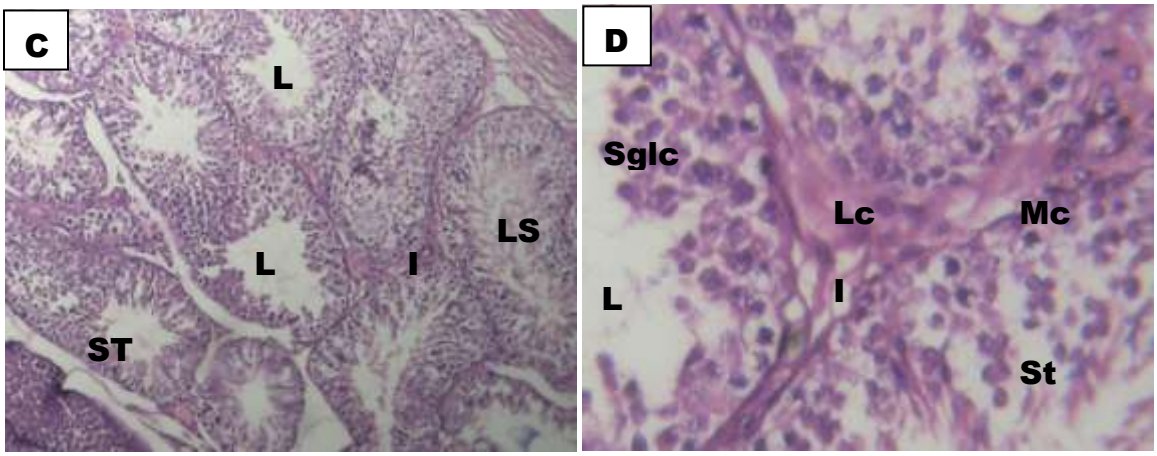

PLATE 2 *- Testes from Non- dumpsites at Magnification C(**X100**) and D(**X400**) stained with H and E technique*
Keys: Sglc *–Spermatogenic lining cells,* ***I*** *– Interstitium,* ***Ls****- luminal semen,* ***Sz****-Spermatozytes,*
Mc *– Myoid cells,* ***Sp*** *–spermatocytes,* ***ST*** *– Seminiferous tubules,****Sg*** *- spermatogonia,L- lumen,*

Histopathology of Ovaries of Rabbits Fed Dumpsite Forage and Non – Dumpsite Forage

Non - Dumpsite – (NDS) - Plate E(X100) and F(X400) of Ovary from NDS revealed normal cellular architecture of theca granulosa lutein cells, vascular, follicular cells, stroma containing reticular and fusiform cells, primary and secondary follicles with distinct area oocytes, cumulus oophorus, zona pellucida, corona radiata, follicular antrum, there is no evidence of cellular abnormality seen.

Dumpsite (DS) - Plate G(X100) and H(X400) of ovary from DS revealed proliferation of granulose cells, alterations in the primary and secondary follicles, disappearance of the corona radiate , zona pellucida and sequential cellular hypertrophy.

Non –Dumpsite Fed Forage Ovaries

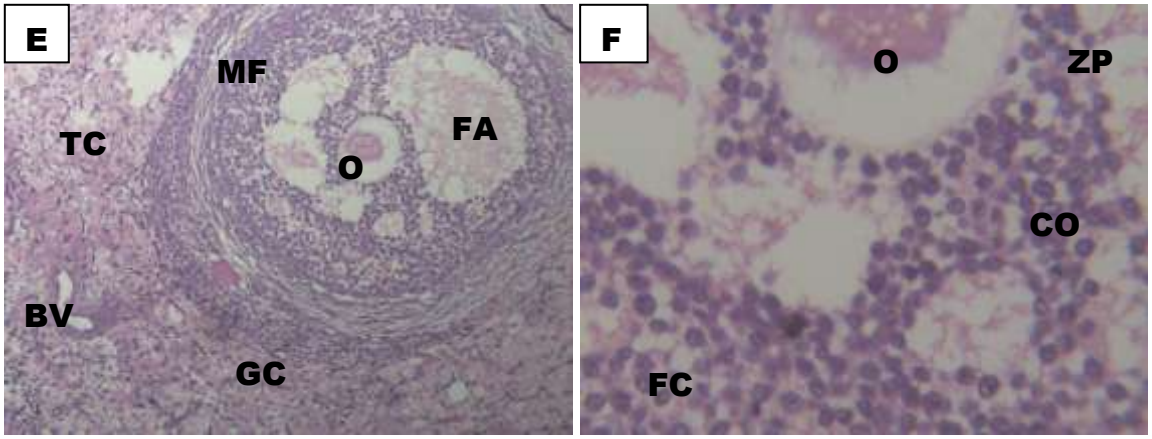

PLATE *3 - Ovary from Non dumpsite at Magnification E (X100) and F (X400) stained with H and E technique*
Keys: GC*-Granulosa cells,* ***GCL****-Granulosa lutein cells,* ***TC****-Theca cells,* ***ZP****-Zona pellucida,* ***O****-Oocytes,* ***CR****-Corona radiate,* ***FA****-Follicular antrum,* ***SP****-Secondary follicle,* ***PF****-Primary follicle, ,* ***FC****-Follicular cells.*

Dumpsite Fed Forage Ovaries

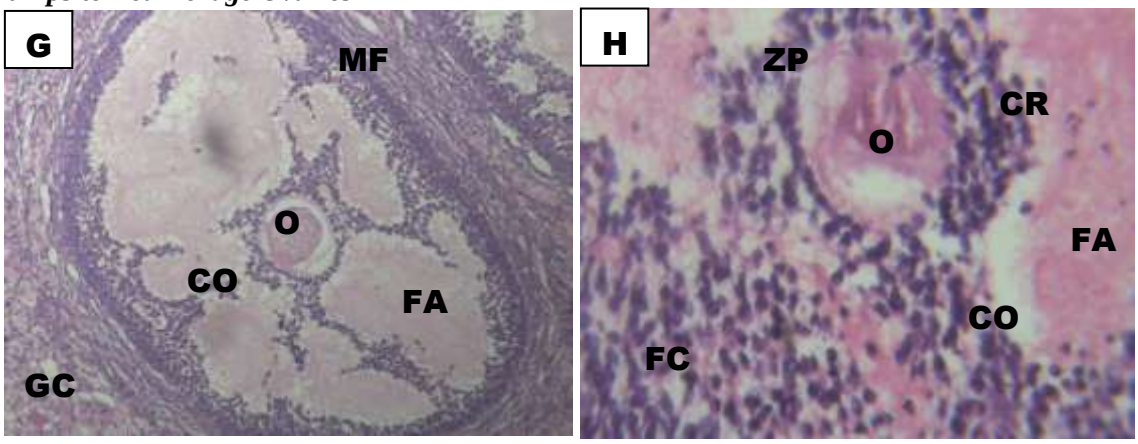

PLATE 4 *- Ovaries from Dumpsite at Magnification G (X100) and H(X400) stained with H and E technique*
Keys: GC*-Granulosa cells,* ***GCL****-Granulosa lutein cells,* ***TC****-Theca cells,* ***ZP****-Zona pellucida,* ***O****-Oocytes,* ***CR****-Corona radiate,* ***FA****-Follicular antrum,* ***SP****-Secondary follicle,* ***PF****-Primary follicle, ,* ***FC****-Follicular cells*

DISCUSSION

Pollution is a world problem and it's potential to influence the health and wellbeing of animal population is increasingly apparent. The concern of heavy metals is that they are not biodegradable and may accumulate in the environment. Animal studies have shown the reproductive toxicity of a number of heavy metals (Meharg *et al*., 2006)

The histhopathological results revealed that for the ovary histology from the dumpsite group there was cellular hypertrophy at magnification of X100 and X400 (plate G & H respectively) while the non-dumpsite group at X100 and X400 (plate E & F) group which revealed normal cellular architecture of theca granulosa lutein cells, vascular, follicular cells, stroma containing reticular and fusiform cells, primary and secondary follicles with distinct area oocytes, cumulus oophorus, zona pellucida, corona radiata, follicular antrum, with no evidence of cellular abnormality seen. While the analysis of the testis results, Plate A(X100) and B(X400) from the dumpsite group revealed moderate cellular abnormality, with area of hypertrophy. For the non-dumpsite Plate C(X100) and D(X400) revealed normal cellular architecture of seminiferous tubules with distinct area of interstitium containing leydig cell, the tubules enclosed the spermatogenic lining cells towards the luminal semen, with no evidence of cellular abnormality seen.

Conclusion

From this study, it has been observed that prolonged feeding of dumpsite forage to animal leads to negative effect on the semen, hormones and also pathological changes in the testis and ovary. It is advised that the breeder should avoid prolonged feeding with forages that contain heavy metals.

ACKNOWLEDGEMENTS

This research was not supported by any organization or funding body, however the authors contributed collectively to carry out this experiment.

REFERENCES

Abdus-salam N (2009). Assessment of Heavy Metal Pollution in Dumpsite in Ilorin Metropolis. *Ethiopian Journal of Environmental Studies and Management* **2** 92-99.

Adeniyi AA (1996). Determination of Cadmium, Copper, Manganese and Zinc in Waterleaf (*Talinium triangulare*) in Dumpsite Environment.

Adeniyi AA, Fashola J and Ekanem AM (1993). A Comparative Evaluation of Heavy Metals in Lagos Dumpsite. Nigeria. Journal of Revision Science 65-69.
Alloway BJ and Ayres DC (1997). *Chemical Principles of Environmental Pollution* (Blackie Academic and Professional) 53-359.
Cameron KC, Di HJ and McLaren RG (1997). Is Soil An Appropriate Dumping Ground for our Waste? *Australian Journal of Soil Research* **35** 995-1035.
Chen Chin Peng and Aminah A (1997). *Calopogonium mucunoides* Desv. Record from Proseabase. In: *PROSEA (Plant Resources of South-East Asia)* edited by Faridah Hanum I and van der Maesen LJG (Foundation, Bogor, Indonesia).
Clarkson TW, Weiss B and Cox C (1983). Public Health Consequences of Heavy Metals in Dumpsite. *Environmental Health Perspective* **48** 13-127.
Cogan U, Yaron Brek M and Zimmermen G (2004). Effect of processing condition on nutritive value of isolated soybean protein. *Journal of Agricultural and Food Chemistry* **16** 196-198.
Cook BG, Pengelly BC, Brown SD, Donnelly JL, Eagles DA, Franco MA, Hanson J, Mullen BF, Partridge IJ, Peters M and Schultze-Kraft R (2005). Tropical Forages. CSIRO, DPI&F(Qld), CIAT and ILRI, Brisbane, Australia.
FAO (2011). Grassland Index. A Searchable Catalogue of Grass and Forage Legumes. FAO.
Fielding D (1991). *The Tropical Agriculturist* 2nd Edition (CTA/ Macmillan Education Limited).
Gbadamosi AJ and Egbunike GN (1999). Studies on cock semen (1) Effects of Frequency of Ejaculation and Breed on Physical Characteristics. *Tropical Journal of Animal Science* **1** 157-164.
Herbert U (1992). Growth and Reproductive Characteristics of Rabbits Fed on Leucaena (*Leucaena leucocephala*) and (*Gliricidia sepium*) foliage. PhD. Thesis.
Herbert U, Ozoje MO and Adejumo DO (2005). Effect of *Leucaena* and *Gliricidia* Leaf Meals on the Seminal Characteristics, Testis Weights and Seminiferous Tubule Diameters of Rabbits. *Animal Research* **54** 173-178.
Hess RA and Renato de Franca LS (2008). Spermatogenesis and Cycle of the Semniferous Epithelan. *Advances in Experimental Medicine and Biology* **638** 1-15.
Joshi HS (2007). Effect of Feeding *Leucaena leucocephala* on Reproduction of Rats. Internet Material.
Kwhite E (2005). Lecture notes on Animal Reproduction Available: http://www.advs.usu.edu/kwhite/lect 10notes. htm.
Nriagu JO (1988). A Silent Epidemic of Environmental Metal Poisoning. *Environmental Pollution* **50** 139-161.
Ogbilewu IP, Umesiobi DO, Okoro VMO and Okoli IC (2007). Validation of concentrates and Forage Legumes on Weaner Rabbit Performance. In: *Animal Agriculture in West Africa (the Sustainability Question*) Proc. of the Annual. Conference. of NSAP.
Omole TA (1982). The Effects of Dietary Protein on Growth and Reproductive Performance in Rabbits. *Journal of Applied Rabbit Research* **52** 83-88.
Osinowo OA (2006). *Introduction to Animal Reproduction-Saphre Academic Services Limited* 1st edition 91.
Pizarro EA (2001). Novel Grasses and Legumes Germplasm: Advances and Perspectives for Tropical Zones. In: *XIX International Grassland Congress, São Pedro, São Paulo, Brazil* 93-100.
Purves D (1985). *Trace Element Contamination of Environment* (Elsevier: Amsterdam The Netherlands).
Shuman LM (1999). Organic Waste Amendments Effect of Zinc Fractions of two Soils. *Journal of Environmental Quality* **28** 1442-1447.
Singh I (2004). *Textbook of Basic Histology* 5th Edition (Medical Publisher Ltd) 279-281.
Togun VA and Egbunike GN (2006). Seasonal variations in the Sperm Production Characteristics of Zebu (white Fulani) Cattle Genitalia in the Humid Tropical Environment. *Middle-East Journal of Scientific Research* **1** (In press).
Umesiobi DO, Iloeje MU and Berepubo NA (1998). In Southern Nigeria 2. Locally Extended Boar Semen Improves its Fertility Study. *Journal of Agriculture and Rural Development* **21**(2) 98-106.

Umesiobi DO, Iloeje MU and Berepubo NA (1999). Influence of sperm dose and time of Insemination on the time of Insemination on the Conception Rate of Sows. *Delta Agricultural* **6** 206-212.
Umesiobi DO, Iloeje MU, Anyanwu, GA, Herbert U and Okeudo NJ (2000). Relationship between Sow Fecundity and Sexual Repertoire of Boar. *Journal of Agriculture and Rural Development* **1**(1) 114-8.
Wild A (1995). *Soil and the Environment: An Introduction* (Cambridge University Press) 109-165.