

## RESEARCH PAPER

### THYROIDECTOMY AND INDUCED HYPOTHYROIDISM: A FACTOR IN THE GENESIS OF HYDROSALPINX

<sup>1</sup>Amadi, K, Sabo, A.M., Idah, O.V., <sup>2</sup>Inegbenebor, U.

Department of Physiology, College of Medical Sciences <sup>1</sup>University of Jos, Jos, Plateau State; <sup>2</sup>Ambrose Alli University, Ekpoma, Edo State, Nigeria.

Corresponding Author: [parkers2004amam@yahoo.com](mailto:parkers2004amam@yahoo.com)

Received: 9<sup>th</sup> July, 2015

Accepted: 28<sup>th</sup> July, 2015

Published: 30<sup>th</sup> September, 2015

#### ABSTRACT

The primary or natural cause of hydrosalpinx seemingly remains elusive; though some reports had indicated that increased intraluminal pressure is responsible for the phenomenon. This study was designed to determine the remote causes of increased intraluminal pressure using animal thyroid hormone as a pressure sensitive hormone. Four groups of sexually mature female Wistar rats (n=25), categorized into (1) Control or euthyroid rats, (2) thyroidectomized (T), (3) thyroidectomized but treated with thyroxine (TTT) and (4) thyroxine-treated or euthyroid rats (TT), were used for this study. For 60 days, the animals were fed with rat chow, while thyroxine (10ug/kg) was specifically administered to each of the rats in TTT and TT groups every alternate days of the experimental period. At the end of the experiment, the animals were sacrificed after blood sample collection, to harvest the uterine horn tissues, which were subsequently processed for histological study; with emphasis on the tubo-uterine junction. Results showed significant cytoarchitectural changes in the experimental groups, while thyroxinemia without thyrotocosis -as indicated by the non-significant T4 level (P<0.040), was observed in the TT group that received physiological doses of thyroxine. Our findings suggest therefore, that hypothyroidism might be a primary factor in the genesis of hydrosalpinx.

**Key Words:** hydrosalpinx, oedema, thyroidectomy, hypothyroidism, thyroxine treatment.

#### INTRODUCTION

The thyroid hormone, as observed in hypothyroidism, following thyroidectomy and thyroxine administration, has been identified as an important factor with the capacity to influence the morphology and function of the uterus, ovary and testis (Amadi *et al.*, 1996a, b, c). It has also been shown that the contractions of the oviductal isthmus are regular and at intervals, spread to the uterine muscle; becoming more intense at the time of ovulation (Glenister *et al.*, 1986).

Muscular contractions are of the greatest importance in egg transport through the ampulla in rats (Blandau, 1979). Otubu (1983) had emphasized the importance of smooth muscle in ovum transport. Any factor or phenomenon that adversely affects the normal morphology or function of the muscle fibre of the tract might obstruct fertilization and thus could result in infertility. The fact that thyroid hormone is a pressure-sensitive hormone (Maruo, 1992) makes it a possible primary, natural candidate of increased intraluminal pressure applied by Otubu (1983) to induce hydrosalpinx.

This speculation arose from several works in our laboratory showing that various levels of thyroid hormone caused various effects on the reproductive tract especially on the musculature. Muscle fibres in the ligament of the ovary, in the infundibular pelvic ligament and in the fimbria, all take part in the rhythmic movements of the internal



generative organs. This study therefore, seeks to throw some light on the remote causes of increased intraluminal pressure in hydrosalpinx using albino rat thyroid hormone models as a pressure-sensitive hormone.

## MATERIALS AND METHODS

**Study subjects:** Four groups of sexually mature female Wistar rats (n=25), categorized into (1) Control or euthyroid rats, (2) thyroidectomized (T), (3) thyroidectomized but treated with thyroxine (TTT) and (4) thyroxine-treated or euthyroid rats (TT), were used for this study. The animals were bred and procured from Animal House of the University of Jos, Jos, Plateau State.

**Thyroidectomized rats (T):** Thyroidectomy to induce hypothyroid state was performed as described by (Amadi *et al.*, 1996a). The animals were anaesthetized by ether damped on cotton wool at the bottom of a desiccators separated from the upper chamber by a wire gauze on which the animals were placed. The rats were tested to have been fully anaesthetized when they did not withdraw their limb to which pinpricks were applied.

The hair on the ventral part of the neck was shaved and sterilized with methylated spirit. A midline incision was made with a scalpel blade and dilated with forceps to expose the pair of thyroid glands, which were then cleared of all adhering tissue except the thyroid artery. Using a thermocautery, the gland was severed from the neck while sealing the blood vessels in the process. The external parathyroids were kept intact. The cut was then sutured by interrupted stitches, while procaine-penicillin powder was applied on the wound, while the injection was administered intraperitoneally for the first day and intramuscularly for four subsequent days.

**Thyroidectomized rats treated with thyroxine (TTT):** This group of animals had the same treatment as described for the thyroidectomized rats, but were additionally given thyroxine (10ug/kg body weight by A.H. Cox and Co Ltd. Barnstaple England) every alternate day by oral route for 60 days. The dose of thyroxine was administered using a 1ml syringe without the needle in the oropharynx.

**Thyroxine Treated rats (TT):** The same dose was given to this group like in the case of the TTT group, but on alternate days for 60 days. This was to achieve thyroxinaemia without thyrotoxicosis as established in a previous work by (Amadi *et al.*, 1999). All the groups, irrespective of their treatment regimen, were fed with rat chow and tap water was given *ad-libitum* throughout the period of the experiment.

**Determination of T4 and TSH level:** Thyroid stimulating hormones (TSH) was assayed using the double antibody RIA procedure of (Katamaya *et al.*, 1992) as specified by WHO international laboratories (kits) for Biological Standards London (courtesy of TADAM MEDICAL CENTER JOS). Free thyroxine (T4) content of all animals was determined using RIA reagent Kits (WHO International laboratories 1993) as described by Manar (1993).

**Histological Studies:** Samples of oviduct and uterine horns from the experimental animals were fixed in 10% formol saline, a microanatomical fixative, as described by (Baker *et al.*, 1994); consisting of 10ml of 40% formaldehyde, 9mg sodium chloride (NaCl) and 900ml distilled water. The fixed specimen was then embedded in paraffin wax. Micro sections of 5 $\mu$  in thickness were cut using a rotary microtome and stained with haematoxylin for 10 minutes, differentiated in acid alcohol, counterstained in eosin for 2 minutes, dehydrated by ascending the alcohol series, cleared in xylene and mounted with Canada balsam. They were then viewed under the microscope with special attention on the features of the Isthmus or the tubo-uterine junction

**Morphometry:** The thickness of the mysalpinx and oviductal epithelium were measured by means of an eye piece with cross hairs; OME: FEZ-1. Hamburg, Germany. The mean of two measurements for an individual case was taken.

**Statistical Analysis:** Statistical comparisons of the oviductal and uterine mucosal folds, epithelial heights, and muscle thickness. TSH and T4 levels subjected to the student's t-test. The standard error of mean (SEM) was calculated for all sets of data.



## RESULTS

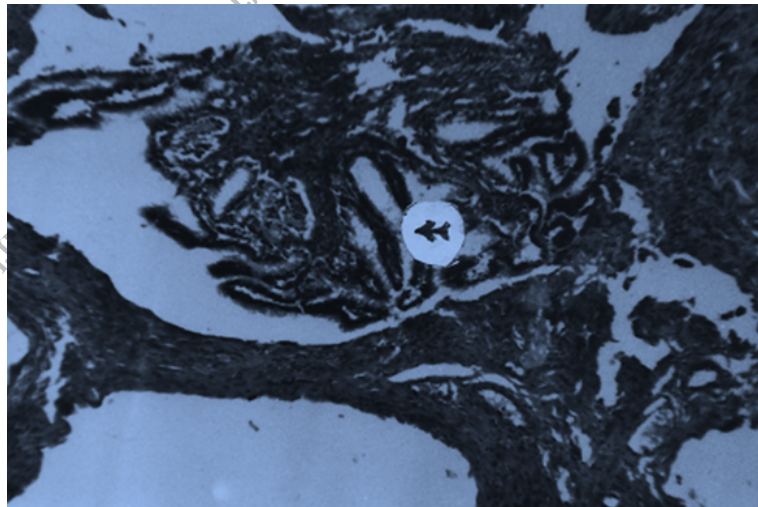
Table 1 is a display of TRH and T<sub>4</sub> levels of the various thyroid models. The table clearly shows thyrotoxicosis in the thyroxine-treated (TT) groups. There were no significant differences in T<sub>4</sub> (P<0.40) when values of control were compared with the TT group; but in heart rate at <P0.05. The table summarizes the measurements in the thyroid models (Morphometry) and shows the morphometric data obtained for the control and experimental groups. Values of comparative significance were noted in the “T” and “TT” sub-experimental groups

**TABLE 1: HEART RATE, TSH AND T4 LEVELS OF EXPERIMENTAL ANIMALS**

Group (n=10)	Heart rate (beats per minute)				Thyroid stimulating Hormone (TSH) ( $\mu$ .ml)				Thyroxine (T <sub>4</sub> ) ng/ DI				P- Value
	C	T	TTT	TT	C	T	TTT	TT	C	T	TTT	TT	
1	148	136	150	149	6.5	1.8	5.6	8.2	3.8	0.6	2.5	4.8	Heart Value CV <sup>3</sup> T: P<0.001
2	140	130	145	140	5.4	1.0	6.7	6.6	4.0	0.6	1.9	1.0	
3	145	135	140	142	6.0	0.7	6.0	5.8	3.5	0.5	4.2	2.2	CV <sup>3</sup> TT: P<0.50
4	150	140	150	160	4.8	1.6	5.5	5.0	3.0	0.8	1.5	3.5	FOR TSH
6	145	120	138	140	5.8	0.8	4.5	6.8	2.8	0.5	2.0	4.0	CV <sup>3</sup> T: P<0.001
Mean	154 $\pm$ 1.70	112 $\pm$ 19.60*	144.6 $\pm$ 2.50	146 $\pm$ 3.8	5.7 $\pm$ 0.3	1.18 $\pm$ 0.2	5.66 $\pm$ 0.4	6.48 $\pm$ 0.5**	2.42 $\pm$ 0.5	0.6 $\pm$ 0.05	2.42 $\pm$ 0.5	3.16 $\pm$ 0.66	CV <sup>3</sup> TT: P<0.001
SD	3.78	43.8	5.55	8.55	0.64	0.49	0.80	1.20	0.51	0.12	1.05	0.46	FOR T4
SE	1.69	19.59	2.48	3.8	0.29	0.22	0.36	0.54	.23	0.05	0.47	0.66	CV <sup>3</sup> T: P<0.001 CV <sup>3</sup> TT: P<0.40

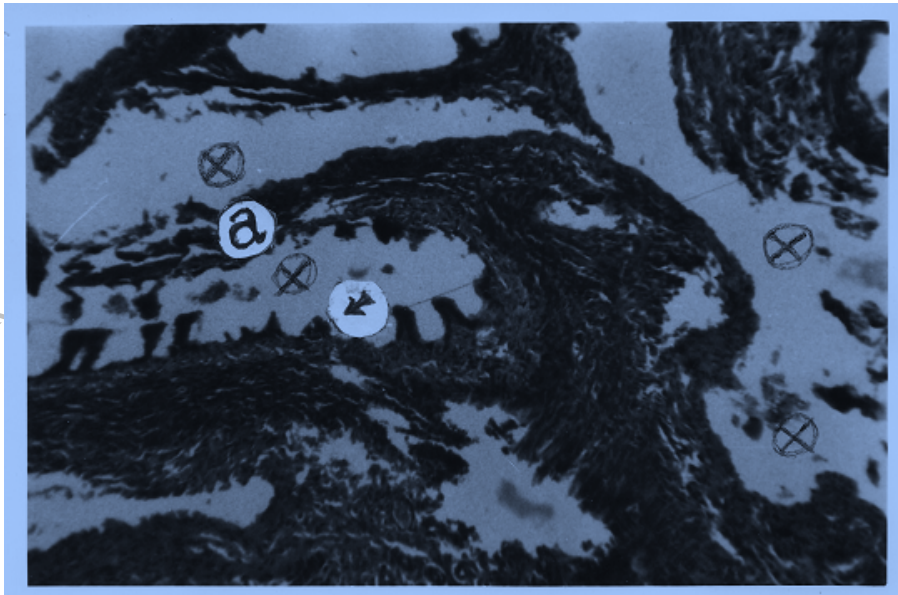
C= Control; T= thyroidectomized; TTT=Thyroidectomized, Treated with Thyroxine; TT= thyroxine – treated; Regard P<0.05 as significant student t- test; V<sup>3</sup>=compared with \*P<0.001; \*\*P<0.01; (Courtesy AJMMS 2008: In Morphology and function of the oviduct~ Effects of Thyroidectomy and thyroxne administration by Amadi *et al* 2008).

On the other, the morphological evaluations of the oviduct as depicted by the photo-micrographic plates below, show that a section of normal oviduct (Control or Euthyroid Rats) presented typical mucosal linings thrown into folds. The epithelial cells were columnar, while the ampullo-isthemic junction (AIJ) is open with a mature follicular cell that could be seen in the uterine area of the ampullo-ischemic junction (AIJ) at 4 0'clock (Plate 1).

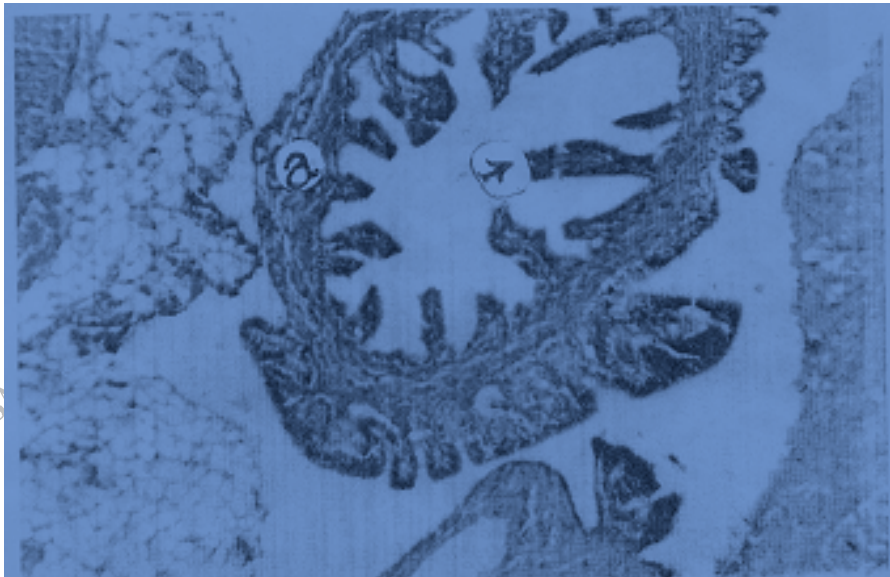


**Plate 1: Photomicrograph of Control Oviduct (x1000) showing normal oviduct with typical mucosal lining thrown into folds (arrows) (see Amadi *et al.*, 2007)**

However, the thyroidectomized (“T”) group’s oviduct section (Plate 2), shows significant reductions ( $P<0.01$ ) in the wall thickness of smooth muscle. The height of the epithelial cells was also significantly reduced from columnar to cuboidal. The mucosal folds became flattened compared to those of euthyroid (control) group. There was oedema in some foci of the stroma and the tube was completely blocked at the ampullo-ischemic junction (AIJ) at 4 o’ clock.

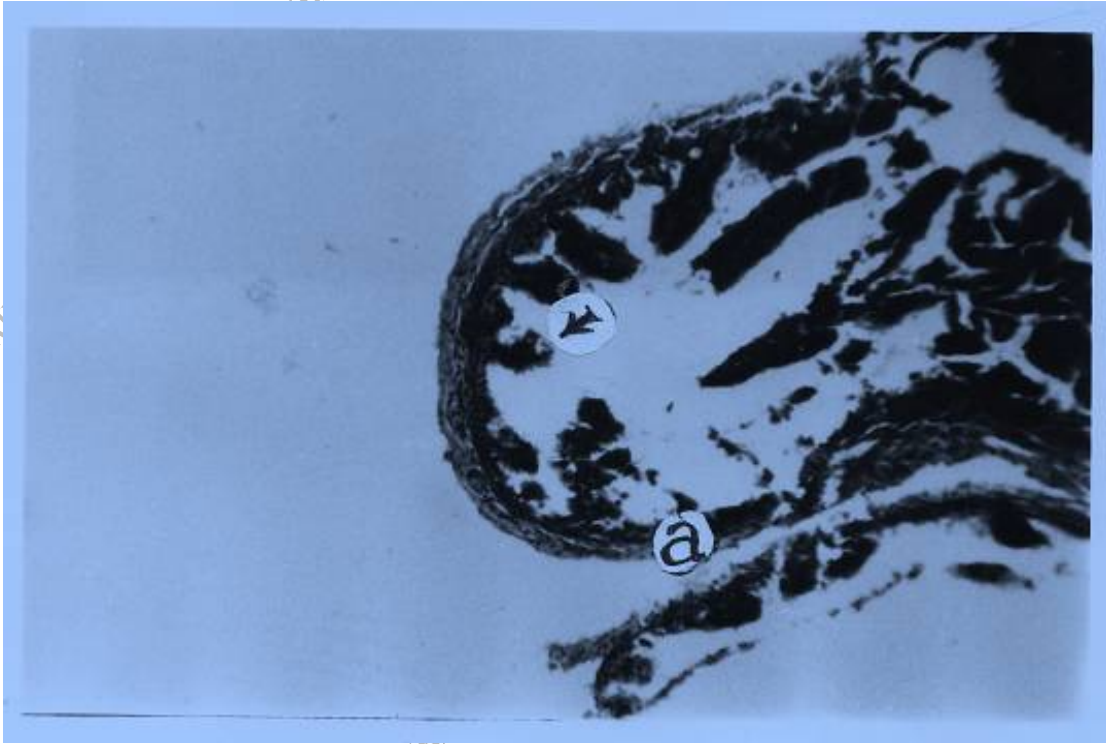


**Plate 2: Photomicrograph of thyroidectomized (“T”) oviduct (x1000) showing significant reduction in the thickness of the smooth muscle (a) and epithelial cell height (arrows). Mucosal folds are flattened with gross oedema formation and erosion of the endometrium, endosalpinx and myometrium (x). Observe the blocked fimbria at 4 O’clock (see Amadi et al., 2007)**



**Plate 3: Photomicrograph of thyroidectomized oviduct with thyroxine replacement (TTT) (x1000) showing increasing epithelial heights (arrow) and thickened (see Amadi et al., 2007)**

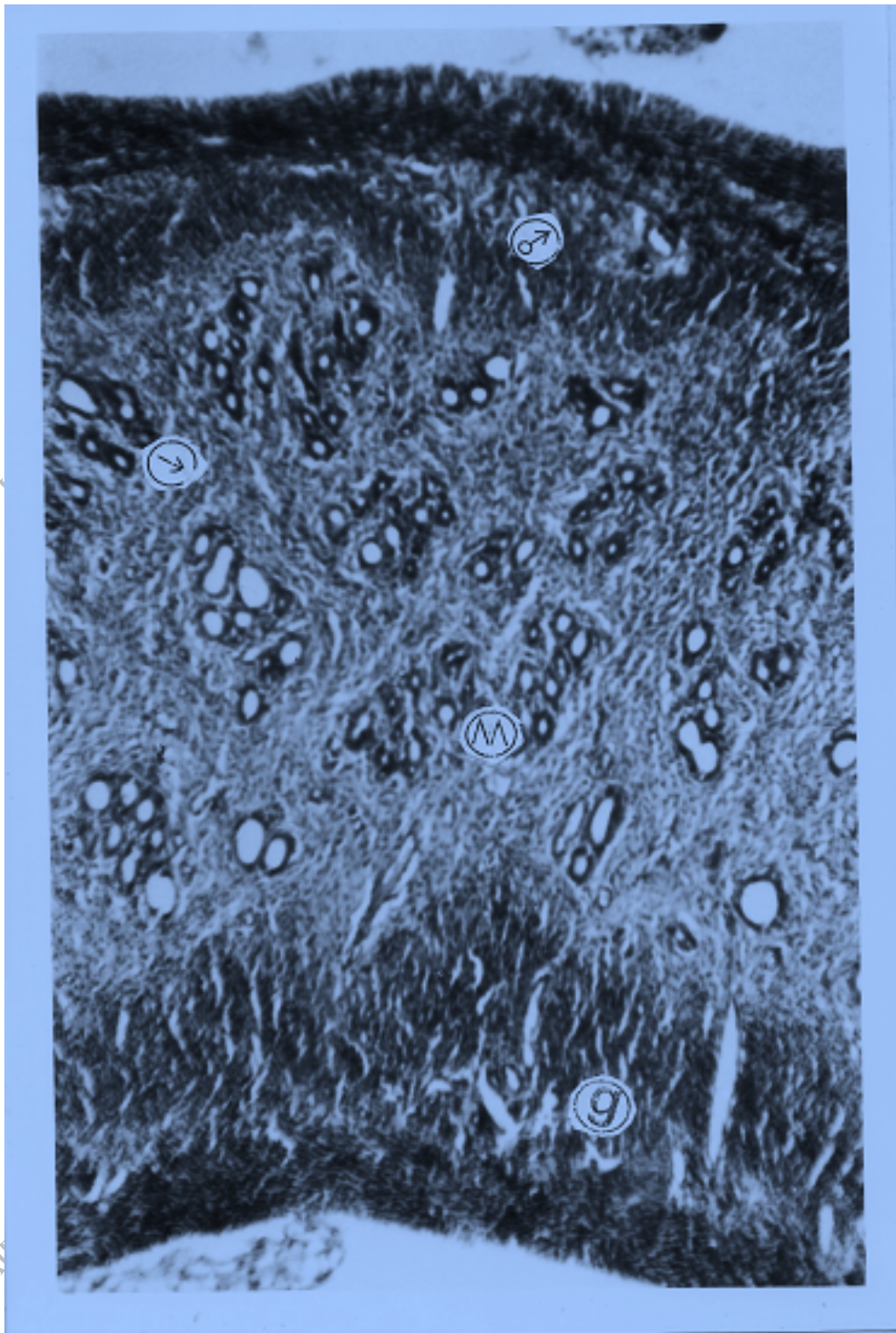
Interestingly, the observations in the oviduct of the thyrosectomized rats treated with thyroxine –TTT, revealed increasing epithelial heights, which was however, not up to the height of the euthyroids (control) in some foci. Similarly, the smooth muscle thickness increased significantly ( $P<0.05$ ) more than the thyrosectomized oviduct sections but there was no significant difference between the mucosal folding and the heights when compared with the control.



**Plate 4: Photomicrograph of thyroxine –treated (TT) oviduct (x1000) showing shrunken muscle layer (a) reduced mucosal folds (arrow)**

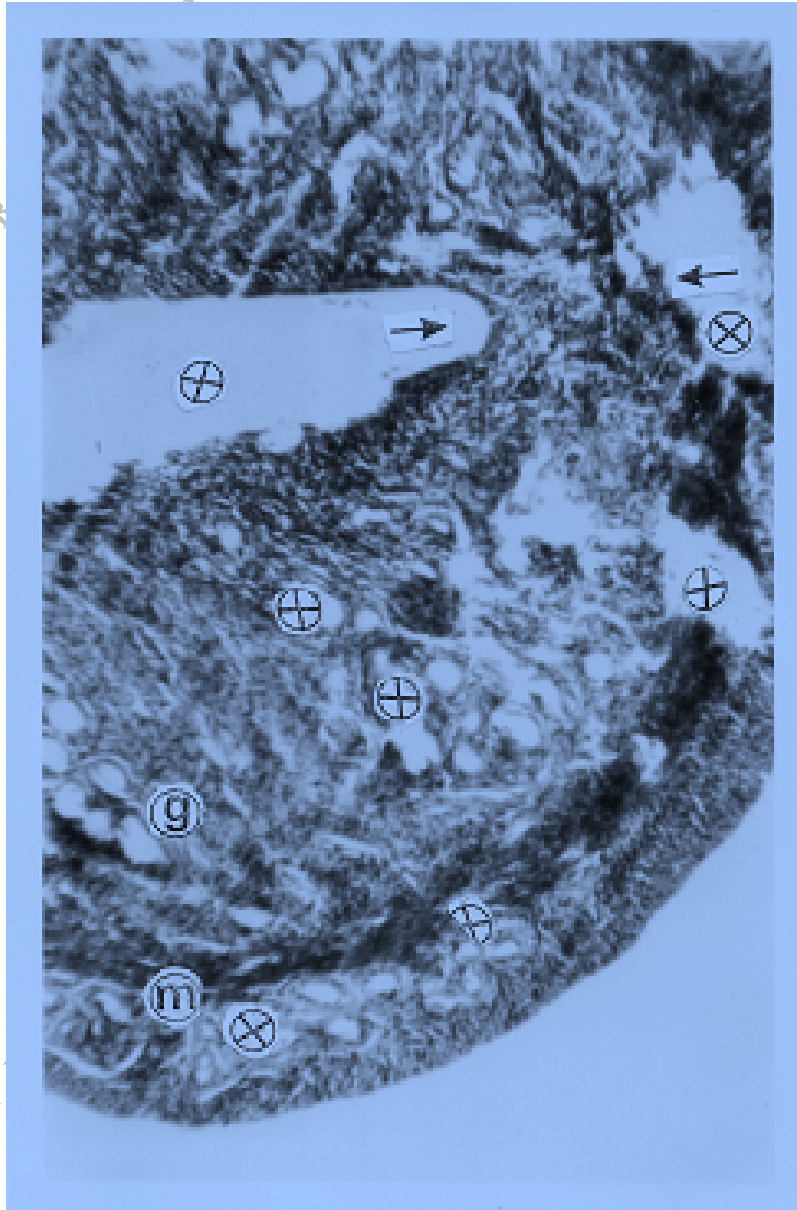
#### **MORPHOLOGY OF THE UTERUS**

The histological section of the endometrium of control uterus in Plate 5 below shows endometrial glands and stroma. The endometrial lining was in the proliferative phase. The glands were uniform and regular. They were lined by a single layer of columnar epithelium with basally located nuclei. The stroma was spindly and compact. The smooth muscle contained several congested small blood vessels of varying sizes and shapes (see plate 5).



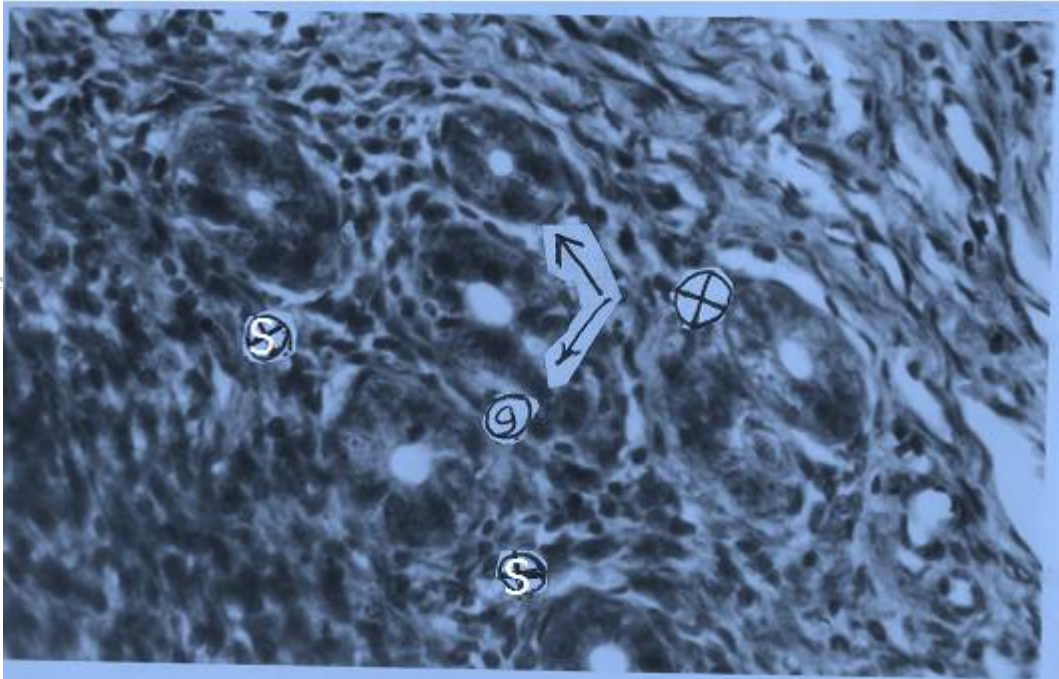
**Plate 5: Photomicrograph of Control Uterus: showing histological section of the endometrium with distinct endometrial glands and stroma.**

The thyroidectomized uterus (Plate 6) presented an increase in the number of proliferative endometrial glands which were cystically dilated and lined by columnar to cuboidal epithelium. Some of the glands showed squamous metaplasia in some foci. The stroma showed massive oedema with round to oval cells and prominent arterioles ( $\rightarrow\leftarrow$ )



**Plate 6: Photomicrograph of thyroidectomized Uterus (x1000) with thickened smooth muscle (m), endometrial glands (g), stroma with massive oedema (X) and tubular occlusion ( $\rightarrow\leftarrow$ )**

The thyroidectomized rats treated with thyroxine (TTT) presented thickened smooth muscle layer and an increase in heights of the endometrial tissue lining. The glands representing a slight increase in the numbers of proliferative gland (→) were of various sizes and shapes, and were lined by a single layer of tall columnar epithelium with basally located nuclei. Some of the epithelial cells were vacuolated (X). However, these glands were cystically dilated (g) containing no secretion within the period of study. The stroma was loose and oedematous with oval, to round cells. Prominent arterioles were also observed, giving a general appearance of cystic endometrial hyperplasia. The thyroxine –Treated (TT) Uterus had reduced endometrial muscle layers.



**Plate 7: Photomicrograph of The thyroidectomized rats treated with thyroxine (TTT) showing numbers of proliferative gland (→), epithelial cell vacuolations (X), cystically dilated glands (g) and loose and oedematous stroma with oval to round cells (S).**

#### DISCUSSION:

The result obtained in this study focused on hypothyroidism by thyroidectomy. In the oviduct and uteri of this group of animals, the cystically dilated glands and loose stroma might have given way to oedema formation in all the thyroidectomized rats. This was confirmed by vacuolations of some epithelia cells and the glands containing no secretion in thyroidectomized oviducts and uteri, with subsequent thyroxine replacement. Oedema formation has been described as excessive accumulation of fluid in tissue spaces due to increased transduction of the fluid from the capillaries as a result of increased intraluminal pressure (Blakiston's Meical Dictionary, 1979) which also resulted in the occlusion of the tube in oviducts and the uterus. The occlusion is suggested to have precipitated the hydrosalpinx of the uterus; and better exhibited in the thyredectomized oviduct. Intraluminal pressure has been reported to cause hydrosalpinx (Otubu 1983) and it is a characteristic feature of occluded tubes.

In the present study hypothyroidism was illustrated as a natural candidate for the genesis of hyydrosalpinx. Hypothyroidism from the present work might probably be a factor in the genesis of hydrosalpinx.



As we had stated earlier (Amadi, *et al.*, 2005, 2007), changes in the mechanical action of the smooth muscle layers of the uterus and the oviduct, that correlated morphology with contractile function, depends on the thyroid hormone status. Also, the state of both the endosalpinx and the myosalpinx of the tube are probably most relevant to the subsequent function of the uterus after treatment of the hydrosalpinx by thyroid hormone therapy. In the hypothyroid group with subsequent thyroid hormone replacement therapy, the anatomical features did not quite attain the control values; due to the fact that with the removal of the thyroid gland there was bound to be morphologic and functional hysteresis before thyroid hormone rehabilitation could take effect within the period of study. The animals in the affected group had to depend on the secondary organs like the salivary gland, gastric mucosa, small intestine, the skin, the breast (and placenta) for trapping iodide (I<sup>-</sup>) necessary for the formation of thyroxine within the period of study (Amadi *et al.*, 2007).

In conclusion, although it is dangerous to project animal experiment on to humans, oedema formation in the oviduct and hydrosalpinx of the uterine tube seen in the present work would reasonably suggest that assay of thyroid hormone levels should not be ignored in therapeutic regimens especially for the female reproductive tract.

#### ACKNOWLEDGEMENT

We deeply thank Mr. Godwin Njoku of Medical illustrated and technological unity (MITU) for the photographs. Also Miss Rashidah is profoundly appreciated for secretarial expertise.

#### REFERENCES

- Amadi, K., Adeniyi, K.O., Nwana, E.J.C. and Otubu, J.A.M. (1996c). The effect of thyroidectomy and thyroxine treatment on the morphology of the testis in the rat. *Molecular Andrology*; VIII (1-2): 7-14.
- Amadi, K., Nwana, E.J.C. and Otubu, J.A.M. (1999). Effects of thyroxine on the contractile response of the vas deferens to prostaglandin E2. *Arch Androl*; 42: 55-62.
- Amadi, K., Adeniyi K.O; Nwana E.J.C. and Otubu, JAM. (1996a). The effect of thyroidectomy and thyroxine treatment on the morphology of the ovary. *Pathophysiology*; 116: 80-84.
- Amadi, K., Adeniyi, K.O., Nwana, E.J.C. and Otubu, J.A.M. (1996b). The effect of thyroidectomy and thyroxine administration on the morphology and function of the rat uterus. *Pathophysiology*; 112:30-33.
- Baker, F.J., Silvertown, R. E. and Luckock, E.D. (1994), Mayer's acid-alum haematoxylin and eosin: a general purpose staining procedure. In: an Introduction to Medical laboratory technology 11<sup>th</sup> Edn. London. p. 357.
- Blakiston's Pocket Medical Dictionary 4<sup>th</sup> Edn (1979). Alfonsa RG ED Mc Graw-hill Book company New York.
- Blandan, R. J., (1979). Tubal sterility patency tests and results of operation. *J Obst Gynae Birth Cwllth*; 80:142-151.
- Glenister, T.W., Hyden, F.E., Kerr, H.G. (1986). Human Reproduction. In: a companion to medical studies Vol. 1(2<sup>nd</sup> Edn) Blackwell scientific publications. Oxford. Pp. 38-67.
- Katamaya, K., Takamaya, A.E. and Mochizuki, M. (1992). The levels of thyroid hormones in patients with menstrual disorders. *Jpn. J. Fertil Steril*; 35:1.
- Manar, A. (1993). UNICEF Team on Goitre in women.
- Maruo, T., Katayama, K., Barnea, E. R. and Mochizuki, M. (1992). A role for thyroid hormone in the induction of ovulation and corpus luteum function. *Hormone Res*; 37 (suppl 2): 12-18.



Otubu, J.A.M. (1983). The hydrosalpinx: Clinical and experimental study of the functional morphology, pathogenesis and muscular activity of occluded tubes Ph.D. thesis submitted to the University of London.

#### **AUTHORS CONTRIBUTIONS**

All the authors participated fully in this study.

