THYROID HORMONES: GATE KEEPERS OF LIFE AND DEATH

Inaugural Lecture

By

Professor Kemakolam Amadi
B.Sc (Hons), M.Sc Chemical Pathology (Ibadan)
Ph.D (Jos), MPSN, MNYAS, MAPS.

Professor of Reproductive Endocrinology
Department of Human Physiology
Faculty of Medical Sciences
University of Jos, Jos, Nigeria.

Thursday March 27, 2014

Mobile: 08036484145
E-mail: parkers2004amam@yahoo.com

THYROID HORMONES: GATE-KEEPERS OF LIFE AND DEATH

PREAMBLE:

My idea of Inaugural Lecture is to expose to the academic community and a wider audience the translational research one has carried out to benefit mankind. Every finding is an already existing TRUTH. That is why Chambers Etymological 20th Century Dictionary described science as "Truth systematized or knowledge ascertained", while Colonel Robert Green Ingersoll 1833 – 1899 described it as "the intellectual wealth of the world".

In Genesis 1: 28, God said to them (male and female) "fill the earth and subdue it". He did not mean: destroy one another or a superior power encircling a smaller nation/power and swallow it up. I believe God meant that He has hidden unquantifiable amount of treasure in the world for man to discover for the use of mankind.

For example, I have always told my students that in communities that practice male circumcision, the surgery is best carried out on the 8th day of life. The existing Truth is seen in Genesis 17:12 – God said to Abraham thus "And he that is eight days old shall be circumcised among you; every man child in your generations". This is because the level of an indispensable blood clotting factor called FIBRINOGEN is highest in the blood; so that a child circumcised on that day does not bleed much (does not lose much blood). In other words I am saying that through the medium of research, nature unveils, man beholds and science probes an unseen world.

The little probe I have carried out, and which is begging to be patented and put into public use is embedded in my treatise titled: Thyroid Hormones: Gate-keepers of life and death. The wording may be a bit technical so as not to lose its meaning within the context of the dissertation.

Thyroid Function in the Mammal with Special Reference to Its Role in Normal Development

The word "THYROID" is adjective and noun" having the shape of a shield; signifying cartilage of the larynx (laryngeal prominence/thyroid cartilage/Adam's apple) or a gland of the trachea.

Question:…………………………………………………………………………………………………..

The Adult Thyroid Axis

The thyroid gland consists of two lobes situated on either side of the trachea, connected by a narrow isthmus which passes just below the larynx. The gland secretes two major compounds which posses significant hormonal activity, thyroxine (T_4) and triiodothyronine (T_3) which profoundly affect the physique and temperament of human beings. That is the body build and a person's nature as shown in the way she/he behaves or reacts to situations or the tendency to get emotional and excited very easily and behave in an unreasonable manner.Both compounds are iodothyronines which are produced as a result of the iodination and subsequent condensation of the tyrosine residues contained in the protein thyroglobulin which constitutes a large portion of the dry weight of the gland. The thyroid is unusual in that it contains relatively large quantities of hormone stored in the colloid material contained in the follicles of the gland.

The rate of secretion of hormone is determined by the plasma concentration of thyroid stimulating hormone (TSH) which is derived from the thyrotroph, one of three types of basophil cells of the anterior pituitary (anterior hypophysis). The thyroid is capable of a small basal secretion which remains in the total absence of TSH. In the human, an excessive thyroid secretion rate may occur as an autoimmune disease as the result of the presence of long acting thyroid stimulator (LATS) which is an immunoglobulin which reacts with thyroid microsomes. There is no evidence for such an autoimmune condition occurring in other species. Plasma concentration of TSH may show a marked diurnal rhythm, superimposed on which there are other, more irregular, fluctuations (Thomas, Abel and Nathanielsz, 1974). The rate of thyroidal secretion is therefore not constant.

However, since T_4 has a long plasma half life (t½), of 6-7 days plasma T_4 concentrations are relatively stable. The thyroid hormones are carried in the blood reversibly bound to plasma proteins: thyroxine binding globulin (TBG), thyroxine binding pre albumin (TBPA), and albumin.

In most species, total plasma T_4 concentrations are usually approximately 100 times as great as total plasma T_3 concentrations (T_4: 60ng/ml: T_3: 0.6ng/ml).

Foetal Thyroid Axis

It is during foetal life and early neonatal development that the hormones of the thyroid axis play their most crucial role and where irregularities of thyroid function may produce the most marked and permanent effects.

The early growth and development of the gland does not appear to be TSH dependent since histological development, colloid formation and storage can occur in the absence of TSH; this protein is present at 29 days of gestation in the human, whereas foetal plasma TSH is not

detectable until about 79 days (Gitlin and Biasuci, 1969). It does however, appear that the ability to trap iodide and hormonal secretion are TSH dependent. Both of these begin to appear at about 10 weeks in the human when TSH secretion commences.

Chronic Foetal Experiments

As a result of the work of Meschia, Cotter, Breathnach and Barron (1965) it has been possible to develop an animal preparation in which catheters may be inserted into the foetal vasculature and maintained patent over several weeks of use. Such preparations in the sheep (Hopkins and Thornburn 1972; Erenberg and Fisher, 1973; Nathanielz, Comline, Silver and Thomas, 1973) and the cow (Nathanielsz, Comline, Silver and Thomas 1974; Thomas, Comline, Silver and Nathanielz, 1974) have been used to examine several aspects of foetal thyroid function since vascular catherterization can be combined with ablation of the thyroid and other endocrine glands.

Many aspects of the physiology of the foetus are disturbed for some time after surgery, including foetal breathing movements (Dawes, 1973), electrolytes (Mellor and Slater, 1971) and carbohydrate metabolism (Shelley, Bussett and Milner, 1975). For these reasons, it is essential that no datacollected during the 48 hours post surgeryare used in any experimental investigation.

Thyroid Hormone

The thyroid gland secretes two major hormones: thyroxine (T_4) and Triiodothyronine (T_3). The only difference between the two is that T_4 has four iodine atoms attached to it and T_3 has only three. Although the thyroid gland secretes mostly T_4 (about 90%), it is T_3 that is considered the more active form of the hormone (Cargill and Vargas, 2002).**Figures 1, 2 & 3:**
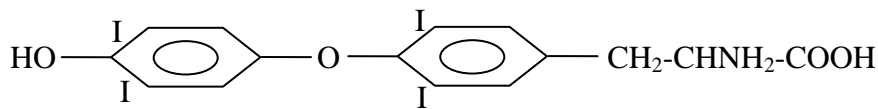

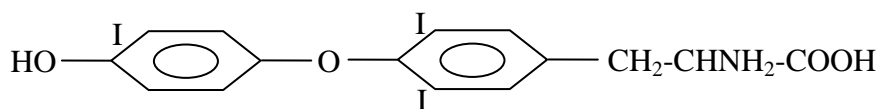**Figure 1:** Structure of L-3,5,3′,5′-Tetraiodothyronine (Thyroxine)

Figure 2: Structure of L-3,5,3′ Triiodothronine

Reverse Triiodothyronine (rT_3) or 3,3′,5′-triiodothyronine has the structure shown in Figure.

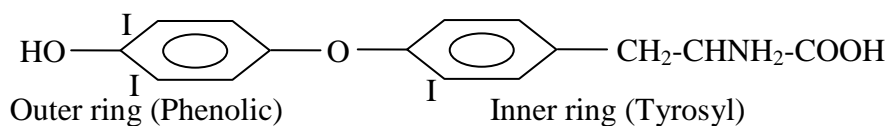Outer ring (Phenolic)   Inner ring (Tyrosyl)

Figure 3: Structure of L-3,3′,5′ Triiodothronine (Reverse T_3)

Hopkins and Thornburn (1972) studied the long term effects of surgical thyroidectomy in the foetal lamb both histologically and hormone assay. These animals which were allowed to go through to delivery tended to born a few days later than usual and none survived for more than

30 hours after delivery. Many failed to establish spontaneous respiration and died soon after birth.

Conversion of T_4 to T_3

Most, if not all the physiological activity produced by T_4 is due to its conversion to T_3 (Williams, 1981) the thyroid secretes much large amounts of T_4 than T_3, but T_4 is more stable in the body than T_3 and is also more firmly bound to plasma proteins so that T_4 is an extracellular hormone while T_3 penetrates tissue fluids and cells readily, since it has a much higher affinity forthe thyroid hormone receptorprotein in the nucleus.

Cargill and Vagas (2002) explained that in a series of complex steps that involve mainly the liver and kidneys, T_4 is stripped of an iodine atom and converted to T_3 at their target cells when needed.

Reverse Triiodothyronine (rT_3)

Thyroxine (T_4) is acted upon by one or other of two monodeiodinatingenzymes. One 5-monodeiodinase removes an iodine atom from the outer ring of the molecule to yield T_3; the other 5-monodeiodinase removes an iodine atom from the inner ring to form reverse T_3 (rT_3). Deiodination is the most important route of metabolism of T_4 and is thought to proceed by serial removal of iodine atoms finally to produce thyronine which is the fully deiodinated skeleton of T_4 (Haffenberg, 1984); which is excreted with its metabolites in the urine (Williams, 1981).

Reverse T_3 (rT_3) 3, 3′, 5′-Triiodothyronine is different from T_3 (L-3, 5, 3′) in that it is derived from T_4 by the loss of an iodine atom from the tyrosyl ring instead of the phenolic ring (Thomas and Nathanielsz, 1980). It has been shown to be capable of inhibiting the calorinogenic action of T_4 (Pittman and Baker, 1959). Thyroglobulin is hydrolysed to give L-3-monoiodotyrosine, L-3, 5-diiodotyrosine (precursors of the thyroid hormones) and 1, 2-monoiodohistidine. These compounds are also released into circulation although they posses no thyroid hormone activity (Nocenti, 1968).

Independence of Foetal Thyroid Axis

The foetal thyroid axis appears to function independently of that of the mother during the latter part of gestation. This is supported by the fact that the placenta is largely impermeable to: T_4(Comline, Nathanielsz and Silver, 1970) T_3 (Thorburn and Hopkins, 1973) and TSH (Erenberg and Fisher, 1973) as shown by tracer studies.

Foetal thyroidectomy results in a virtually complete disappearance of T_4 from the blood plasma (T_4 concentration (<0.7ng/ml) – with a simultaneous elevation of foetal plasma TSH concentration (300 – 1500µ/ml), while both maternal T_4 and TSH concentrations are essentially unchanged (Erenberg and Fisher, 1973). Thus it would appear that the foetal pituitary is under the same negative feedback influence from plasma T_4 as in the adult.

Thyroid Hormones and Parturition (The process of giving birth)

In the foetal calf there is an inhibition exerted on the thyroid axis in the last two days ofgestation which results in a depression of foetal plasma T_4 and TSH concentrations. It is probable that this inhibition is secondary to the processes responsible for the initiation of parturition, since administration of exogenous cortisol or ACTH to the foetus will induce parturition that is accompanied by the same pattern of thyroidal changes. That is to say the thyroid hormone level in the foetus is a critical determinant of initiation of parturition and thus the life or death of the foetus. Thornburn and Hopkins (1973) originally suggested that the failure of fetuses to maintain

their plasma T_4 concentrations before parturition might be the result of a decreased central drive on the thyroid axis. In particular, the maturation of foetal temperature receptors might result in a heat stress with a combined inhibition of thyroid function and activation of the adrenal axis which is known to result in delivery (Liggins, Fairclough, Grieves, Kendall and Knox, 1973). Thomas, Horn, Krane, Bass and Nathanielsz (1977) have shown that if parturition is induced in the sheep by infusion gradually increasing amounts of cortisol into the foetus, so mimicking the normal increase in cortisol secretion which precedes parturition, then foetal plasma T_4 concentration falls. In some of these animals there was also an elevation of foetal plasma T_3 concentration which suggests that cortisol, a potent enzyme inducer, may have a role to play in the changes in peripheral deiodinase activity which occurs at this time.

The Role of Foetal Thyroid

Attempts to produce a suitable experimental thyroid deficiency raise several problems. First, the extent to which different species grow and mature in utero varies considerably. Many of the developmental stages which occur in utero in the sheep and the human occur post-netally in the rat or rabbit. Thus, credible experimental models in these animals advocate adult forms so as to exclude inducing adverse neonatal effects. This is especially true for the development of the CNS (Eayrs, 1961). Secondly, many methods of inducing thyroid deficiency result in other harmful effects which may themselves retard foetal development. If the onset occurs during adult life the effects may be reversed by T_4 administration for example, human cretinism, which is characteristic of areas of iodine deficiency can only be prevented by maternal iodine supplementation during gestation or thyroid or iodine replacement therapy commenced immediately after birth.

Thyroid Function in the Newborn

The challenges experienced by the foetus during parturition makes this period probably the most hazardous experience during pre- or post-natal life. The foetus responds to the stresses of the pressure of uterine contractions, temporary hypoxia and hypercapnia, acidosis, cold exposure and the cessation of a steady nutrient supply by an explosive activation of neural and endocrine protective mechanisms, there is a considerable rise in the plasma concentration of neurohypophyseal and adenohypophyseal hormones as well as a general activation of the sympathetic nervous system.

In the thyroid axis, there are considerably elevated concentrations of plasma TSH and T_4 immediately post-natally. Some of this elevation is probably the result of cold exposure (Fisher and Oddie, 1964). However, in the experimental animal, plasma TSH concentrations often begin to rise before the foetus emerges from the birth canal suggesting that other factors may play a contributory role. Amadi (2004) attributed it to the sudden closure of the ductus arteriosus which causes turbulence in blood flow and thus dissipation and upsurge in levels of free plasma thyroid hormones (T_4 and T_3) potentiated by reduced half life (t½) of T_4 that changes faster to T_3; the rise of which transiently alters cardiovascular homeostasis consequent on low pH and elevated free fatty acid concentrations, that tend to displace thyroid hormones from their binding proteins observed by Hollander Scott, Burgess, Rabinowitz, Merimee, and Oppenheimer, 1967) causing

preeclampsia, but which quickly readjusts to status quo ante; by a reversal process, initiated by the pressure dampers in the aortic arch and the carotid glum (Sinus).

The Thyroid Hormones in the Adult

The catalogue of evidence, some listed above have made neonatal thyroidectomy or ablation contraindicated. Hence modern physiology experiments have turned to adult thyroidectomy where the parameters stabilize within hours (Tata, 1964; Adeniyi and Olowookorun, 1989; Amadi, Nwana, Otubu, 1996, 1999, 2005, 2007). This treatise therefore is based on adult euthyroidism, hypothyroidism (thyroidectomy) and hyperthyroidism (thyroxinaemia without thyrotoxicosis).

Brian, Sonia and Miguel (2005) have reported that we live in a dangerous environment, continuously immersed in a medium containing 21% oxygen, a highly reactive molecule. Fortunately for us, this impediment to life has been turned back on itself and has actually been harnessed by the cell to generate the stored chemical energy necessary to meet the major demands of cell physiology. Mitochondria are the organelles providing this protection, compartmentalizing and controlling the oxidation of metabolic fuels to produce ATP, the high energy phosphate used to maintain ion gradients, synthesize new molecules, actively dispose of old molecules, and perform mechanical work. It is vital for the cardiac cell which consumes about 10% of total oxygen but stores very little of it; to continuously adjust the fine balance between energy production and energy consumption and to limit the ill effects of the byproducts of metabolism which include reactive oxygen species (ROS). Biological systems can therefore operate at the edge of dynamic instability. Our data modify the central hypothesis that ion channels on mitochondrial inner and outer membranes are key participants in the decision between cell life and death.

Brian *et al.,* (2005) have reported that emerging evidence indicates that in cardiomyocytes for example, mitochondrial ion channels activated by reactive oxygen species can induce a mitochondrial "critical" state which can scale to cause electrical and contractile dysfunction of the cardiac cell and, ultimately, the whole heart. Reactive oxygen species can induce activation of mitochondrial ion channels only in adequate level of thyroid hormones; as hypothyroid states induce NIL or NON-SIGNIFICANT mitochondrial channel activation (Amadi, 2004). Oxygen production and utilization are directly proportional to the thyroid hormone status of a system. (Amadi *et al.,* 2006).

There is a strong evidence that an increase in matrix Ca^{2+} ion is important for stimulating oxidative phosphorylation at several sites including the Ca^{2+}- sensitive dehydrogenases of the Kreb's Cyle (Balban, Bose., French and Territo, 2003; Cortassa, Aon., Marban., Winslow and O'Rourke 2003).

Both the electrical and Ca^{2+} handling subsystems of the myocyte (the power house of all other systems) are strongly influenced by the energy state of the mitochondria only in physiological levels of thyroid hormones. We have established a Ca^{2+} ion receptor dependence on thyroid hormones for the regulation of cellular functions (Amadi, Sabo, Adelaiye and Sagay, 2005). That is to say there is an integrated cardiac mitochondrial energy metabolism and Ca^{2+} dynamics, dependent on the thyroid hormone status of the system or individual. Thus, the decision between life and death is a tripartite cross talk of mitochondria, Ca^{2+} ion receptors with thyroid hormones as the gate-keepers.

MORPHOLOGY AND DEVELOPMENT OF THE REPRODUCTIVE TRACT

The Female Reproductive Axis:

The Uterus and Genesis of Hydrosalpinx: Probably apart from Leiomyoma uteri (fibroid) one of the problems facing gynaecologists is hydrosalpinx which is oedema of the uterus (womb). In his work at Hammersmith Hospital University of London. Otubu (1983) by applying intraluminal pressure, created/ induced hydrosalpinx in the rabbit. This was to find out the cause of the phenomenon for possible intervention. A pressure- sensitive hormone as thyroid hormone (Maruo, Katayama, Barnea and Mochizuki, 1992) was investigated by our research team as a probable primary, natural candidate of the raised intraluminal pressure 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 ligaments of the ovary, in the infundibular pelvic ligament and in the fimbria, all which take part in the rhythmic movements of the internal generative organs (Amadi, Adeniyi, Nwana and Otubu, 1996). In 2005 Amadi *et al.,*by creating hypothyroidism via thyroidectomy in the rabbit found out the following features: the tubo-uterine junction showed gross oedema formation, erosion of the endometrium, endosalpinx, myometrium and in the heights of mucosal folds, epithelial cells with an overall picture of cystic stromal hyperplasia compared with the euthyroid state **(Plates: 2; 1).**
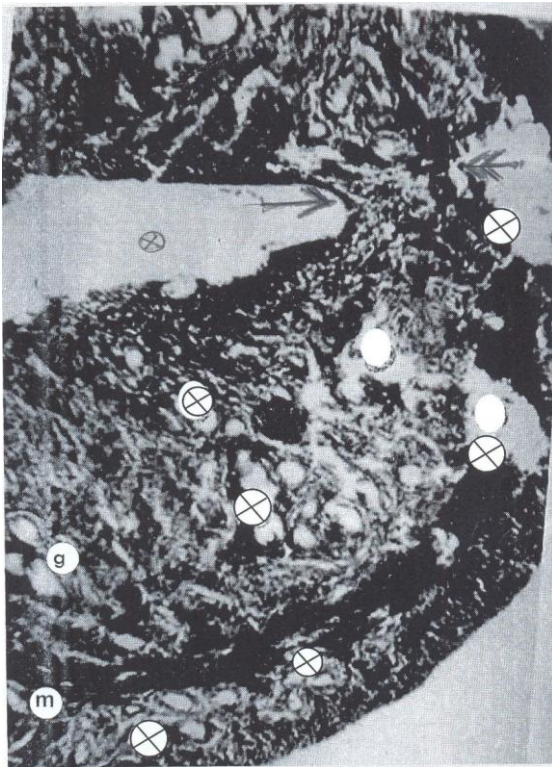
Plate 2: Thyroidectomized Uterus
- Smooth muscle layer appeared thickened (m)
- Increase in number of proliferative cystically dilated endometrial glands lined by columnar to cuboidal epithelium (g)
- Stroma showed gross oedema ⊗
- Overall cystic endometrial hyperplasia
- Gross oedema and erosion of endometrium, endosalpinx and myometrium.
- Myometrial occlusion →←
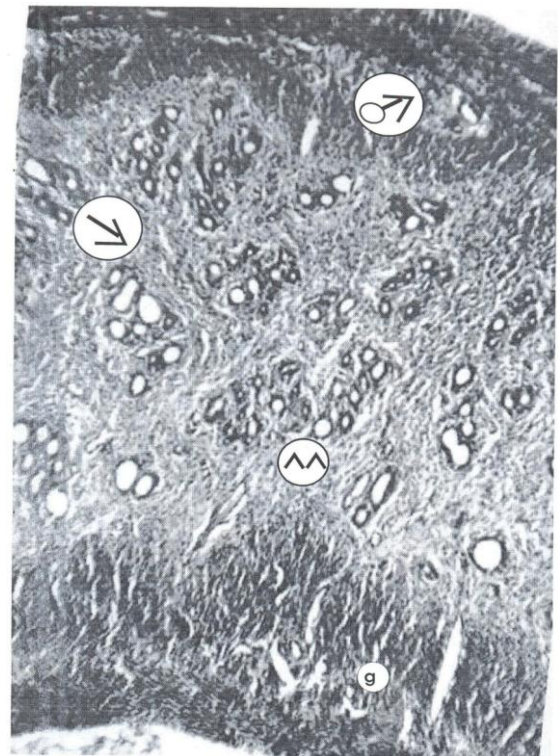- Mag : x 1000
  (Amadi, Adeniyi, Nwana, Otubu, 1996)

Plate 1: Control Uterus
- Endometrial lining in proliferative phase (∧∧)
- The glands uniform and regular, lined by single layer of columnar epithelium with basally-located nuclei (g)
- Stroma spindly and compact (↘)
- Smooth muscle contain several congested small blood vessels of varying sizes and shapes (♂)
- Mag : x 1000
  (Amadi, Adeniyi, Nwana, Otubu, 1996)

In 2007, extending the work to the oviducts of forty (40) sexually mature female Wister albino rats in different thyroid hormone states, Amadi *et al.,* demonstrated beyond reasonable doubts the following features in hypothyroid states:

- There was significant reduction in wall thickness of smooth muscle.
- Epithelial cells height reduced from columnar to cuboidal.
- Mucosal folds flattened
- Fimbrial end blocked at 4 0'clock; implying that egg-transport is halted and the life of the oocyte is terminated.
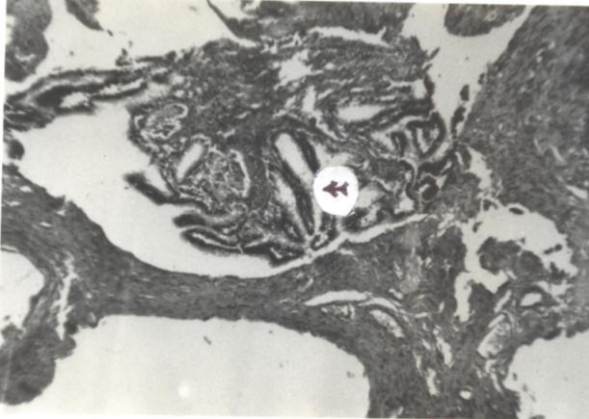
Plate 1: Photomicrograph of Control Oviduct (C)
Features:
- Normal Oviduct with typical muscosal lining thrown into folds ⊕
- Epithelial cells columnar ⊕
- Proximal (fimbrial) and open, showing developing oocyte within the uterus, towards the columnar folds at 4 O'Clock.
- Mag : x 1000
  (Amadi, Nwana, Otubu, 2007)
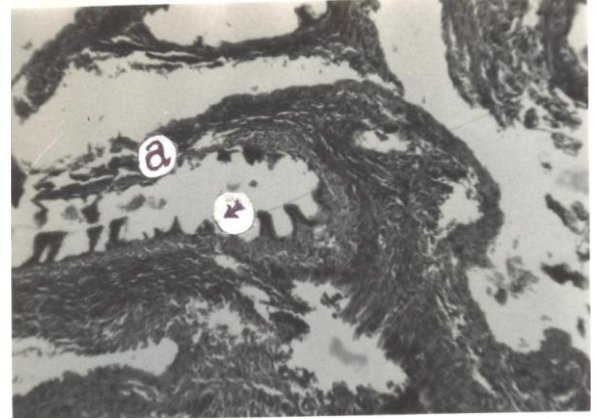
Plate 2: Photomicrograph of Thyroidectomized Oviduct (T)
Features:
- Significant reduction in wall thickness of smooth muscle **ⓐ**
- Epithelial cell height reduced from columnar to cuboidal ⊕
- Mucosal folds flattened
- Gross oedema formation and erosion of the endometrium, endosalpinx and myometrium.
- Fimbrial end blocked @ 4 O'Clock.
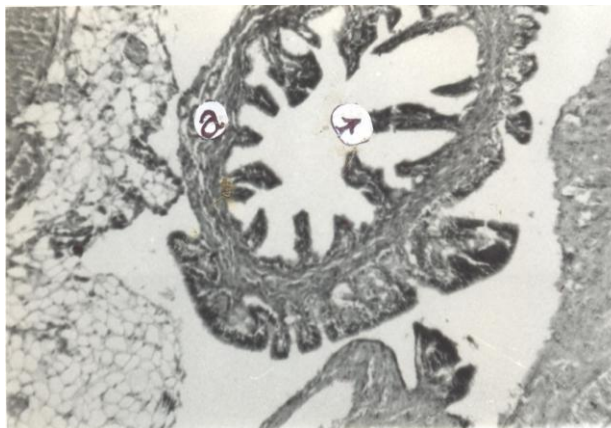- Mag : x 1000
  (Amadi, Nwana, Otubu, 2007)

Plate 3: Photomicrograph of Thyroidectomized Oviduct with Thyroxine Replacement (TTT)
Features:
- Epithelial height increasing once again ⊕
- Smooth muscle thickness increased significantly ⓐ
- Mucosal folds returned
- Oviduct (TS) centrally placed among surrounding fimbrine
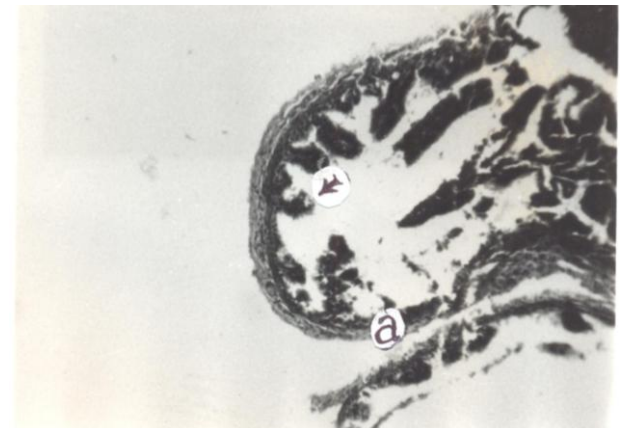- Mag : x 1000
  (Amadi, Nwana, Otubu, 2007)

Plate 4: Photomicrograph of Thyroxine -Treated Oviduct (TT)
Features:
- Shrunken muscle layer ⓐ
- Mucosal folds reduced to one layer ⊕
- The heights of the two layers differed significantly compared with control and T oviducts
- Fimbrial end open showing developing oocyte @ 2 O'Clock
- Mag : x 1000
  (Amadi, Nwana, Otubu, 2007)

If there is implantation of the fertilized egg in the oviduct itself,that would be ECTOPIC gestation which calls for surgical emergency for its removal because foetal developed is impossible and ends in abortion that is termination of life (compare with other thyroid states).

The blocking of the proximal (fimbrial) end observed in plate 2 could have given rise to raised intraluminal pressure which might have been responsible for the gross oedema and anatomical destruction of the entire tube. Oedema formation has been described as excessive accumulation of fluid in tissue spaces due to increased transduction of fluid from the capillaries as a result of increased intraluminal pressure (Blakiston, 1979); consequent on depressed muscular activity. These features are characteristics of occluded tubes that induce hydrosalpinx as illustrated in our finding.

Muscular activity has been shown to provide the propulsive force in the instantaneous movement of ovum surrogates (Hodgson *et al.,* 1977). Delay in the mechanism of transport of ova at the ampullo-isthmic junction (AIJ) is thought to be controlled by contractions of the smooth muscle cells rather than any sphincteric action (Otubu, 1983). The changes we observed in the endosalpinx, stroma and myosalpinx include oedema atrophy, fibrosis, hypertrophy and decreased Ca^{2+} deposits (Table 1). These changes might affect the mechanical action of the smooth muscle layers of the uterus and the oviduct and thus correlated morphology with contractile function; the two which depend on the thyroid hormone status of the animal (Amadi, *et al.,* 2005, 2007). 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^-) necessary for the formation of thyroxine within the period of study (Amadi, Nwana and Otubu, 2007). Although substantial caution must be taken in projecting animal experiments on to humans, the gross erosion of and oedema formation across the endometrium, endosalpinx and the myometrium of the entire tube observed here would reasonably suggest hypothyroidism as a primary candidate in the genesis of hydrosalpinx and thyroid hormone levels should not be ignored in the management of reproductive tract disorders.

Table 1: Means (± SEM) of TSH, T_4 and Ca^{2+} Level in Experimental Rats

Animal Groups	TSH	T4	Ca2+
	U/ml	Ng/dl	Mmol/L
Control (n = 10) (C)	6.5 ± 0.8	3.9 ± 1.0	8.0 ± 0.04
Thyriodectomized n = 10 (T)	10.8 ± 0.02	0.08 ± 0.4	2.10 ±0.02**
Thyroidectomized treated with thyroxine (n = 10) (TTT)	5.9 ± 0.6	4.0 ± 0.4	7.6 ± 0.03
Non Thyroidectomized treated with thyroxine (Non TT; n = 10)	12.2 ± 1.8	18.6 ± 8.07	9.2 ± 0.08

* P < 0.05; **P < 0.01, in comparison with control

Amadi, Sabo, Adelaiye & Sagay, 2005)

Morphology of the Ovary (aka "The Power-Generating House of the Female")

Figures 1, 2 of our study on the ovary, speak robustly on the thyroid hormone status of an animal with respect to ovarian development. In Figure 1 (control euthyroid animals) there are several corpora lutea of varying sizes and shapes, several growing follicles and thick-walled blood vessels in fibrocollagenous stroma.
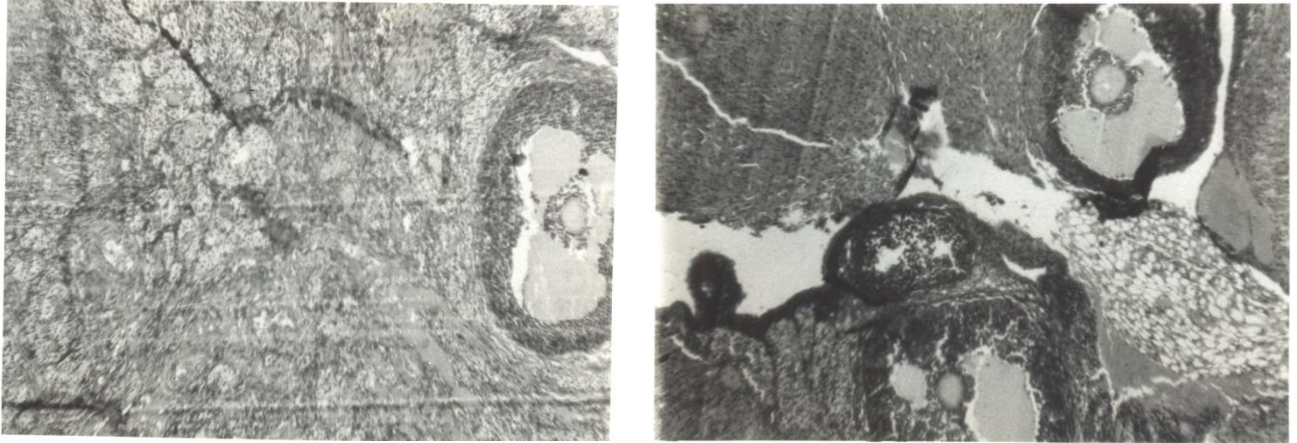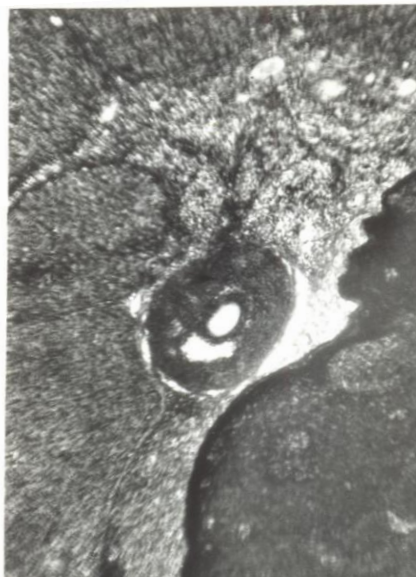

Plate 1: Ovary of Control Rat shows
- Severalcorpora Lutea of varying sizes and shapes
- Several growing follicles and thick-walled
- Bloodvessels in firbocollagenous stroma

Plate 2: Ovary of Thyroidectomized Rat
- The Corpora albicantia and Corpora Lutea greatly reduced in size
- Slight increase in stroma components
- Lose stroma with degenerating follicles (ateretic)
- Strikingly thin-walled blood vessels resulting from hypotrophy
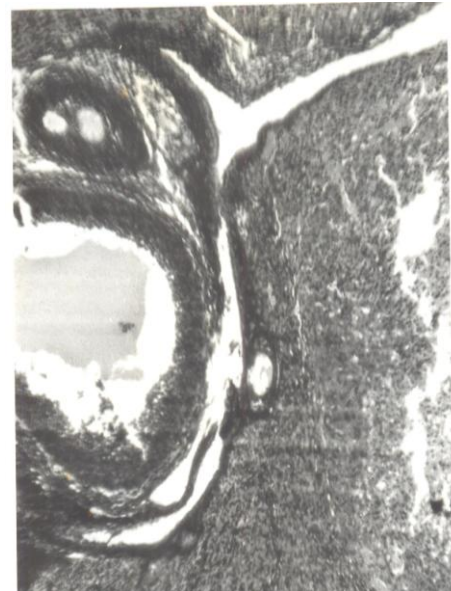
Plate 3: Ovary of Hypothyroids with Thyroxine Replacement
- Shows prominent Corpora
- Albicantia and Lutea cysts close to control

Hypothyroid rats (Figure 2) exhibited corpora albicantia and corpora lutea greatly reduced in size, the stroma loose, with degenerating (atretic) follicles and strikingly thin-walled blood vessels (Amadi, Adeniyi, Nwana and Otubu, 1996). An atretic follicle can never give rise to a mature ovum (the egg of life).

Thyroxine replacement (Figure 3) returned the picture to control state. This implies that an adequate (physiological) circulating level of thyroid hormones is one of the essential factors for normal morphological differentiation of the ovary.

The Male Reproductive Axis

Thyroid hormones have been known to induce morphological changes in organs of the body. However, before the advent of Amadi *et al.,*(1996) there was no unanimity in the literature on studies on the reproductive hormonal axis of the male using the albino rat model.WhileKalland Vera, Peterson and Swerd-Loff (1978) reported significantly smaller seminal vesicles and ventral prostates due to hypothyroidism; Bruni *et al.,*(1975) found no difference in testicular weights of hypothyroid animals when compared to euthyroids. Other workers have reported significantly larger testes relative to body weights in hypothyroid animals.

It was thus compelling to throw some light on the controversy arising from conflicting data obtained by the above workers on the reproductive abnormalities in hypo-or hyperthyroidism.

Our findings are displayed in Table 1 and Figure 1 − 4 (Amadi, Adeniyi, Nwana and Otubu, 1996). (1) Control; (2) Thyroidectomized (hypothyroid) (3) Thyroidectomized with thyroxine replacement); (4) Thyroxine treated (thyroxinaemia without thyrotoxicosis).

Table 1 shows the mean body and testicular weights and the ratio of testicular / body weights of the experimental rats, Hypothyroidism significantly (P < 0.01) depressed the body weight while mild hyperthyroidism (thyroxinaemia without thyrotoxicosis) increased the body weight significantly (P < 0.05). The difference in weights of the testes of the various groups was not significant.

Table 1: Mean ± SEM Body and Testes Weights of Experimental Rats at Sacrifice.

Animals	Body Weight Initial	(gm) Final	Testes Initial	Weight (gm) Final	Ratio of Testes /Body Weight
Control (n = 10)	130 ± 10.0	250 ± 10.2	-	3.4 ± 0.2	0.012
Thyroidectomized (n = 10)	132.2.5	120.5 ± 8.0**	-	3.3 ± 0.3	0.027**
Thyroidectomized rats treated with thyroxine (n = 10)	130 ± 5.6	248.6 ± 11.2	-	3.3 ± 1.0	0.013
Thyroxine-treated (n = 10)	130 ± 10.2	300 ± 12.0*	-	3.6 ± 1.2	0.012

Values are means ± SEM. Numbers of rats studied are shown in parameters.
*P<0.05 x **P<0.01 compared with value for control rats.

(Amadi, Adeniyi, Nwana, Otubu 1996).

The ratio of the weights of the testes to body weights was however significantly larger in the hypothyroid rats (large for nothing testes), compared with the other groups. May I call your attention to the arrest of spermatogenesis in this group. Figure 2 that is to say the spermatozoa of this group are useless they may appear phynotypically normal but genotypically abnormal. This was confirmed by co-habiting adult females with the hypothyroid males for up to three months but they proved to be physiologically "GAY COUPLES".Whenhowever the same females were left in the same cage with just one control male for two weeks and then separated each female rat thereafter had upwards of six litters. Kindly note that no amount of concoctions erroneously given to these females would solve the infertility except thyroid hormone therapy as seen in Figure 3.

Figure 1: Control:- These are numerous closely packed seminiferous tubules of varying sizes and shapes. Spermatogenesis is at all levels in all the tubules. The interstitial cells of leydig are present but not prominent.

In the hypothyroid rats (Figure 2) there is arrest of spermatogenesis in the majority of the tubules. The outcome is oligospermia and the chances of fertilizing an ovum to initiate life are very slim. We also observed that the testicular / body weight ratio (Table 1) was significantly ($P < 0.01$) higher in hypothyroidism than in age-matched euthyroid controls. These big-for-nothing testes are a primary cause of male infertility (Amadi, Adeniyi, Nwana and Otubu, 1996).
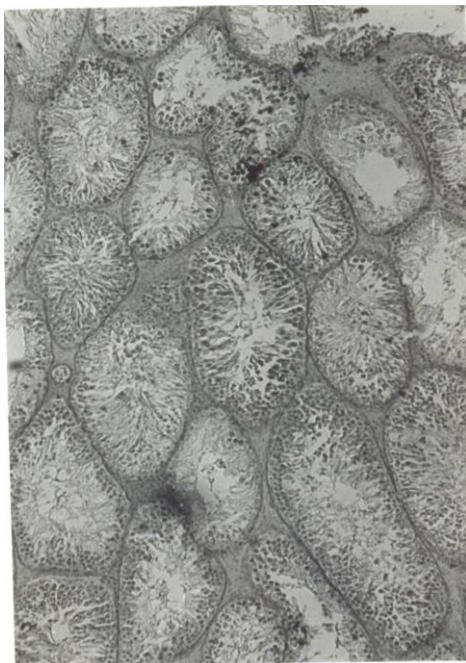

Figure 1: Testis of Control Rats
- Numerous seminiferous tubules. Spermatogenesis at all levels.
- Interstitial cell of Leydig present. (Amadi, Adeniyi, Nwana, Otubu 1996).

Figure 2: Testis of Thyroidectomized Rat.
  The photomicrograph shows
- Arrest of spermatogenesis in the majority of the tubules.
- Spermatogenesis in some tubules up to secondary spermatid levels.
- Interstitial cells of leydig absent. (Amadi, Sabo and Sagay, 2006)

Figure 3: Testis of TTT reveals:
- Several seminiferous tubules of varying sizes and shapes
- Spermatogenesis approaching control
- Spermatogenesis at all levels in majority of tubules.
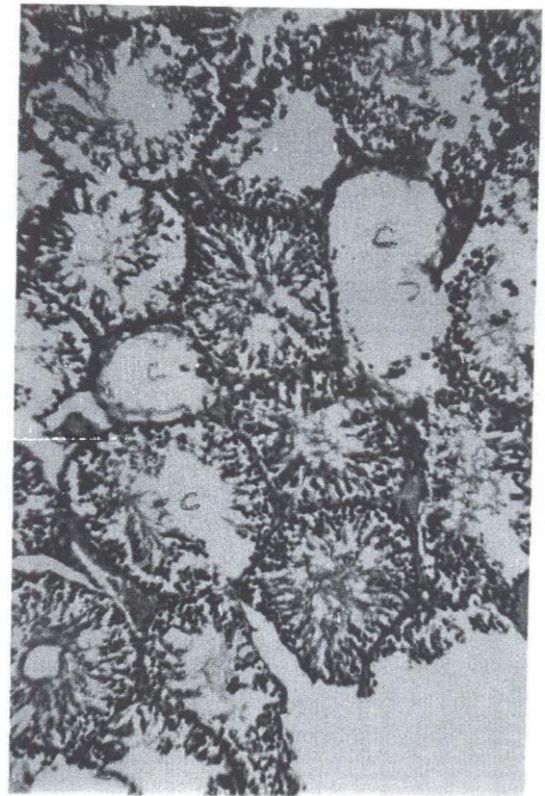 (Amadi, Sabo and Sagay, 2006)

Figure 4: Testis of Thyroxine-treated Rat.
- Beyond 60 days thickening of basement membrane.
- Spermatogenesis to spermatid level.
- Presence of early calcification. (C)
  Orig. mag. X 400.
  (Amadi, Sabo and Sagay, 2006)

CONTRACTILITY OF THE REPRODUCTIVE TRACT
The Role of Thyroid Hormone as the Primary Trigger of Reproductive Tract Contractility
In consummation of their anatomic cum physiological destiny, the oviduct, the myometrium (uterus) and the vas deferens (ductus deferens) undergo contractile activity. The uterus for instance is the organ of gestation which receives and holds the fertilized ovum during the development of the foetus and becomes the principal agent in its expulsion during parturition.

The oviduct or fallopian tube or uterine tube is the duct serving to transmit the ovum from the ovary to the exterior or fertilized egg to an organ as the uterus.

The vas deferens / ductus deferens is the portion of the excretory duct system of the testis, which runs from the epididymal duct to the ejaculatory duct. Synonym "the exclusive motor way of the spermatozoon". Llewelly –Jones (1986) had reported that the uterine muscle is never completely relaxed. Even, in between contractions a resting tone of 6 to 12 mmHg is found. Some factors have been documented to directly or indirectly influence the contractility of the reproductive tract. These could be classified as spontaneous contractility, electrical stimulation, prostaglandins, other hormones and drugs.

The smooth muscle of all visceral organs is characterized by instability of its membrane potential by showing continuous irregular contractions independent of its nerve supply Ganong (2009).

Garfield *et al.*,(1978) had reported that hormonal regulation could play an essential role in alteration of Ca^{2+} transport, in myometrial cells, Amadi *et al.*,(2005) observed a dependence of Ca^{2+} on thyroid hormone for the regulation of cellular functions especially uterine contraction.

The series of events leading to the removal of inhibition and thus activation of uterine smooth muscle prior to or during parturition remained undetermined. No mechanism either was established of events occurring in muscle that account for this change until Amadi, Sabo and Sagay came on stage in 2006; to decode the process. We investigated isolated non-pregnant uterus, the oviduct and the vas deferens of two hundred Wistar albino rats and fifty female rabbits animal models in different thyroid regimens. Isometric contractions were monitored via a force-displacement transducer (Grass FT 03) and recorded on a Grass Polygraph Model 7D, Grass instrument Quincy MA) (Courtesy Professor JAM Otubu's personal polygraph).

This passive force was found to give optimal active tension in length-tension experiments because an isolated tissue under these conditions show minimal spontaneous activity (Hollingsworth, 1974). Contractile activity was monitored for 15 minutes each time with fresh oxygenated buffer at 36.7^0C for all the thyroid states. Dose-response curves of some drugs used on the reproductive tract: such as oxytoxin, acetylcholine and prostaglandin E_2 were obtained also. Figures 1, 2, 3, 4, 5, 6, 7, 8. Although Lewelly-Jones (1986) had reported that uterine muscle is never completely relaxed, the findings of Amadi *et al.*,(2006) suggest that the hypothyroid uterus is completely relaxed and did not show any spontaneous contraction even with electrical stimulation. Oxytoxin (ocin), Acetylcholine (Ach) or prostaglandin – E_2 (PGE_2) – induced simulations did not elicit appreciable contractions either. The contractions were significantly (p < 0.001) diminished or totally inhibited in all cases when compared with other models.
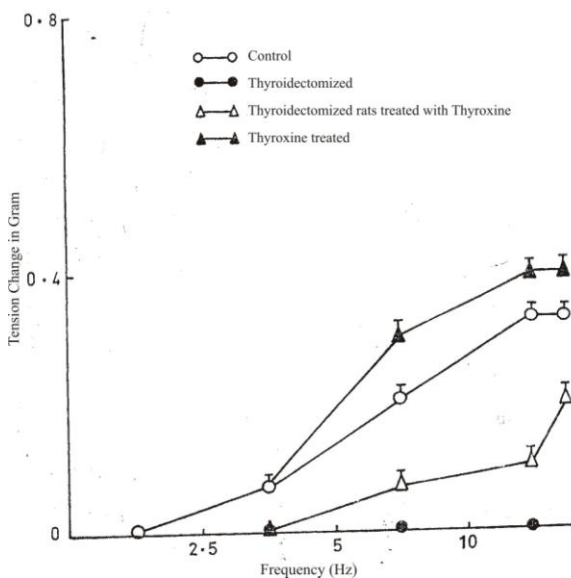

Figure 1: Spontaneous Contractility of Oviduct
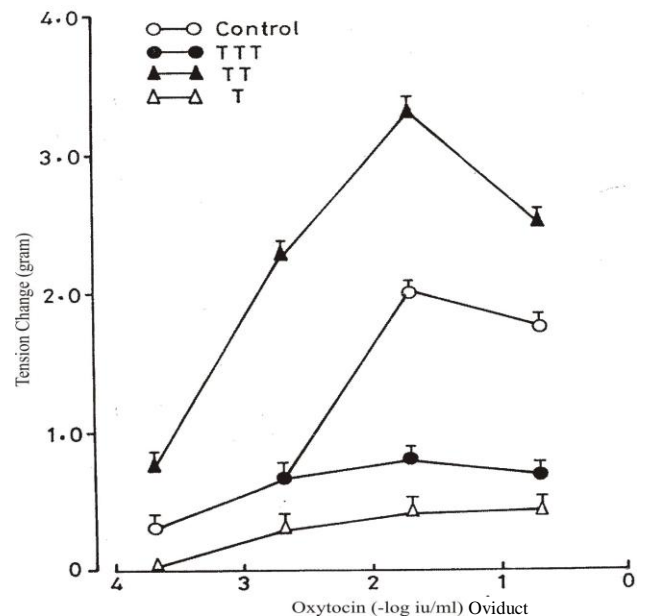
(Amadi, Sabo and Sagay, 2006)

Figure 2: Oviductal Contraction of Various Thyroid Models in Oxytocin
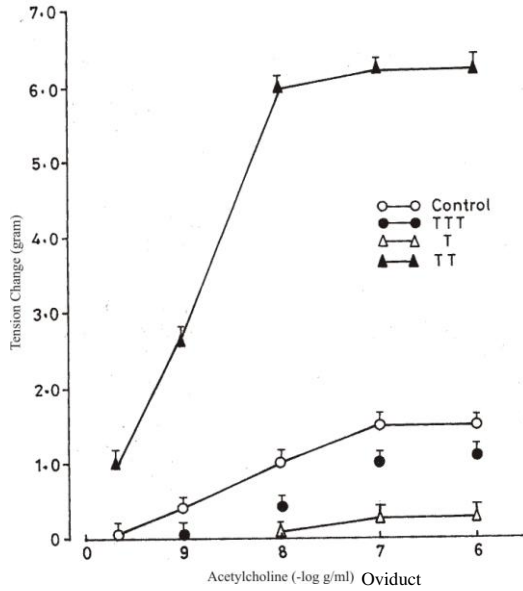
(Amadi, Sabo and Sagay, 2006)

Figure 3: Responses of Oviduct to Ach Figure
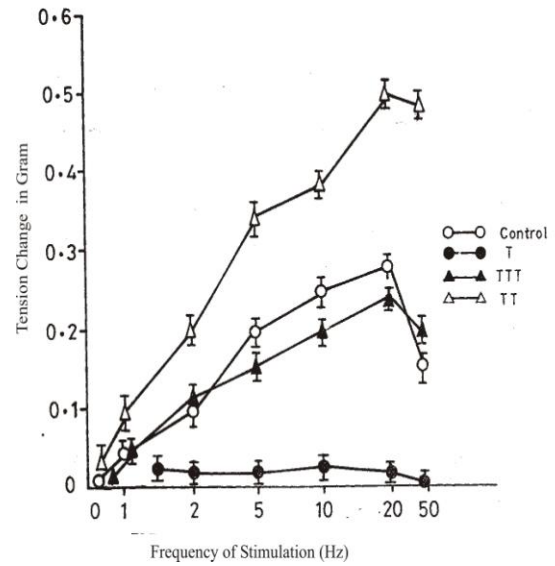
(Amadi, Sabo and Sagay, 2006)

Figure 4: Intrinsic Contractility of Myometrium in Various Thyroid Regimes.

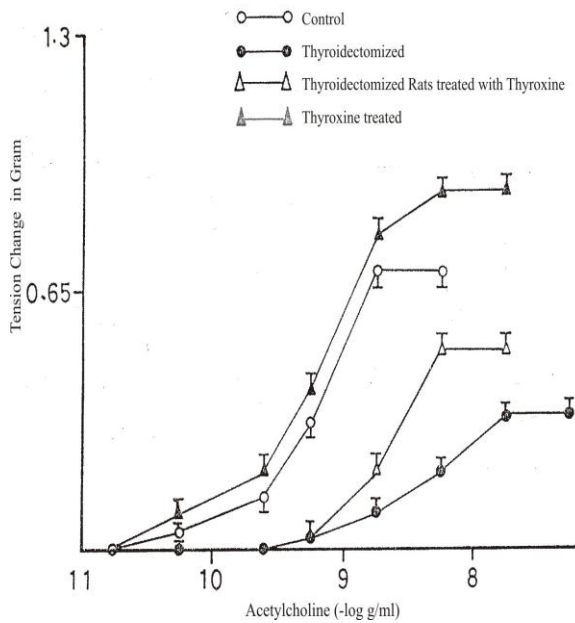(Amadi, Sabo and Sagay, 2006)

Figure 5: Uterine Contractile Responses to Ach.
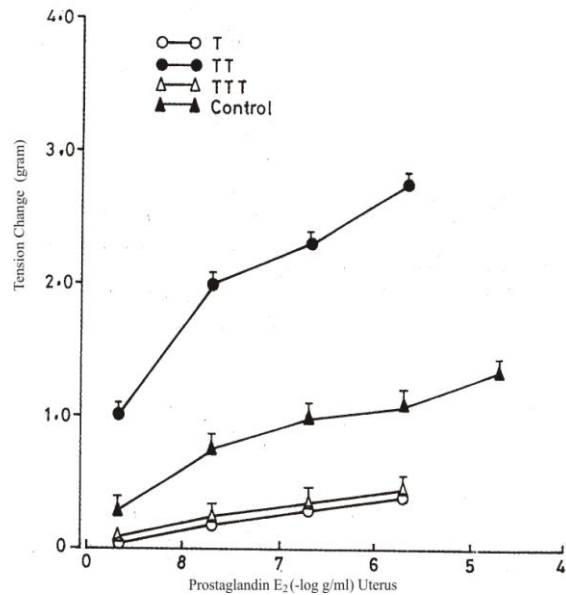
(Amadi, Sabo and Sagay, 2006)

Figure 6: Contractile Responses of the Myometrium to Prostaglandin E_2 (PGE_2)
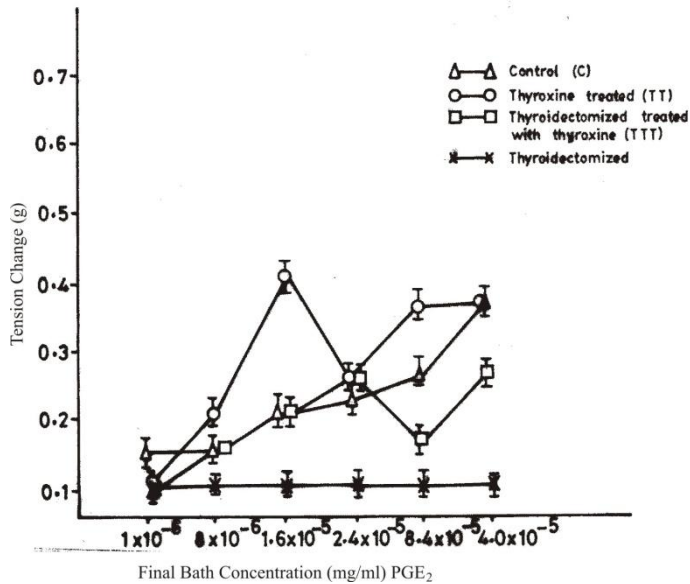
(Amadi, Sabo and Sagay, 2006)

Figure 7: Responses of Vasa Deferentia in Various Thyroid States to PGE2 Following Inhibition of EDRF (Nitric Oxide) & Perfusion in Ca^{2+}- free Physiology Salt Solution.
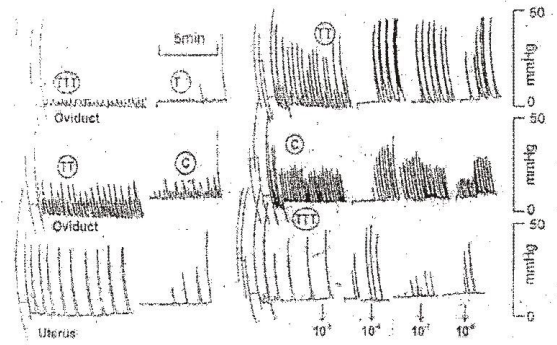
(Amadi, Sabo and Sagay, 2006)

Figure 8: Spontaneous Contractility of Progesterone-treated Oviduct in: Control (C): Thyroidectomized (T): Thyroidectomized-Treated with Thyroxine (TTT) and Thyroxine-Treated (TTT)
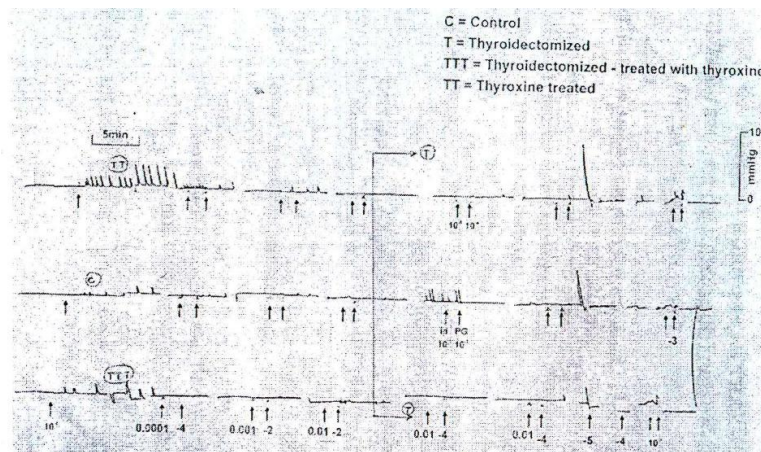

Figure 9 : Oestrogen Response to Electrical Stimulation in Thyroidectomy and Thyroxine iμ/ml

Thyroidectomy (hypothyroidism) appears to interfere with the membrane potential or tone of the smooth muscle of the reproductive tract, by not eliciting action potential and hence no contraction vis-à-vis the observation by Ganong (1993) that the smooth muscle of all visceral organs is characterized by the instability of its membrane potential, from the fact that it shows irregular contractions. Ruegg (1986), Siegman (1987) had it that prostaglandins either directly or by potentiating oxytocin, lower the threshold of the excitation wave to pass from fibril to fibril so that a contraction of the whole muscle fibre and adjacent fibres occurs. Our study (Amadi *et al.,* 2006) however shows that absence of thyroid hormone suggested muscular atony by lack of contraction. Goldstone and Ford (1998) have shown that hypothyroidism leads to muscular rigidity. Bird and Robertson (1976) had earlier reported that exogenous oxytocin induces uterine contraction. However the fact that hypophysectomized women go into labour normally argues against oxytocin being of importance in its initiation (Ishikawa and Fuchs, 1978). Another

16

groupof workers Tasarik *et al.,*(1990) found that progesterone reduced uterine contractility by hyperpolarizing the myometrial cell. They also observed that the effects of oestrogens on uterine contractility are controversial but that circulating oestrogen modifies the action of progesterone. We have shown that neither oestrogen nor progesterone produced any contraction in thyriodectomized (hypothyroid) group (Figure 9 & 10), but treatment with thyroxine elicited robust contractions in all the parameters studied. Thyroid hormone appears to activate the Ca^{2+} - oxytocin- prostaglandin pathway which leads to uterine and vas deferens contraction.Prostaglandinsstimulate contractions of the myometrium (Tasarik *et al.,*1990) and mediate the stimulatory effect of thyroid simulating hormone (TSH) on thyroid adenylate cyclase (Landan *et al.,*1993) or conversely act in a negative feed back directly on adenylate cyclase. It is found that PGE_2 stimulates myometrial contractions by causing Ca^{2+} secretion and movement in situ. Both actions depend on serum thyroxine levels (Amadi, Nwana and Otubu 1999). Oxytocin is also known to bind to receptors and induce the release of prostaglandins that potentiate "oxytocin –induced" uterine contractions (Riehl, 1981; Olsen *et al.,* 1990). Oxytocin was not however found to have an effect on PG production in non-pregnant rat uterus as shown by the hypothyroid group but in thyroid hormone medium. (Amadi, Nwana and Otubu, 1999). Our findings tag thyroid hormone the primary trigger for reproductive tract contraction via Ca^{2+} activation and mobilization by activating the Ca^{2+}-Oxytocin-PG pathway. We thus recommend that thyroid hormone level should be monitored as a therapeutic base in the administration of drugs that stimulate the contraction of the reproductive tract.

Figure 1: shows the magnitude of thyroxine- induced phasic contraction during calcium loading; Table 1: Hormone & Ca^{2+} levels.(above) Table 2: Haemostatic parameter in different thyroid hormone states.
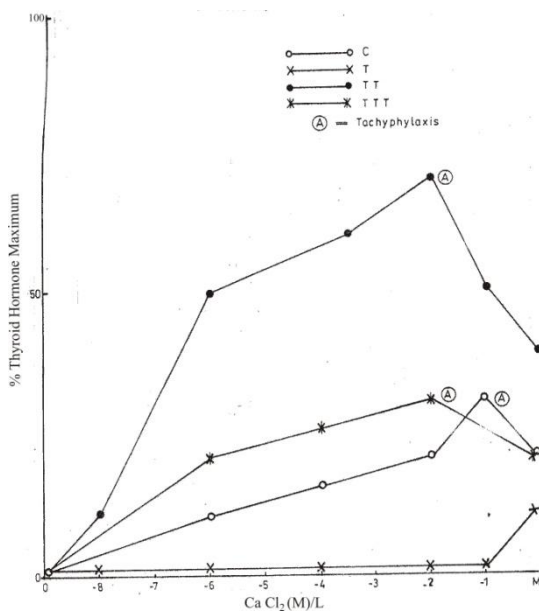

Figure 1: Magnitude of T$_4$ -induced Phasic Contractions During Ca^{2+} -Loading
        Amadi, Sabo, Adelaiye & Sagay, 2005)

Table 2: Effect of Thyroidectomy and Thyroxine on Haemastatic Parameters.

Parameter (Mean ± SEM)	Thyroxine - Treated (TT)	Thyroidectomized (T)	Thyroidectomized Treated with Thyroxine (TTT)	Control (C)
BT (min)	*2.6 ± 0.60	**8.6 ± 0.28	4.8 ± 0.41	4.2 ± 0.14
CT (min)	3.2 ± 0.25	**8.0 ± 38	5.8 ± 0.26	5.4 ± 0.28
PT (Sec)	5.8 ± 0.45	**10.6 ± 1.30	6.4 ± 1.20	7.2 ± 0.35

BT = Bleeding time, CT = Clothing time, PT = Prothrombine time, N = 10 (Total number of Animals).
* = $P < 0.05$ in comparison with control, ** = $P < 0.001$ in comparison with control.
Amadi, Sabo, Adelaiye & Sagay, 2005)

It is thus the activation of Ca^{2+} regulated by the level of thyroid hormones that modulates cellular activity. It is known that calcium channel activation rather than the associated increase in Ca^{2+} concentrations is the key factor regulating secretion (Nemeth and Scrapa, 1986).

We have shown from our work titled "Dependence of Ca^{2+} Ion Receptors on Thyroid Hormone for the Regulation of Cellular Functions/ Thyroid Hormones Activate Ca^{2+}- Receptors" (Amadi *et al.,*2005) that thyroid hormone is a calcimimetic which could provide antiresorptive therapy by inhibiting calcium loss. Thyroid hormone thus targets calcium receptors in cells such as smooth muscle of the reproductive tract and red blood cells (the fluid of life) and might provide novel therapies for some diseases. It might find utility in bone and mineral disorders, especially if it is counter-balanced by an appropriate calcilytic drug or agent.

Thyroid Hormone on Contractility of the Vas Deferens and Modulation of Erectile Function

We studied one hundred adult male Wistar albino rat models of different thyroid hormone status viz: (1) Euthyroid controls (2) Hypothyroid (3) Hypothyroid with Thyroxine replacement and (4) Euthyroid with thyroxine treatment. All exogenous thyroid hormone treatment was at physiological doses (10µg/Kg body wt).

Spontaneous Contraction of Vas Deferens

Hypothyroid state (induced by thyroidectomy) caused a significant decrease in the frequency of contraction of the vasa differentia when compared with the contraction obtained for the other groups (Figure 1).
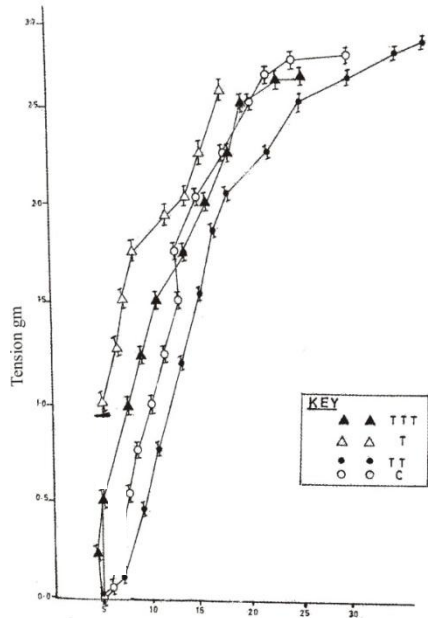
Figure 1: Spontaneous contractions of Vasa Deferentia in Various States: (i) euthyroid (control); (ii) thyroidectomized (T); (iii) thyroidectomized treated with thyroxine (TTT), (iv) thyroxine-treated (TT).

Mean ± ISD frequency of contraction (Beats /min) of Vasa Deferentia n = 5 rats in each group.
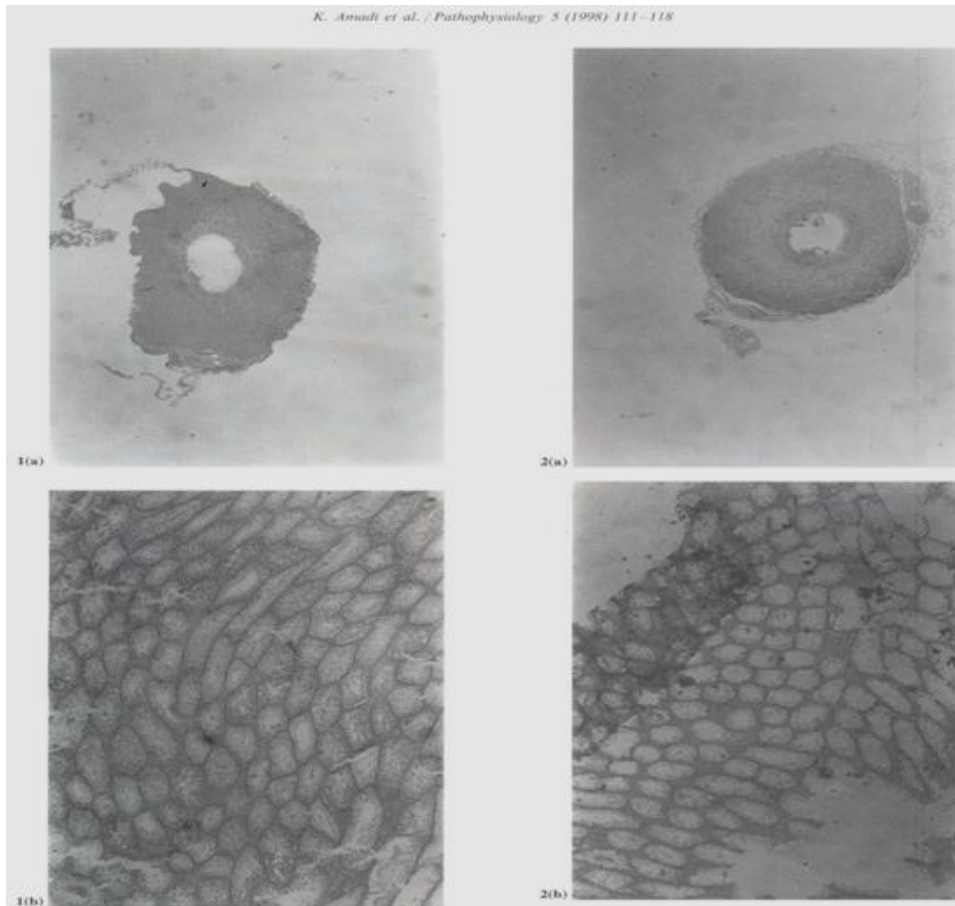
Plate 1: Photomicrographs of (a) Cross-section of ductus deferens from control rats (x 400). (b) Cross-section of epididymis from control rats (x 400).

Plate 2: Photomicrographs of(a) Cross-section of ductus deferens from thyroidectomized rats (x 400). (b) Cross-section of epididymis from thyroidectomized (x 400).
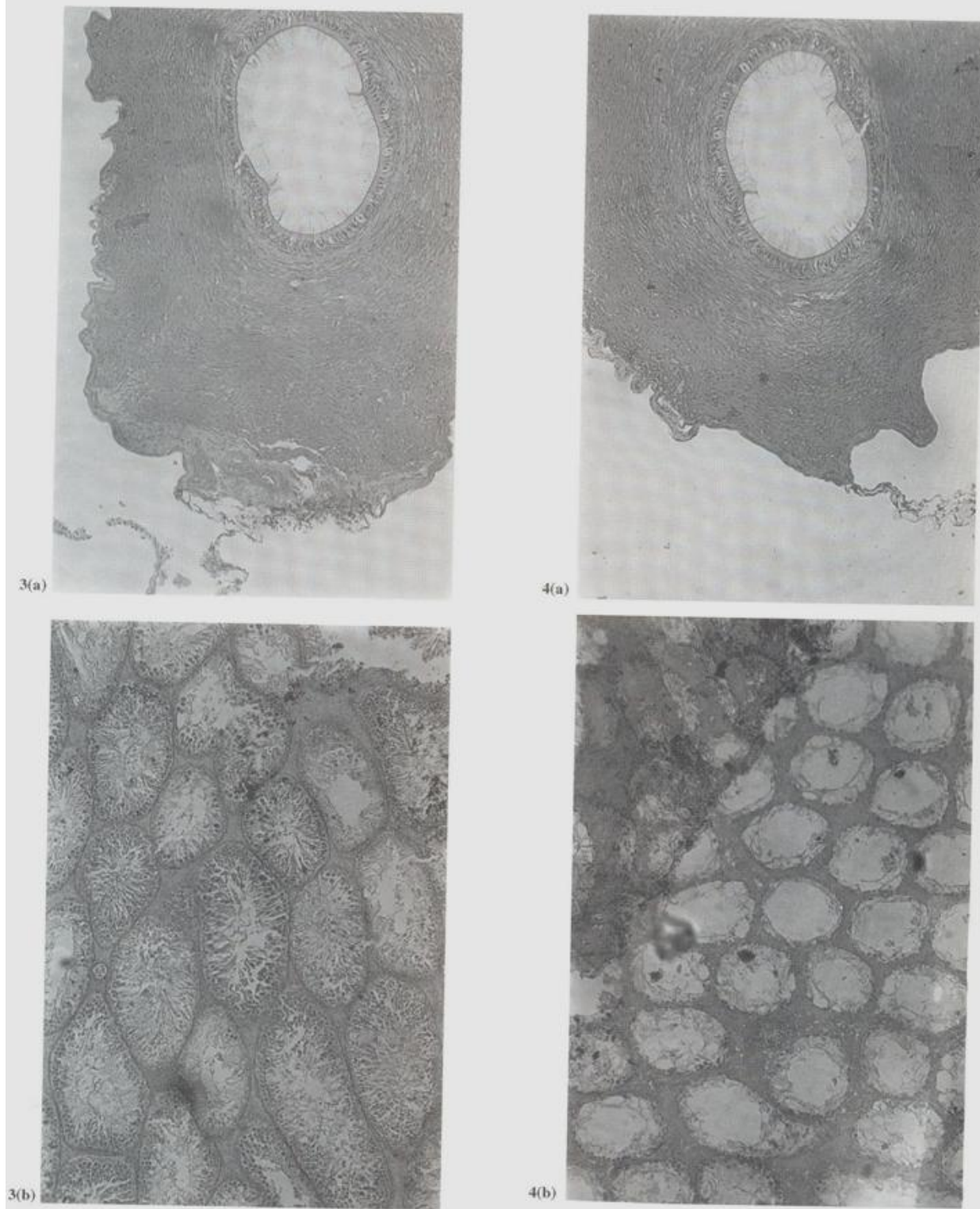
Plate 3: Photomicrographs of (a) Cross-section of ductus deferens from thyroidectomized rats treated with thyroxine (x 400). (b) Cross-section of epididymis from thyroidectomized rats treated with thyroxine (x 400).

Plate 4: Photomicrographs of (a) Cross-section of ductus deferens from thyroxine-treated rats (x 400). (b) Cross-section of epididymis from thyroxine- treated rats (x 400).

This group of rats also developed the least maximum response (P < 0.01) to stimulation and the tension at which they started to contract was also significantly (p < 0.01) higher than the others. Plates 1 (a, b) and 3 (a, b) show clearly that the morphological features of the smooth muscle layers, the epithelium and epididymis are well defined in control and hypothyroid with subsequent thyroid hormone replacement. In the hypothyroids, however there was early irregularity of folding of the epithelium (Fig 2a) the folding are known as "bumps" which cause irregularly irregular sperm motility thereby changing their progressive vectorial motility into a scalar movement that is not favourable for the fertilization of the approaching ovum, (Amadi, Adeniyi, Nwana and Otubu, 1998) needless to say that this is a factor in male infertility; and by extension lack of sexual vigour.

Thyroid Hormone: The Modulator of Erectile Function

Erectile dysfunction is a known cause of impotence, abnormal sperm count, its morphology and thus infertility in the male. Padma -Nathan $et\ al.,$(2001) found that erectile dysfunction is associated with behavioural risk factors like cigarette smoothing, excessive intake of alcohol and other age-related medical conditions. Rajfer $et\ al.,$(1992) have reported phosphodiesterase-type 5 (PDE_5) to be a critical component of the Nitric oxide -cyclic Guanidine Monophosphate (NO-CGMP) signilling pathway responsible for the modulation of smooth muscle tone in the penis. The authors noted that inhibition of PDE_5 magnifies the NO-mediated response to sexual stimulation, increasing intracelullar concentrations of cGMP and thus maintaining penile erection. Sildnenafil, an inhibitor of PDE_5 is an effective treatment for erectile dysfunction (Padma-Nathan $et\ al.,$1998; Goldstein $et\ al.,$1998; Dinsmore $et\ al.,$1999). The inhibition of PDE_5 would therefore be expected to result in improved contractile force, Prostaglandin E_1 (PGE_I) solutions for intravenous injection therapy have been reported to be superior to or at least equal in efficacy when compared to other erectogenic agents (Lea, Bryson and Baffour, 1996). The problem of erectile dysfunction is still prevalent despite attempts by scientists to find a total cure for it. The question arises whether the problem could be due to enzyme or hormonal malfunction, its imbalance or deficiency. We took a leap on thyroid hormone (T_4) for a possible intervention in the erectile or contractile activity of the rabbit corpus cavernosum treated with PGEI and with sildenafil after inhibiting EDRF. It has been stated that the most important chemical mediator of cavernosal relaxation is Nitric oxide (NO) Endothelial derived relaxation factor (EDRF) released directly from nonadrenergic, non cholinergic (purinergic) nerve endings and from endothelium (Utkan $et\ al.,$ 2001).

Forty sexually mature male New Zealand Rabbits of four thyroid status, were used, as applied by Utkan $et\ al.,$(2001). The corpora cavernosa tissue comprising four strips of corpus cavernosum smooth muscle of each rabbit, euthanized by stunning, were dissected out of the penis, having made a ventral incision on the right corpora, to dissect the tunica and strips of the corpus cavernosum tissue measuring 2 x 2 x 15mm were studied under standard organ bath conditions. The strips except hypothyroid group were exposed for 15 minutes to 10M- NG Monomethyl- L-arginine (L-NMMA) a specific inhibitor or Nitric oxide (NO) synthesis as applied by Ebeigbe and Aloamaka (1999).

This endothelium –derived relaxing factor (NO) has been reported to regulate vascular and smooth muscle tone (Kin $et\ al.,$1995, Lot, 1993).

The strips were then subjected in groups inturn to graded doses of PGE_I and Sildenafil ($10^{-6} - 3$ x 10^{-4}M) and contractile responses recorded as shown in Figures 1 and 2 which highlight the

following: thyroid hormone or thyroxine treatment produced robust, constrictoractions without preconstriction after de-endotheliazation, suggesting thyroid hormone as an endogenous excitatory factor mediating constrictor actions of arginine analogues in vessels and corpus cavernosum smooth muscle. This was strongly indicated by the non-response of PGE_I in hypothyroidism. Both thyroxine treatment regimes exhibited identical response as control at a concentration of 2.4 x 10-5 M/L. This tachyphylaxis thus prescribes the maximum beneficial dose in thyroid hormone treatments for erectile function. A similar safety margin is shown in figure 2 in Sildernafil treatment. Contraction in hypothyroidism on the contrary was just lifted from the base line, depicting Sildernafil action to be dependent on thyroid hormone level in the vicinity of the erectile tissue of the penis (Amadi, Sabo and Sagay 2006).

Let me emphasize interesting findings that thyroid hormone repairs erectile dysfunction. In hypothyroidism or thyroidectomy there was no contraction of the vas deferens in PGE_I despite inhibition of Endothelium Derived Relaxation Factor (EDRF) by 10M NGL - NMMA. There was delayed but feeble short-lived contraction or increase in tone of the corpus cavernosum, the inhibition of PDE_5 by Sildernafil not withstanding. Inspite of these inhibitions, thyroid hormone treatment in the affected groups gave robust contractions proportionate to the concentration of PGE_I and Sildenafil respectively. Work on prostaglandin E_2 (PGE_2), an analogue of PGE_I has shown its contraction of the vas deferens smooth muscle to be dependent on the thyroid hormone status of the animal (Amadi *et al.,* 1996, 1999). May I submit as follows:

1) The Muscle tone produced by PGE_I in thyroxine treatment and the lack of contraction in thyriodectomy suggest that thyroxine is implicated in the erectogenic activity of PGE_I.
2) It would also appear that the endogenous excitatory factor mediating the constrictor actions of arginine analogues and PGE_I is thyroid hormone.
3) It is note worthy that the responses in the thyroidectomized group with subsequent thyroid hormone replacement did not quite attain the control values let me remind you that this was probably because, after removal of the thyroid gland, there was bound to be contraction hysteresis before thyroxine rehabilitation took effect within the time of study. The group had to depend on the secondary organs like salivary glands, liver, intestine, gastric mucosa and testis for trapping iodide for T_4 formation.

In hypothyroidism, the non contractile responses of the erectile tissue neither treated with EDRF inhibitor nor with Sildenafil, an inhibitor of PDE_5, were similar to response after the inhibitions. That would imply that hypothyroidism depletes EDRF and thus inhibits the Nitric oxide- cGMP pathway or potentiates PDE_5 action. Sildenafil did not also produce increase in tone or tension of the hypothyroid rabbits. Thyroxine on the other hand inhibits PDE_5 to activate the NO-cGMP 2nd messenger probably, involving the thyroid-adenyl cyclase by linking to the regulatory G-protein. This means that the effectiveness of Sildenafil in inhibiting PDE_5 is dependent on thyroid hormone. (**Figure 1 & 2**)
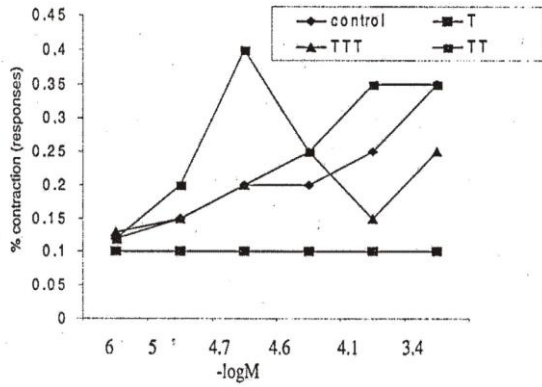
Figure 1: Responses of Vasa deferentia in various thyroid states to PGE1 following inhibition of Nitric oxide.
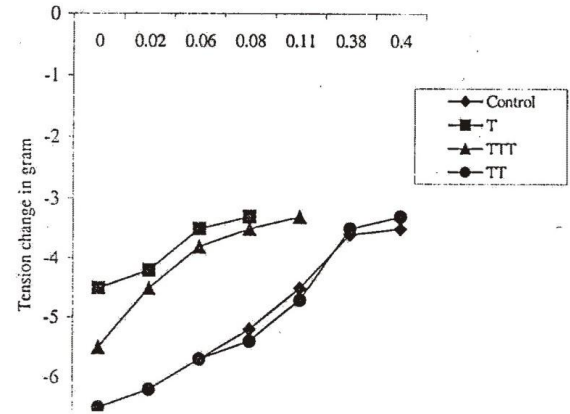(Amadi, Sabo & Sagay 2006)

Figure 2: Responses of erectile tissue of rabbit penis to sildenafil.
(Amadi, Sabo & Sagay 2006)

As penile erection is a haemodynamic process involving the relaxation of smooth muscle of corpus cavernosum; depletion of EDRF by hypothyroidism factored into the feeble contraction or non relaxation of the muscle due to atony. (Amadi *et al.,* 1999). The lack of or low tone prevents increased flow of blood into the trabecular spaces of the corpora covernosa, which (inflow) should increase both the vascular and muscle tone to make the penis turgid and with enough buckling pressure for "action". This fact was buttressed by lower blood cell count or quantity of blood flow (rheology) in hypothyroid animals (Table 3).Hypothyroidismis observed to be a factor in erectile dysfunction through mechanism of de-endothelization or depletion of EDRF. Thyroid hormone on the contrary repairs erectile dysfunction.

Table 3: Haematocrit, Red Blood Cell Count and Mean Arterial Pressure of Experimental Rabbit Mean X SEM

Experimental Animals	Haematocrit (%)				Red Blood Cell (x 106) mm^{-3}				Mean Arterial Pressure (mm11$_2$)			
	C	T	TTT	TT	C	T	TTT	TT	C	T	TTT	TT
1	41	34	40	42	5.4	2.2	4.8	6.5	20	15	21	22
2	40	25	38	40	4.9	3.4	5.0	7.0	2.5	14	19	20
3	42	30	40	41	5.0	4.0	5.8	20	20	15	20	21
4	39	38	41	42	5.2	3.0	5.0	6.0	20	16	16	20
5	45	32	14	43	4.8	4.5	4.5	6.8	20	15	15	25
X	41.4 ±1.0	29.8± 1.57	*40.0 ± 0.55	41.4 ± 0.51	5.06 ± 0.10	*3.4 ± 0.28	4.76 ± 0.11	6.42 ± 0.23	21.01 ± 0.01	15.01 ± 0.10	18.2 ± 0.01	21.6 ± 0.02

C = Control: T = Thyroidectomized; TTT = Thyroidectomized-treated with thyroxine, TT = Thyroxine-treated Number of animals in parenthesis. CV*T, P < 0.001 for Haematocrit, CV*T & P<0.001 for RBC V* = Compared with, P<0.05. (n = 5)

POSTPARTUM HAEMORRHAGE: A Thyroid Hormone Gate Keeping?

Post-partum haemorrhage is not an infrequent cause of death in Nigeria. The trafficking across the placenta, of the most abundant serum antibody IgG, an essential component of humoral immune response has been described as a catabolism -β_2 cross-talk (Brambel *et al.,*1992). IgG homeostasis has not been established for certain. Based on our earlier work on hypothyroid subjects that presented with prolonged bleeding, clotting and prothrombin times (Amadi *et al.,*2005) we investigated the possibility of thyroid hormone being a factor in post-partum haemorrhage (PPH). Having obtained ethical clearance and informed consent of fifty pregnant mothers, ex-vivo placental models of different thyroid states were taken as well as maternal and cord blood, within five minutes post partum. The blood, microvasculature and endothelial cells were "washed" and pulse-chased in a medium containing unlabelled IgG for varying times of 0 – 40 minutes at 37^0C in physiological saline at varying pH 6.0 – 7.2. The amount of IgG trafficked was evaluated by immunohistochemical staining with anti-FEA$_I$ an endosomal marker, of human leucocyte endothelial cells (HULEC- 5A) which were stained with this marker. (British Drug House, Poole, England),which tags to neonatal constant fraction receptor (FcRn) or IgG receptors.

The IgG density (IgGρ) in PPH was significantly (P < 0.01) deficient in the sera assayed hypothyroid while the mean physiological plasma thyroxine (T$_4$) level was found to be 57 ± 0.05

ng/ml of serum in 48 of the 50 cases studied, 2 or 4% of the cases that had PPH exhibited T_4 levels below the threshold of 2.5 – 5ng/ml or mean of 2.3 ±0.8ng/ml which "opens the gate" to postpartum haemorrhage (Amadi 2004- Ph.D Thesis submitted to the University of Jos).

The findings suggested two major thoughts to reproductive physiology and to her daughter: Obstetrics & Gynaecology:

1) Thyroid hormones as gate keepers to inhibit PPH.
2) The hormones as a factor in IgG trafficking which now appears to be a catabolism -β_2-Thyroid Hormone tripartite Cross-talk (Amadi *et al.,*2013).

This could have some clinical applications by including thyroid hormone screening in ante-natal clinics in evaluation of impairment of IgG trafficking across the placenta; for application of therapeutic antibodies for passive immunization and targeted immunotherapy.

The Influence of Thyroid Hormones on IgGρ and APGAR Score
Our series of studies looked into the level of serum thyroid hormone in relation to IgG population and APGAR Score of the neonate. APGAR score or rating is s qualitative estimate of the condition of an infant 1- 5 minutes post partum. It is derived by assigning points: 0, 1, 2 to the quality of heart rate, respiratory efforts or crying power, colour of the skin, muscle tone and reflexes and expressed as the sum of these points; the best score being 10 (Blakiston, 1979). Several neonates respond severally differently to these parameters. I have discussed earlier the role of IgG in immunity. Let me add that the human foetus receives a passive immunization by the selective passage across the placenta of maternal IgG (Gitlin *et al.,*1964). These workers reported that other immune globulins are not transplacental Brambel *et al.,*(1960) had earlier reported the active transpacental transport which seems to be controlled by the maternal level of IgG that thus stabilizes the foetal IgG level (Gitlin, 1971). Muralts (1975) however suggested that all four IgG subgroups can cross freely the placental barrier.

Earlier work in our laboratory had implicated hypothyroidism with depressed muscle tone but the hormone switches on Ca^{2+} receptors for vigorous contractions (Amadi, Nwana and Otubu, 2007). We therefore investigated if there could be any relationship between IgGρ and muscle tone which is a parameter in APGAR scoring in euthyroid and hypothyroid states. In this study ex-vivo term placenta, maternal and cord blood have been used to analyze the materno- foetal transfer of IgGρ in maternal and cord blood by tagging the antibody with fluorescence dye and comparing the populations with APGAR score of the neonates Table 1, 2a & 2b; 3a & 3b and Figures /Plates 1, 2 and 3. Our findings were that the IgGρ increased in umbilical cord blood in euthyroid women; but a sharp decline (P < 0.01) in hypothyroid mothers irrespective of mode of delivery, barring complications. It was however noted that the hypothyroid mothers had protracted dystocia and one of them had postpartum haemorrage that was arrested by the obstetrician with anticoagulants, antibiotics and uterine curettage. The PPH was due to the retention of some products of conceptus consequent on sluggish myometrial contraction because "an empty contracting, uninjured uterus does not bleed" (Sagay 2008) personal communications.

These findings appear reasonable since it is generally assumed that the pressure of the uterine contractions during parturition lead to filtration of IgG into the foetal circulation (Payne, 1969; Cochran 1972; Turmero, 1974). The strong uterine contractions of euthyroid mothers probably

enhanced a high IgG density (IgGρ) filtration into the cord circulation; but the weak contractions of the hypothyroid myometrium would not filter much IgG across the materno-foetal circulation.

The findings in our investigations might justify our suggestion that thyroid hormone screening be incorporated into ante-natal clinics to spare hypothyroid pregnant women of protracted labour and post-partum haemorrhage. Moreover, it directly mirrors the pregnancy outcome with respect to the quality of life of the neonate.

Much as the resistance to the passage of the neonate through the birth canal filters IgG into the foetal circulation, the thyroid hormone level of the mother appears an obvious natural determinant of the neonatal passive immunization (IgGρ) and its APGAR score. (Amadi *et al.*,2013 in press).Onthyroid hormone screening among other factors in antenatal care depends the future of the maternal cum neonatal health and maternal -neonatal health of the future.

Table 1: APGAR Score Model

Parameters	Score		
	0	1	2
i. H ~ Heart Rate	Nil	<100/m	>100/m
ii. A ~ Activity (Reflexes)	Nil	Grimance	Vigorous
iii. R ~ Respiration	Nil	Weak cry	Strong cry
iv. M ~ Muscle Tone	Limp	Slight	Active
v. C ~ Colour of Skin	White	Purple with Blue extremities	Pink all over
Total	**Zero**	**5**	**10**

Table 2a & 2b:

(a) Relationship of IgG Density (IgGρ) of Hypothyroid Mothers to Their Neonates' IgGρ and APGAR Score.

Parameters:	Mothers (n = 5)	Neonates (n = 5)	APGAR Score
IgGρ	0.25	2.5	7
(mg/100ml Blood)	0.50	3.0	8
	0.30	2.0	6
	0.25	2.5	7
	0.25	2.5	7
$\overline{X} \pm$ SEM	0.76 ± 0.44	2.5 ± 0.16	7

(b)

Maternal serum T_4 Level ng/dL
0.6
0.6
0.5
0.8
0.5
$\overline{X} \pm$ SEM $0.6 + 0.05$

T_4 = Thyroid hormone in serum.
(Amadi *et al.*,2013)

Table 3 a & b:

(a) Relationship of IgG Density (IgGρ) of Euthyroid Mothers to Their Neonates' IgGρ and APGAR Scores.

Parameters:	Mothers (n = 15)	Neonates (n = 15)	APGAR Score
IgGρ	11.0 (n = 5)	10.0	10
(mg/100ml Blood)	10.5 (n = 2)	11.5	7
	11.5 (n = 2)	12.0	10
	10.0 (n = 1)	10.5	8
	10.5 (n = 5)	11.0	10
$\overline{X} \pm SEM$	10.70 ± 0.35	11.0 ± 0.50	9

(b) Thyroid hormone (T_4) Levels in Euthyroid maternal Serum (ng/dL), (n = 10).

(n = 2) 3.8	
(n = 2)4.0	
(n = 2) 3.5	
(n = 2) 3.0	
(n = 2) 2.8	
$\overline{X} \pm SEM$	3.42 + 0.05

(Amadi *et al.*,2013)

Plate1: LS Umbilical cord and Ex - vivo term placenta of euthyroid mother. IgG (reddish patches) more on foetal side (f) than maternal side (m), showing vectorial mobility x4000.
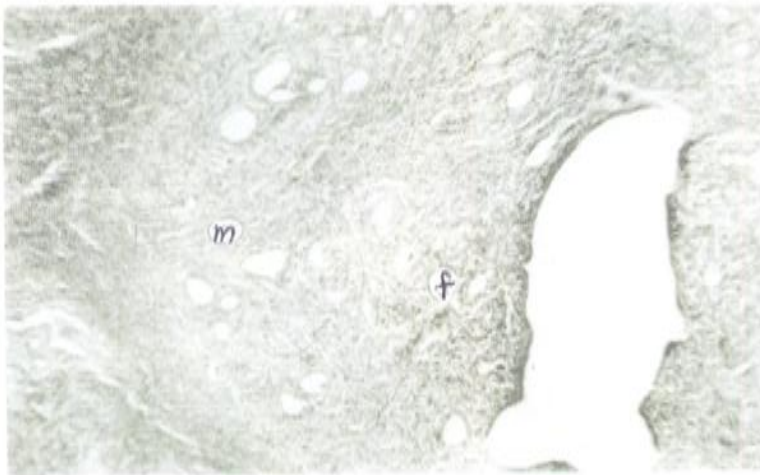

Plate 2: T S placenta of hypothyroid mother showing IgG (fluorescent white spots distributed more or less evenly on both maternal end (m) and foetal side (f) X 4000 showing scalar mobility.
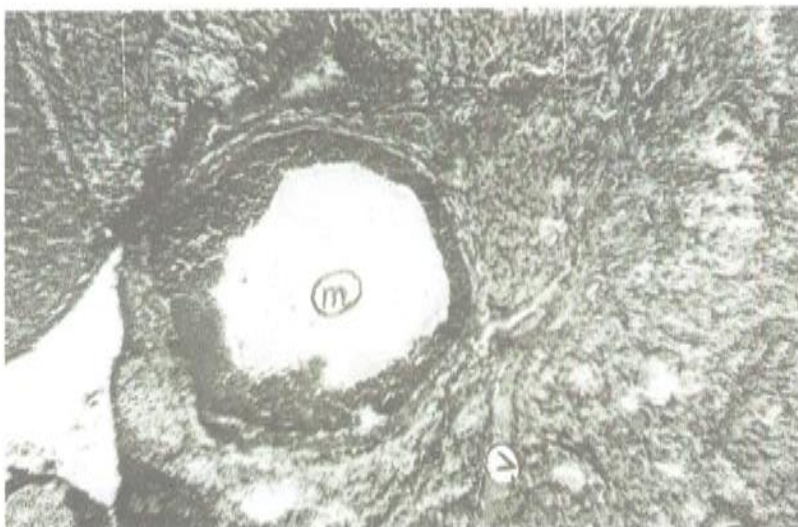

Plate 3: T S Term placenta showing part of vasculature (v) surrounding a central mucous area. (m) x4000.

27

THYROID HORMONE: A "Prime Suspect" in Human Immuno Deficiency Virus (HIV/AIDS) Patients.

Human Immunodeficiency Virus (HIV) infection is pandemic world wide. Researches on seropositive patients have shown that thrombocytopaenia, and severe anaemia manifest as abnormally high erythrocyte sedimentation rate (ESR) (Sullivan 2002). It has been reported that circumcision reduced the incidence of HIV/AIDS infection (World congress on HIV/AIDS, Australia 2007). Beyond circumcision however there might be some constitutional factor that converts HIV infection to clinical AIDS. This study aimed to look at the thyroid hormone status being a possible primary factor that aggravates the disease process in HIV/AIDS. The hormone has been reported to be crucial for optimal immune function (Souba and Smith 1996). It was reported also that hypothyroidism cause oedema of the uterine tube; which (oedema) predisposes the victim to susceptibility to non-specific infections, HIV inclusive (Amadi, Adeniyi, Nwana and Otubu, 1996). Moreover a number of theories have been put forward in an attempt to explain variations in the development of clinical AIDS after an initial HIV infection. These theories include concurrent infection with viruses like Cytomegalo Virus (CMV) and hepatitis B-virus infection.

The study was carried out on two hundred AIDS patients aged between 20 – 40 years. Fifty volunteer healthy subjects who tested negative for HIV or AIDS were used as controls. The informed consent of the patients and subjects were obtained. They were very enthusiastic because of their anxiety to seeing if our research findings could lead to a cure of their predicament. They were matched age to age, duration of detected infection and gender groups. All the males that participated in the investigation were circumcised in early infancy. Thyroid stimulating hormone (TSH), free plasma thyroxine (T_4), Total protein, Albumin, Globulin, immune complex or complement fixation and Bence Jones proteins were assayed from the venous blood /urine samples obtained under aseptic conditions. Thin blood films made from the buffy coat layer and from packed red cells respectively of the samples anticoagulated in 3.8% sodium citrate were examined.

The buffy coat contains lymphocytes also known as CD-4 cells or thymocytes (T-cell) (Souba and Smith 1996).

Table 1 and Figures 1, 2, 3, 4 & 5 summarized the results obtained (Amadi, Sabo, Ogunkeye and Oluwole, 2008).

Table 1: Summary of TSH. T4 Protein, albumin and globulin levels in seronegative and seropositve HIV/AIDS.

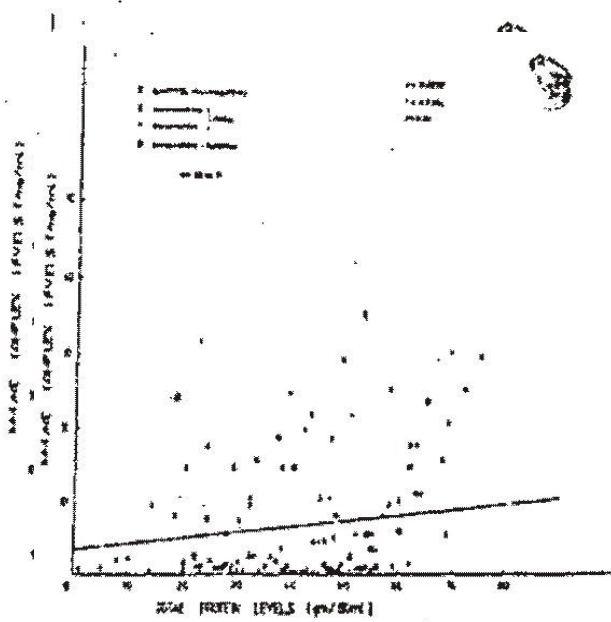| Groups	TSH (μ/ml)	T4 (μg/dl)	Total protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)	CD$_4$ cells (mm^3)	IC$_3$ (mg/ml)
Seronegative	12.1 ± 0.02	8.0 ± 0.05	12.0 ± 0.001 (n = 15)	7.95 ± 0.001 (n = 15)	4.09 ± 0.005 (n = 10)	10,000 ± 8.4	Males 0.74 ± 0.58** (n = 58)
							Females 0.24 ± 0.34** (n = 25)
Seropositive HIV/AIDS patients	30.5 ± 2.01**	2.45 ± 0.54	9.50 ± 0.10** (n = 15)	3.65 ± 0.56* (n = 15)	5.865 ± 0.586* (n = 10)	102 ± 2.8	Males 0.03 ± 0.02 (n = 15)
							Females 1.53 ± 0.744** (n = 25)

Figure 1: Correlation between soluble immune complex levels (mg/ml) and serum total protein levels (g/100ml) in seronegative and seropositive HIV/AIDS
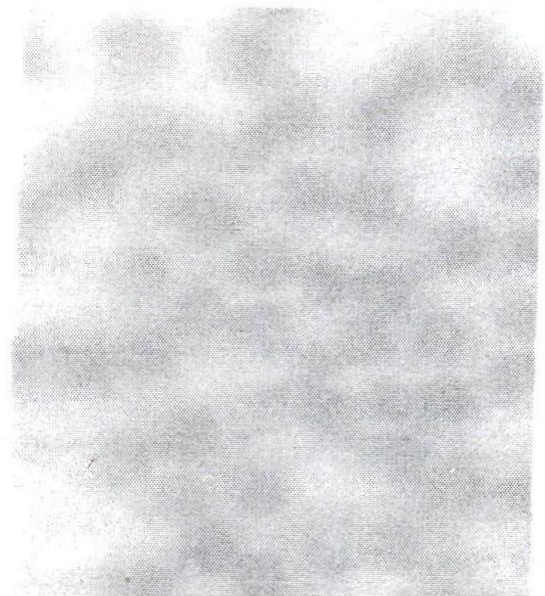

Figure 2: Blood picture of seronegative control. Giemsa stain. Red blood cells appear robust and fully haemoglobinized.
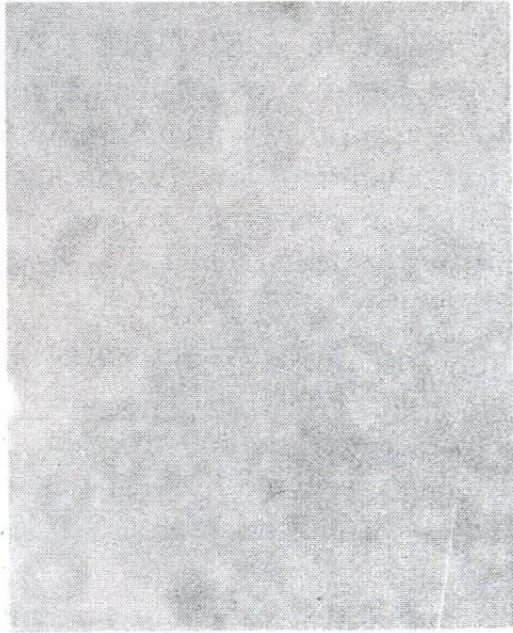
Figure 3: Thymus lymphocytes of seropositive HIV/AIDS patient. Giemsa stain. The lymphocytes appear hollow (ghost cells). Nuclei of the blood corpuscles split into lobules eccentrically marginalized
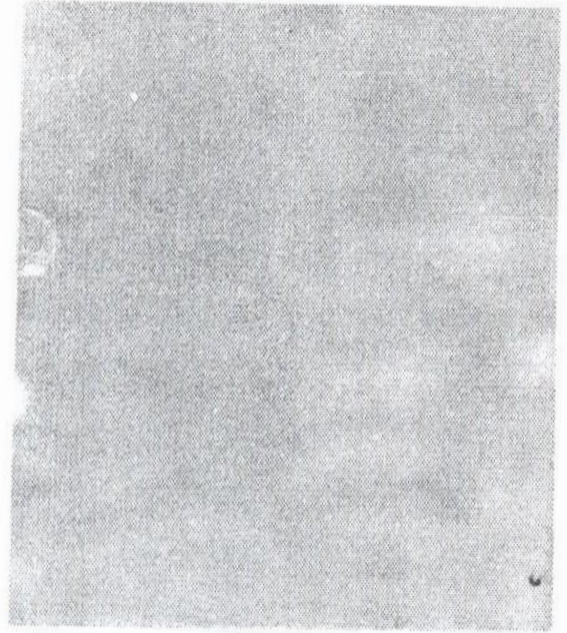

Figure 4: Thymocytes of seronegative control. The nuclei and cell membrane are intact (Giemsa stain).
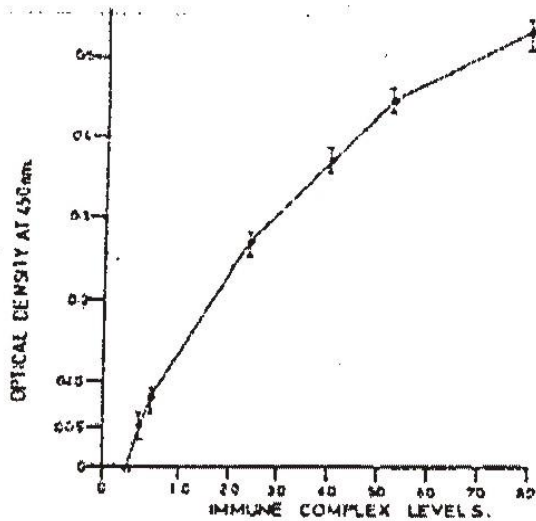

Figure 5: Immune complex aggregating gamma globulin standard curve

Fontes *et al.,*(2003) reported differences in mineralocorticoid or thyroid function among groups of HIV patients. Ketsamathi *et al.,*(2006) reported hypothyroidism in some Thai HIV – infected patients and found a few of their studied population with subclinical hypothyroidism. We found consistent hypothyroidism in the seropositives studied as typified by high thyrotropin (TSH) and low thyroxine (T_4) level. Overt hypothyroidism has been defined as a high TSH and low T_4 levels; subclinical hypothyroidism as a high TSH level (Betran *et al.,*2006).Suchpatients cannot make their own thyroid hormone and thus cannot compensate for the reduced thyroid hormone levels and hence their thyrotropic hormone level (Judith and Harold, 2001). That is to say that the immune system is in a state of regulatory chaos and cannot keep the host healthy and alive.

The significant depletion of T_4 in HIV/AIDS patients suggest that hypothyroid state predisposes the host cells to nonspecific infections; HIV inclusive by the mechanism of transdifferentiation or identity deception (Amadi *et al.,*2008). This could sustain a different cell line progressively depleted of thyroid hormone and vulnerable to viral assault. Annarosa *et al.,*(2005) had reported stem cell transdifferentiation in the adult organism, which is capable of generating mature cells beyond their own tissue boundaries, although they did not explain the causative factor for this phenomenon. From the distortion of T-lymphocytes (Figure 3) in hypothyroid seropositives, the hypothyroid state could be the pedestal for easy transdifferentiation as a result of "stem cell deception" from normal euthyroid cells to immundeficient "ghost" cells readily susceptible to viral insult (Amadi, Sabo, Ogunkeye and Oluwole, 2008) Proper levels of thyroxine are reported crucial for optimal immune function (Souba and Smith, 1996). Blood specimens showed depressed mean CD_4 cell population ($102 \pm 2.8mm^{-3}$) for seropositive clinically AIDS patients; but normal count of the same cells ($10,000 \pm 8.4mm^{-3}$) for seronegatives (Table 1) which might be an evidence of a gross immune suppression in the patients.

Alternatively most of the globulins have no antibody activity and were globulins but not gamma (ɣ) globulins or antibodies. It is known that all antibodies (ɣ - globulin fraction) are immuglobulins but not all immunoglobulins are antibodies.

The high globulin levels in hypothyroid seropositives (Table 1) confirm the observation by some workers that hyper immunized subjects synthesized $5 - 10$ times all classes of immunoglobulins (Fontes *et al.,*2003). This accounts for impaired cell-mediated immunity (CMI) which is more relevant to viral insult as HIV. Low plasma albumin and total protein (Table 1) have been incriminated as responsible for poor lymphocyte transformation consistent with hypothyroidism and presence of Bence Jones proteins (BJP) which is a diagnostic feature in the urine of many patients with malignant immunocytomata (Baku 1974) implies a degree of dedifferentiation (Judith and Harold, 2001). Protein including albumins and globulins are indispensable for body defence against infection. Protein depletion or its caloric malnutrition depresses immune responses of the subjects (Scrimshaw *et al.,* 1968, Reinhold, 1969, Theophilopoulos *et al.,* 1976, Haskova *et al.,* 1978, Amadi, Angela and Salimonu 1990). Starvation and undernutrition impair immunoglobulin (Ig) and serum albumin levels, cause diminished mean absolute concentrations of total proteins, albumin, α_2 and βglobulins but little effect on gamma (ɣ) globulin concentrations (Surks *et al.,* 1972, Samuel *et al.,* 1979; Amadi, Angela and Salimonu, 1990).

My explanation goes to say that either the seropositive might not develop enough antibodies to form immune complexes as found in seronegatives or due to less population of antigenic determinants of the complexes and their diminished ability to recognize and produce specific

antibodies against the antigen. The complexes might have density less than 19 Sedberg Unit (19S) proteins. Complexes of density less than 19S proteins fix complements poorly or that the antibodies were oligovalent and thus cannot sustain life or to say the least good health. Some workers have shown that the antibodies involved in immune complex formation are normal divalent molecules (Osunkoya *et al.,* 1972). Let me add that in as much as thyroid hormones are indispensable for the proper function of both the myeloid and lymphoid organs/tissues, their deficiency (hypothyroidism) cannot sustain CMI which is the "firing squad" against HIV/AIDS.

Work is in progress on screening for thyroid hormone intervention in preventing mother to child transmission (PMTCT). Once again I affirm that on thyroid hormone screening in antenatal clinics depends the future of the maternal cum neonatal health and maternal-neonatal health of the future. Thus by this screening "the challenges faced by motherhood that these little ones may live" (Sagay 2010), may be mitigated or fall back to physiological norms especially in pregnancy so that labour could be reduced to that of a "Hebrew Woman's".

Conclusion / Summary:
In the reproductive tract thyroid hormone gate-keeping is responsible for;
- Cell to cell existence
- Cell to cell dialogue and morphology
- Contractility of tissues, organs or organ systems
- Contractility of the myometrium in parturition that summons the neonate to life or to death
- IgGρ& immunity
- IgGρ&APGAR score or the quality of life in a neonate.
- Prevention of PPH
- Resistance to HIV/AIDS
- Possible vehicle for PMTCT.

Professor A.B. Adeliaye, a clean gentleman who finished up the supervision and restored the "center of gravity"of Physiology Department from below to above ground level.

Professor G.A. Ubom, former Dean of Postgraduate School who stood for truth and fairness that gave birth to the defence of my Thesis.

My wife Christie – a stoic. You have always stood by me especially in times of travail you have kept your vow of "for better for worse" and not FOR BETTER FOR US I love you.

My children: Duke, Chidi, Tochi and Chinelo for bearing all the hardships with the mind of a child and the heart of a lion.

My colleagues in the department who stood by me and for their assistance on the bench especially Mr. D.D. Dakat (RIP).

Professor B.N. Okeahialam, a man that lights a candle where ever he goes and whose simple life style of truth, humility and honesty translates efficacy into decent behavior.

Professor O.O. Ogunkeye a pillar ofintegrity and courage for your encouragement and support at the bench.

Mrs. J.A. Otubu, a woman evangelist by her way of life and help to people especially those rejected. Thank you noble lady.

Professors B.O Amure, B.O. Osunkoya, L.S. Salimonu, A.I.O Williams, R.A. Elegbe, D.D Oyebola, A.F. Bolarinwa and O. Ladapo all of University of Ibadan for your mentoring.

Finally to all adherents to TRUTH "the intellectual wealth of the world" – Colonel R.G. Ingersoll.

Lastly, finally and very sincerely to this dignified, respected audience: Thank you very much.

REFERENCES

1) Amadi, K. (2004). The Effects of Thyriodectomy and Thyroxine Administration on the Reproductive Tract of the Wistar Albino Rat and the Rabbit Ph.D Thesis Submitted to the University of Jos.

2) Amadi, K., Adeniyi, K.O., Nwana, E.J.C., Otubu, J.A.M. (1996). The Effect of Thyroxine Treatment on the Morphology of the Testis in the Rat, Mol. Androl; 8: 7 – 13.

3) Amadi, K., Adeniyi, K.O., Nwana, E.J.C., Otubu, J.A.M. (1996a). The Effect of Thyriodeectomy and Thyroxine on the Contractile Responses of the Myometrium to Prostaglandin E_2 and Potassium Chloride. Trop. J. Obstet Gynaecol; 13 (1): 67 – 70.

4) Amadi, K., Adeniyi, K.O., Nwana, E.J.C., Otubu, J.A.M. (1996a). The Effects of Thyriodectomy and Thyroxine Administration on the Morphology and Function of the Rat Uterus. Pathophysiology; 112: 30 – 33.

5) Amadi, K., Adeniyi, K.O., Nwana, E.J.C., Otubu, J.A.M. (1996b). The Effects of Thyroidectomy and Thyroxine Treatment on the Morphology of the Ovary Pathophysiology: 116: 80 – 82.

6) Amadi, K., Adeniyi, K.O., Nwana, E.J.C., Otubu, J.A.M. (1998). The Effects of Thyroidectomy and Thyroxine Treatment on the Morphology and Contractility of the Vas Deferens to Electrical Stimulation in Vitro Pathophysiology; 5: 111 – 118.

7) Amadi, K., Angela A.E. Bassey and Lekan, S. Salimonu (1990). Immune Complex and Parasitaemia Levels in Casein –Depleted Mice with P. berghei Infection Jour Med Trop, 3: 36 – 39.

8) Amadi, K., Idah, O.V., Sabo, A.M. (2013). The Relationship of Gamma Immunoglobulin Density (IgGρ) and APGAR Score in Normal Term Pregnancy. Intl. J. Basic, Applied and Innovative Research; 2: 45 – 50.

9) Amadi, K., Nwana, E.J.C., Otubu, J.A.M. (1999). Effect of Thyroxine on the Contractile Responses of the Vas Deferens to Prostglandin E_2. Arch. Androl., 42: 55 – 62.

10) Amadi, K., Nwana, E.J.C., Otubu, J.A.M. (2007). Morphology and Function of the Oviduct: Effects of Thyroidectomy and Thyroxine Administration. Afr. J. Med. Med. Sci., 36: 353 – 360.

11) Amadi, K., Sabo, A.M. Idah, O.V., Galam, N.Z., Dabum, L.J.(2013). The Influence of Thyroid Hormone on Gamma Immunoglobulin Density (IgGρ) and APGAR Score Obstet. Gynecol (in Press).

12) Amadi, K., Sabo, A.M., Adelaiye, A.B., Sagay, A.S. (2005). Dependence of Calcium ion Receptors on Thyroid Hormone for the Regulation of Cellular Functions. Nig. J. Physiol. Sci., 20 (1 – 2): 95 – 100.

13) Amadi, K., Sabo, A.M., Ogunkeye, O.O., Oluwole, F.S. (2008). Thyroid Hormone: A "Prime Suspect" In Human Immune Deficiency Virus (HIV/AIDS) Patients? Nig. J. Physiol. Sci., 23 (1 – 2): 61 – 66.

14) Amadi, K., Sabo, A.M., Sagay, A.S. (2006). The Role of Thyroid Hormone as the Primary Trigger of Reproductive Tract Contractility. J. Med Trop; 8 (2): 31 – 42.

15) Amadi, K., Sabo, A.M., Sagay, A.S. (2006). Thyroid Hormone; The Modulator of Erectile Function in the Rabbit Nig. J. Physiol, Sci, 21 (1 – 2): 83 – 89.

16) Balaban, R.S., Bose, S., French, S.A., Territo, P.R. (2003). Role of Ca^{2+} in Metabolic Signalling Between Cardiac Sarcoplasmic Reticulum and Mitochindria in Vitro. Am. J. Physiol. Cell Physiol 284: C 293.

17) Blakiston's Pocket Medical Dictionary 4th Edn. Alfonsa R.G. Ed. McGraw- Hill Book Company N.Y. 1979.

18) Brain, O'Rourke., Sonia, Cortassa and Miguel, A. Aon (2005). Mitochondrial Ion Channels. Physiology, 20: 303 – 315.

19) Cochran, T.E. (1972). Foetal and Maternal Immunoglobulin Concentration at Delivery and Post Partum. J. Obstet. Gynae. Brit. C. WLth; 79: 238 – 243.

20) Comline, R.S., Cross, K.W. Dawes, G.S. and Nathanielsz, P.W. Cambridge University Press.

21) Comline, R.S., Nathanielsz P.W. and Silver (1970). Passage of Thyroxine Across the Placenta in the Foetal Sheep. J. Physiol, 207:3-4P.

22) Cortassa, S., Aon, M.A., Marban, E., Winslow, R.L. and O'Rourke, B. (2003). An Integrated Model of Cardiac Mitochondrial Energy Metabolism and Ca^{2+} Dynamics. Biophys J. 84: 2734 – 2755.

23) Daniel, D.S., Daniel, U.D., Upinder, S. *et al.,*(1991). Expression of IgG Fc Receptor Antigens in Placenta and on Endothelial Cells in Humans: An Immunohistochemical Study. A.J.P., 138 (1): 175 – 181.

24) Dawes, G.S (1973). Breathing and rapid eye movement sleep before birth, In Foetal and neonatal physiology. Proc. of the Sir Joseph Barcroft Centenary Symposium on Foetal and Neonatal Physiology, PP4-62. (Eds.)

25) Eayrs, J.T. (1961), Age as a Factor Determining the Severity and Reversibility of the Effects of Thyroid Deprivation in the Rat. J. Endocrn., 22. 409 – 419.

26) Erenbery, A. and fisher, D.A. (1973). Thyroid Hormone Metabolism in the Foetal and Neonatal Physiology. Proceedings of Sir Joseph Barcroft Centenary Symposium on Foetal and Neonatal Physiology, PP.508-526. (Eds) Comline, R.S., Cross, K.W., Dawes, G.S., and Nathanielsz, P.W. Cambridge University Press.

27) Fisher, D.A. and Oddie, T.H (1964). Neonatal Thyroid Hyperactivity Response to Cooling. Am. J. Dis. Child., 107: 574 – 581.

28) Gitlin, D., Kumate, J., Urusti, J., Morales, C. (1964). The Selectivity of the Human Placenta, in the Transfer of Plasma Proteins from mother to foetus. J. Clin Invest, 43: 1938 – 1951.

29) Gitlin. D. and Biasucci, A. (1969). Ontogenensis of Immunreactive Thyroglobulin in the Human Conceptus, J. Clin. Endocr. Metab., 28:849-853.

30) Hopkins P.S and Thorburn, G.D. (1972). The effects of Foetal Throidectomy on the Development of the Ovine Foetus. J. Endocr., 54:55-66.

31) Jones, W.R. Immunoglobulins in Foetal Serum. J. Obstet. Gynae. Brit. C. WLTh. 76: 41 – 46 (1969).

32) Liggins, G.C., Fairdough, R.V., Grieves, S.A., Kendall, J.Z. and Knox, B.S. (1973), the Mechanism of the Initiation of Parturition in the ewe. Rec. Prog. Horm. Res., 29, 111-159.

33) Mellor, D.J. and Slater, J.S. (1971). Daily Changes in Amniotic and Allantoic Fluid during the Last Three Months of Pregnancy in Conscious Unstressed Ewes with Catheters in their Foetal Fluid Sacs. J. Physical 217; 573-604.

34) Meschia, G., Cotter, J.R., Breathnach, C.S., and Barron, D.H., (1965). The Haemoglobin, Oxygen Carbondioxide and Hydrogen Ion Concentrations in the Umbilical Bloods of Sheep and Goats as Sampled via Indwelling Plastic Catheters. Quart. J. exp. Physiol., 50:185-195.

35) Micklem, K., Stross, W.P., Willis, A.C., Cordel, J.L., Jones, M., Mason, D.Y. (1990). Different Isoforms of Human Fc R_{11} Distinguished by CDW32 antibodies. J. Immunol., 144: 2295 – 2303.

36) Nathanielsz, P.W., Comline, R.S., Silver M., and Thomas, A.L. (1973). Thyroid Function in the Foetal Lamb During the Last Third of Gestation. J. Endocrn. 58, 535-546.

37) Nathanielsz, P.W., Comline, R.S., Silver M., and Thomas, A.L. (1974). Thyroid Function in the Foetal Calf J. Endocrn. 61: Ixxj

38) Payne, R. (1969). Cord IgG and Duration of Labour. Lancet, 1; 372.

39) Shelley, H.J., Bassett, J,M., and Milner, R.D.G. (1975). Control of Carbohydrate Metabolism in Foetus and Newborn. Brit. Med. Bull., 31:37-43.

40) Tata, H.R., Biological Action of Thyroid Hormones at the Cellular and Molecular Levels In: Action of Hormones on Molecular Processes. G. Litwark and D. Kitichenvsky Eds. 1 Wiley. New York. Pp. 32 – 65. 1964.

41) Thomas, A.L., Comline, R.S., Silver, M. and Nathanielsz, P.W. (1974). Plasma Thyrotrophin Concentrations in the Foetal Calf. Experimentia; 30: 1226 – 1228.

42) Thomas, A.L., Horn, C., Krane, E.J., Bass, F.G. and Nathanielsz, P.W. (1977). Cortisol Induced Suppression of Plasma Thyroxine and Elevation of Plasma Triiodothyronine in the Foetal Sheep at 130 – 134 days Gestation. J. Endocr. 72: 32 – 33p.

43) Thomas, A.L., MH, and Nathanielsz, P.W. (1974). Variations in Plasma Thyrotophin Concentration in the Conatal Calf and its Relationship to Circadian Periodicity, J. Endocr., 62:411-412.

44) Thorburn, G.D. and Hopkins, P.S. (1973), Thyroid Function in the Foetal Lamb. In: Foetal and Neonatal Physiology, Proc. of the Sir Joseph Barcroft Centenary Symposium on Foetal and Neonatal Physiology PP488-507. (eds) Comline, R.S., Cross, K.W., Dawes, G.S. and Nathanielsz, PW: Cambridge University Press.

45) Turmero, J.A. (1974). Antibody Transfer During Labour. Am. J. Obstet Gynaecol; 119: 486 – 491.

46) Yang, S.L., Kleinman, A.M., Rosenberg, E.B., Weil, P.Y. The Effect of Labour and Mode of Delivery on Immunoglobulin Concentration in the Neonate. Am. J. Obstet Gynaecol. 109: 78 (1971).