FORM AND FUNCTION: THE INSEPARABLE TWINS IN VETERINARY MEDICINE

An Inaugural Lecture delivered at the University of Ibadan

on Thursday, 19th February, 2009

by

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The Vice-Chancellor, Deputy Vice-Chancellor (Administration), Deputy Vice-Chancellor (Academic), Registrar, Librarian, Provost of the College of Medicine, Dean of the Faculty of Veterinary Medicine, Dean of the Postgraduate School, Deans of other Faculties, and of Students, Directors of Institutes, Distinguished Ladies and Gentlemen.

Preamble

It is indeed for me a great honour, to be called upon to deliver this lecture, the 8th in the 2008/2009 series of inaugural lectures on behalf of the Faculty of Veterinary Medicine, and the 27th to be delivered by professors of this Faculty since 1976 when Professor Desmond H. Hill, the founding Dean of the Faculty delivered the first. This lecture also is the third to be delivered by my department (Veterinary Anatomy). The first was delivered by Professor S. F. Amakiri in 1984, followed by Professor T. A. Aire in 1986. In spite of being the third inaugural lecturer, I am by no means the third professor to be produced by the Department. That third chair was occupied by Professor Oyewale Adeyemo in 1993. I am thus the fourth of five professors to be produced by the department. Professor S.K. Onwuka and I are still in the department while the others have found their ways to other climes. Indeed, the issue of 'turn-taking' in the delivery of inaugural lectures in the faculty is a settled tradition as one's turn is determined by one's seniority in the attainment of the professorial rank. However, Mr. Chairman, this is the second inaugural lecture from our residential address. There was no seniority consideration when in December 2005, Professor Gbemisola Oke gave the first—a case of what men can do, women can do earlier.

Inaugural lecturers are expected to give an account of their contributions to their fields within one hour and in as simplified a form as possible. Thus, if one's contribution is as varied as mine is, in a field as challenging as veterinary medicine, the main task would be to determine what is to be expunged and what is to remain, and then, to weave a thread to link those that remain.

During this lecture therefore, I intend to give a glimpse of my career and highlight the purple patches of my work and in

the process acknowledge my mentors, those with whom I have collaborated and those who have assisted me along the way. Before I go any further in discussing my work, Mr. Vice-Chancellor, I crave your indulgence to look, no matter how cursorily or superficially, at the main component of the title of this lecture, which simply implies that the scope of my research, which though has its roots in morphology, has wide applications in veterinary medicine.

Morphology or Anatomy

Morphology is the branch of biology that deals with the form and structure of organisms without consideration of function. The term anatomy, which stems from the Greek word "*Anatemnein*" meaning "to dissect", "to cut apart" (Budras et al. 2002), also has similar application but wider dimension in that it goes beyond dissection or description of form to the interrelationship between form and function as well as application to clinical practice. Anatomy is divided into macroscopic (gross) anatomy, histology (microanatomy), and developmental anatomy (often called embryology). Anatomy is reserved for the oldest and most encompassing area called macroscopic anatomy. In macroscopic anatomy, gross dissection assumes a pride of place as the first method of gaining insight into the organ or tissue.

In our department here at Ibadan, our subjects at the undergraduate level include gross and microscopic anatomy, embryology, neuroanatomy, avian anatomy, comparative aspects of anatomy as well as applied or clinical anatomy. All these are geared towards a comprehensive understanding of structure and its relationship to function. Because the surgeon, however competent, and other health professionals (pathologists, clinicians, e.t.c.) cannot practice their art without a working knowledge of anatomy, the latter can be described as the basis for medical practice and the health sciences.

The term histology is derived from the Greek word *histos*, meaning tissue (web), and *logia* (knowledge). The discovery, development, and use of the light microscope in its various forms helped scientists to make great advances in the field of histology and general structure of cells (Heath and Young

2000). Embedded in the scope of histology is ultrastructure, where the use of the electron-microscope comes into the study of cells or tissues. Earlier, before the advent of ultrastructural studies as enabled by electron microscopy, cell morphology (histology and cell physiology) and cell biology were treated as separate entities (King 1966). Ultrastructure constitutes the bridge between histology and cytology (cell biology). An ultrastructural study therefore makes cell structure and cell function interrelated parts of a whole rather than antagonists. An overview of most experiments and studies involving the use of electron microscope presents the goal of the experiments and uses structural profiles to deduce function.

During the past few decades, especially in human healthcare delivery, there has been an explosion of new techniques for imaging anatomy in living patients. Examples range from endoscopy and laparoscopy to computed tomography (CT) and Magnetic Resonance Imaging (MRI) together with newly emerging technology of 3D or three-dimensional visualization. The other types of investigative methods are used to develop observations, hypotheses, and conclusions in cell and molecular biology as well as observe behaviour of cell organelles, and cellular and molecular structures (Wolfe 1993). Some of these include centrifugation, gel electrophoresis, blotting techniques, chromatography, polymerase chain reaction, cell culture, antibody production, DNA sequencing, DNA fingerprinting, DNA hybridization, and DNA cloning. As of now, cytology is the rave—with immunocytochemistry playing on the stage with loud ovation. I have been part of this rave as will be shown later in this lecture.

The Beginning

During my pre-clinical years, I discovered my flair for anatomy and the reason is simple. Every fine artist or architect can be described as an anatomist of some sort as he/she deals basically with structures. I have always had a flair for fine art and this was utilized to advantage during my pre-clinical years. I realized early in the anatomy class that my best strategy for understanding and easily recollecting things in the dissecting room was to do a sketch of the details and label instantly. That

way, it became permanently engraved in my memory. Dr. Tom Aire, who obviously was similarly endowed and with the benefit of having expressed the talent more extensively, would instantly spot my sketches on his supervisory rounds in our dissection classes. After a few suggestions here and there, my sketches often became the standard for the rest of the class. This unknowingly was the beginning of a special mentoring relationship between Professor Aire and I. The mentoring was suspended as I graduated into the clinical years, only to be reactivated after my NYSC and a short stint at the Ogun State Veterinary Services as Veterinary Officer II. Professor Aire invited me to join the staff of veterinary anatomy department when a vacancy existed. My good friend, Professor C.A.O. Adeyefa was the one who sought me out in Abeokuta to deliver the urgent message. At that time, there was no 'GSM' and every one was at the mercy of 'NITEL'.

I joined the university service in the Department of Veterinary Anatomy in March, 1980 and I delved into teaching—the best way I could—with all the artistic flair and enthusiasm. Nobody hinted me that the best teaching methods alone cannot take one far. So, I returned to Professor Aire who laughed at my ignorance of what it took to progress in academics and he initiated me into the world of scientific research. This started from his ongoing research work on the stages of the seminiferous epithelium of the African giant rat (Aire 1980). The work was classical, following in the mould of what had earlier been done by Clermont on the seminiferous epithelium of the human testis in 1963. I jumped at it and in the process began to learn techniques of microstereology, accurate sectioning, serial sectioning, photography, and more importantly, literature search. I enthusiastically embraced this nurturing. I was later introduced to areas of further work which led to my meeting with Professor S.S. Ajayi of the Department of Wildlife and Fisheries Management. As a pioneer worker on the African giant rat, he focused on the domestication of the rat which ordinarily is a wild rodent. My immediate duty was to meet with him and get as much information as I could. I found him to be very warm and receptive.

Mr. Vice-Chancellor Sir, before I introduce the African giant rat, I wish to highlight the aspects of my research which I intend to discuss in this lecture. They are:

- Breeding pattern of the giant rat as influenced by season, in aid of its domestication.
- Characterization of the epididymis of the giant rat, also aimed at complementing the process of domestication.
- Our initial efforts in the study of the female African giant rat.
- Developing new methodologies in our research that can replace those that are often difficult to utilize or purchase.
- The significant outcomes of our studies on gossypol usage especially in malnutrition and parasitism.

My main focus is related to how structure in each of these studies has elucidated important functional implications.

The African Giant Rat

The African giant rat (*Cricetomys gambianus,* Waterhouse) known in Yoruba as *Okete*, is a wild rat widely spread in sub-Saharan Africa (Rosevear 1969). The big size of this rat makes it one of the most striking of Africa's rodents. They have been found to be fairly tame and docile in captivity even though shortly after being captured, they tend to exhibit such escape reactions as violent struggles and hitting head and tail against the cage.

Fig. 1: The African giant rat *(Cricetomys gambianus,* Waterhouse*)*

Their ability to adapt to various environmental conditions, social acceptance in Nigeria (especially southern Nigeria), their value as a delicacy among rural populations, and considering the need to meet the increasing demand for animal protein among other considerations recommended this wild rodent for domestication.

Since Ajayi pioneered its domestication here in Ibadan (Ajayi 1975), I decided to study the biology of its reproduction (Oke 1988) as a means of providing adequate and needed information and baseline data on both the morphology and physiology of its systems to aid the domestication process. In addition, we felt that the rat may become a valuable laboratory animal because of its large size and availability in West Africa, thus becoming useful in the assessment of some pathological conditions or the efficacy of various pharmacological substances on different organ systems both in the study of diseases and during drug screening programs (Holtz 1972).

Seasonal Studies in the Giant Rat

Attempts to breed this rodent on a large scale were not successful because detailed knowledge of its reproductive biology was scanty and sometimes incorrect. For example, Rosevear (1969) suggested that the rat in the wild is a seasonal breeder. Ajayi (1975), whose work was based on the rat in captivity, on the other hand, reported that they reproduced throughout the year and did not show any seasonal peak in breeding.

In an attempt to resolve this, I investigated the reproductive organs of the male during the two major seasons (wet and dry) of Nigeria using morphometric methods (Oke 1985). My findings showed that values for the dry season (November – February) were consistently higher than values obtained for the wet season in all cases except the epididymal epithelial height, bulbo-urethral-gland weight, and prostate-gland weight (tables 1, 2, and 3), even though there were no significant differences between the seasons in most of the values obtained.

Table 1: Mean Testicular Weight, Volume and Tubular Diameter of Giant Rats within Seasons

Live weights of animals (g)	Season	No. of animals	Weight of testis* (g)	Volume of testis* (g)	Tubular diameter (μm)
1165 ± 51·15 (1430–1027)	Rainy season (Mar–Oct)	26	10·9 ± 0·78 (13·30–8·73)	9·60 ± 0·75 (14·00–7·20)	212·85 ± 8·32 (242 160)
1158 ± 60·10 (1286–828)	Dry season (Nov–Feb)	26	12·11 ± 1·10 (13·50–9·06)	11·74 ± 1·09 (15·20 8·70)	228·64 ± 6·23 (248–192)
Significance of seasonal difference			$P > 0·05$	$P > 0·05$	$P > 0·05$

*Figures are for both left and right testes.
All values are mean ± SE (range).

Table 2: Mean Epididymal Weight, Epididymal Height, and Epididymal Tubular Diameter of Giant Rats within Seasons

Season	No. of animals	Epididymal weight* (g)	Epididymal epithelial height (μm)	Epididymal tubular diameter (μm)
Rainy season (Mar Oct)	26	2·26 ± 0·18 (3·48–1·12)	51·40 ± 1·68 (60 41)	216·45 ± 6·28 (268–184)
Dry season (Nov–Feb)	26	2·81 ± 0·18 (3·54–1·52)	42·30 ± 3·30 (60–30)	242·82 ± 10·80 (300–205)
Significance of Seasonal difference		$P > 0·05$	$P > 0·05$	$P > 0·05$

*Figures are for both left and right epididymides.
Values are mean ± SE (range).

Table 3: Mean Weights of Accessory Sex Glands of African Giant Rats within Seasons

Season	No. of Animals	Weight of seminal vesicles* (g)	Weight of bulbo-urethral glands* (g)	Weight of prostate glands* (g)
Rainy season (Mar–Oct)	26	10·97 ± 1·64 (17·7–12·43)	0·29 ± 0·06 (0·92–0·10)	1·06 ± 0·18 (2·16–0·14)
Dry season (Nov–Feb)	26	11·72 ± 0·93 (15·80–8·90)	0·27 ± 0·05 (0·48–0·06)	0·89 ± 0·21 (2·30–0·15)
Significance of seasonal difference		$P > 0·05$	$P < 0·05$	$P > 0·05$

*Figures are for all components of organs.
Values are mean ± SE (range).

These findings which corroborated Ajayi (1975), showed that the male giant rat does not appear to be a seasonal breeder.

Characterization of the Giant Rat Epididymis

In furtherance of the need to contribute to the domestication and breeding of the giant rat, we also sought to characterize the epididymis (Oke *et al.* 1988, 1989; Oke and Aire 1990). The epididymis, one of the major paths of a male gamete to its ultimate role in breeding, is important in the thrust towards improving the breeding capacity of animals as its complex nature also presents numerous challenges to investigators, like cytologists, biochemists, pathologists, clinicians, and morphologists.

We found the epididymis of the giant rat to be similar to that of the laboratory rat in both shape and relative sizes of the caput, corpus, and cauda regions (fig. 2), although there are species variations in cell specialization along the epididymal duct.

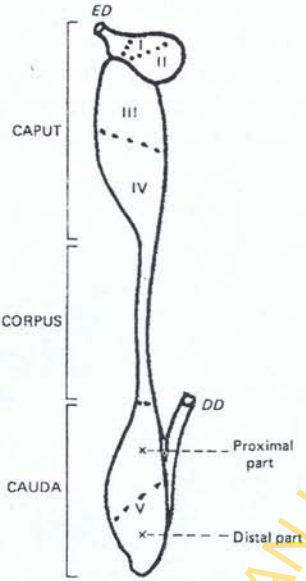

Fig. 2: Regions and zones of the epididymis of the giant rat

Five distinct zones of the epididymis, based on epithelial height, cell types, and structure were observed. These zones, apart from the efferent ductules are zone I (initial segment), zones II, III, and IV (middle segment), and zone V (terminal segment). Zone V has two major subzones, proximal (Va) and distal (Vb).

The lining epithelium of the entire epididymis is a pseudostratified columnar ciliated epithelium. It is characterized by four main cell types which are present in various combinations in the different zones. These are the principal cell, the basal cell, the intra-epithelial lymphocytes, and the clear cell. Apical cells, as seen in the rat, mouse, and guinea-pig (Hoffer and Greenberg 1978) are absent in the giant rat.

The Principal Cell

The principal cell is present in all segments and zones and extends from the basal lamina to the lumen of the duct, into which its stereocilia project. Principal cells constitute the tallest of the epithelium and are the basis for the epithelium of the caput being very tall, thereby reducing the luminal diameter. It

exhibits clumps of mitochondria (fig. 3) in zone I, numerous vacuoles and large numbers of dense bodies (fig. 4) in zone II, and one or two large vacuoles in the supra nuclear region of zone V. The mitochondrial clumps located close to an extensive Golgi region are unusual and peculiar to this animal.

Fig. 3: Photomicrograph of the epithelium of the initial segment of the epididymis (Zone 1). P (Principal cell); B, basal cell. Note the clumps Mitochondria (Mt).x1280.

Among the features that stand out in the supranuclear region of the principal cell in the caput region especially in zone II are large Golgi complexes and numerous cisternae of endoplasmic reticulum studded with ribosomes and arranged in parallel lamellae or whorls (fig. 5). These features led us to suggest the occurrence of protein secretion in the giant rat caput epididymis, especially in zone II, consistent with reports available in many species (Nicander and Malmquist 1977) and other cells (Allison and Davies 1974).

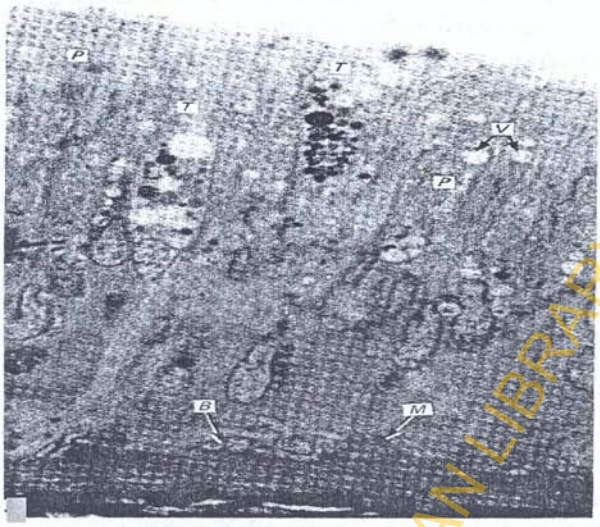

Fig. 4: Zone II epithelium showing P, principal cell; T, truncated cell, with dense bodies and vacuoles V); B, Basal cell; M, macrophage-like cell. x1280.

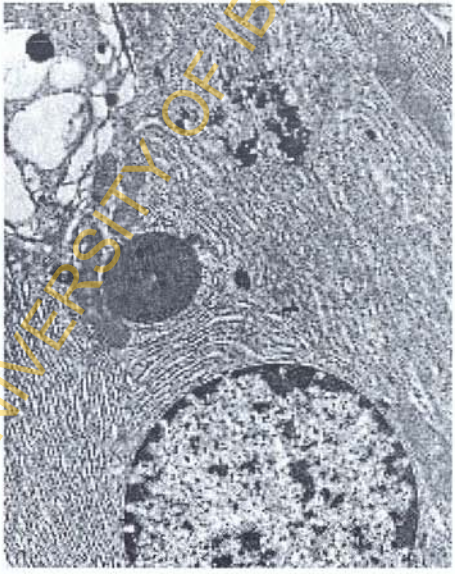

Fig. 5: EM of rough endoplasmic reticulum occurring in parallel lamellae and in whorls. x7,248.

The ultrastructural evidence for secretion in different zones of the caput epididymis was provided by Oke and Aire (1990) using the giant rat. Absorption of fluid and particulate matter has been attributed to the efferent ductules and epididymal epithelium, especially in the first part of the epididymis where Crabo (1965) estimated that over 90% of the fluid leaving the testis in the bull or boar is reabsorbed. I have been able to provide morphological correlation for these notable functions in the giant rat (Oke 1988).

The Basal Cell

This cell type is present but few in all segments. It is generally small in size, but smaller in zone III, having large nuclei and lucent cytoplasm (fig. 3). They lie on the basal lamina in close association with intra-epithelial lymphocytes and macrophage-like cells. In the terminal segment (zones Va and Vb) the basal cells become progressively flattened cranio-caudally. Yeung *et al.* (1994) reported close proximity and morphological similarity between basal cells and peritubular macrophages in man. They also showed that these basal cells expressed a tissue-fixed, macrophage-specific antigen. This is consistent with the hypothesis that links the basal cell with the intra-epithelial macrophage in relation to the ultimate fate of the basal cell in the epididymal epithelium.

The Intra-epithelial Lymphocyte (IEL) and Macrophage-like Cell (MLC)

This cell type is also present in all segments and zones of the epididymis. IELs with characteristic pale cytoplasm and deeply heterochromatic nuclei are found in large numbers (fig. 4). The number of IELs and MLCs increases remarkably in the middle segment (zones II, III, and IV). They are mostly basal in location while some migrate upward (fig. 6) in the epithelium. IELs and heavily laden MCLs occur in the terminal segment in large numbers.

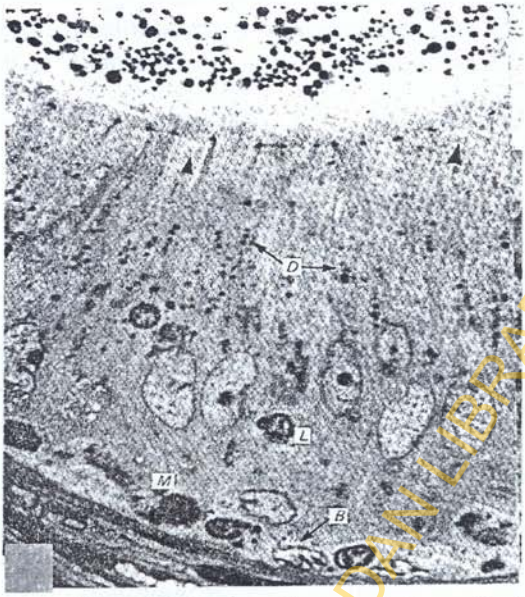

Fig. 6: Epithelium of zone III showing a few subapical vacuoles (arrowhead) and dense globules (D) in the principal cells. B, basal cell; M, macrophage-like cells; L = IELs. x1280.

It is interesting that the giant rat epididymal epithelium contains a large number of intra-epithelial lymphocytes and macrophage-like cells. Sinowatz *et al.* (1979) have reported the occurrence of similar cells in the monkey, man, and bovine epididymides. Their role in the epididymal epithelium is linked to phagocytosis of spermatozoa or fragments of spermatozoa (spermiophagy). They have also been thought to provide a first line of defence against local, mainly viral, antigen (Beagly and Husband 1998) and the segregation of sperm antigens from the general circulation (Dym and Romrell 1975). In intestinal epithelial cells, cytotoxic IELS have been implicated in epithelial cell turnover, bringing about apoptosis (Beagly and Husband 1998).

The Clear Cell

The clear cell is present only in the terminal segment (zone V), where it appears as the dominant feature. The clear cell is slender in the proximal portion (zone Va) (fig. 7) but very broad, often flask-shaped in the most distal portion of the terminal

segment (fig. 8). This cell is broader and lighter-staining than the principal cell. It contains numerous vacuoles of varying sizes and numerous dense bodies which indent the nucleus.

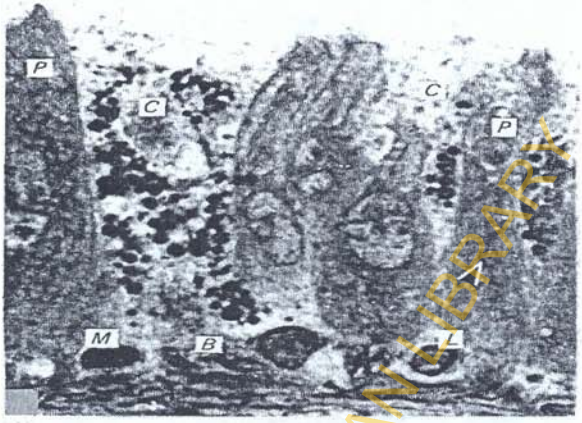

Fig. 7: Subzone I in the proximal aspect of the terminal segment of the epididymis. Principal cell (P), clear cell (c), lymphocyte (L), basal cell (B), macrophage-like cell (M). Note the presence of long numerous mitochondria (arrowhead). x1280.

Fig. 8: Subzone 6 of the terminal segment of the epididymis. Note the presence of large vacuoles (V) in the principal cells. The clear cell (c) becomes very broad; the entire epithelial height is low. G, Golgi zone. x1280.

The giant rat is similar to the rat and hamster in possessing large numbers of clear cells in the terminal segment. The clear cells, which seem to occur only in a few mammals is thought to be the source of glycerylphosphoryl choline (GPC) which together with sialoproteins may account for the hyperosmotic epididy-mal plasma and sperm maturation (Rajalakshmi 1985).

Apart from secretion, phagocytosis, and the movement of spermatozoa from the testis to the exterior, acquisition of fertilizing ability by the sperm cells is also accomplished in the epididymis through changes in size, electric charge, shape, metabolic properties. and capacity for motility. Motility of spermatozoa is acquired as the germ cells traverse the duct, a phenomenon believed to be facilitated by an increase in intra-sperm content of cyclic AMP during epididymal transit (Hoskins *et al.* 1978) combined with the building of a specific forward-motility protein.

The development of sperm motility, and indeed the normal structure and function of the epididymis is dependent on hormones, specifically androgens—which reach the epididymis in the rat testis fluids and the systemic blood circulation. Androgens bind to androgen binding protein (ABP) of which the Sertoli cell is the source (Hagenas *et al.* 1975). In attempting to determine the relationship between the giant rat and the laboratory rat, Adeyemo and Oke (1990) analysed testicular and epididymal proteins by gel electrophoresis (SDS-PAGE). Our findings (fig. 9) showed that the giant rat epididymis demonstrated copious bands suggested as ABP. However the non-recognition of giant rat ABP by anti-rat ABP antibody indicated that fundamental genetic differences could exist between the laboratory rat and the giant rat, especially in this regard.

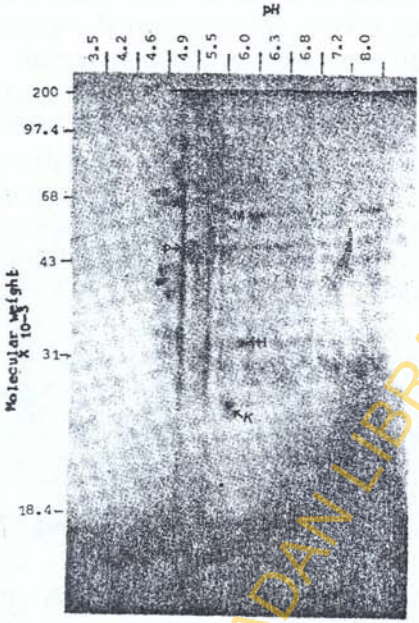

Fig. 9: Cytosolic fraction of the African giant rat epididymis analysed by the 2-DG electrophoresis and stained with Coomasie blue. M = albumin; P = protein suspected to be GR ABP; H and K = proteins characteristically found in this tissue

From all of the above, it is quite clear that the specialization of the epididymal duct along its length is not only morphological but also biochemical and physiological. This is why those who perform biochemical studies on materials from the epididymal duct or pathologists who may be inclined to examining only one portion of this duct need to be aware of these differences—which can no longer be regarded as trivial matters of interest to morphologists only. In addition, its intimate involvement in spermatozoa acquiring motility and fertilizing ability also offers ideal sites for action of male antifertility agents.

Initial Studies on the Female African Giant Rat

Vice-Chancellor Sir, studies so far described have focused on the aspects of the male giant rat in reproduction and hence in breeding. In the course of the research, the need to investigate

the female aspects became increasingly apparent—if the vision of domesticating this otherwise wild rodent was to be realized. Fortuitously, my first female masters student proved to be so outstanding in her performance that she was assigned the work towards her M.Sc degree. Most interesting however is the fact that at the commencement of the research, she was Dr. (Miss) Bankole; by the time she was completing it, she had become Dr. (Mrs) Oke—by marrying my nephew, Engineer Dapo Oke. The worth of her work is such that it formed the framework for another generation of brilliant scholarly pursuit by Dr. A.K. Akinloye whose Ph.D thesis is on more in-depth investigation of the female giant rat reproductive system.

Our investigation involved the characterization of the oestrus cycle in the female using vaginal smears (Oke and Oke 1999). It revealed that the oestrus cycle length is between 4 and 5 days. In addition to the four stages of the oestrus cycle (proestrus, estrus, metestrus, and diestrus), we also identified three intermediate stages (late diestrus/early proestrus, late proestrus/early estrus, and late metestrus/early diestrus) (fig. 10).

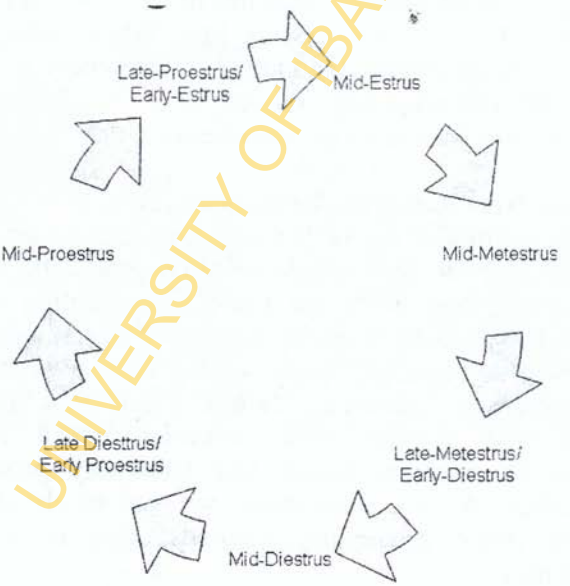

Fig. 10: A chart showing different stages of oestrus cycle in the female African giant rat

The vaginal smears obtained during proestrus were mucoid and contained mainly nucleated epithelial cells while those obtained during estrus were gritty and contained mainly superficial cells. Metestrus smears were characteristically dry and contained leucocytes and superficial cells while those obtained during diestrus were pus-like with a lot of mucus and numerous neutrophils. The totality of the findings established that vaginal exfoliative cytology can also be used to determine the estrous or heat period to facilitate productive mating in the giant rat as in other animals.

This particular finding, along with others from the male research efforts were translated into practical application at breeding, first from our colony in the Faculty and then on a few farm facilities outside. It is however disappointing to note that such proven success in the laboratory could not be replicated nor sustained on the field because of a vital missing link—input of extension workers. Even though the crucial role of this cadre of workers has been long recognized in agricultural practice, its equivalent is required in almost every sphere of science and technology as many research findings never make their way into the real world due to this missing link. Whilst advocating for government to encourage manpower development in this area, attention of entrepreneurs should also be drawn to this potentially lucrative source of opportunities.

Developing New Research Methodologies

For reproducibility of research results, researchers are taught to adhere very closely to standard research protocols. There are, however, occasions when the prevailing situation dictates a departure. It is frequently impracticable to follow some standard practice because of the peculiarity of the Nigerian circumstances viz—temperature, electricity failure, and lack of certain chemicals and reagents. This situation called for being innovative in research design and laboratory protocols—in tissue studies. As a consequence, we had to develop novel alternative tissue processing methods, two of which are described here.

Use of "Adi-agbon" (Coconut oil) as Clearing Agent in Neural Histology

As a way of providing a useful substitute to the relatively expensive and imported clearing agents used in histological tissue processing, we investigated the use of *adi-agbon,* an oily extract of the endosperm of the coconut fruit (*Cocoa nucifera.* L) as a clearing agent for embryonic soft tissues (Shokunbi *et al.* 1992). An ideal clearing agent for paraffin processing of embryonic tissue should satisfactorily de-alcoholize the tissues, be miscible with molten paraffin wax, and should not cause excessive hardening and shrinkage of the specimen. Such a compound should be non-toxic and readily available at modest cost. However, none of the conventional clearing agents for paraffin processing fulfilled all these criteria.

We were able to establish that *adi-agbon* adequately de-alcoholizes embryonic soft tissues, permitting paraffin wax infiltration and easy sectioning. There was some tissue shrinkage, which was not apparent on gross examination due to the compactness of the tissues and reduction in extra-cellular spaces (fig. 11).

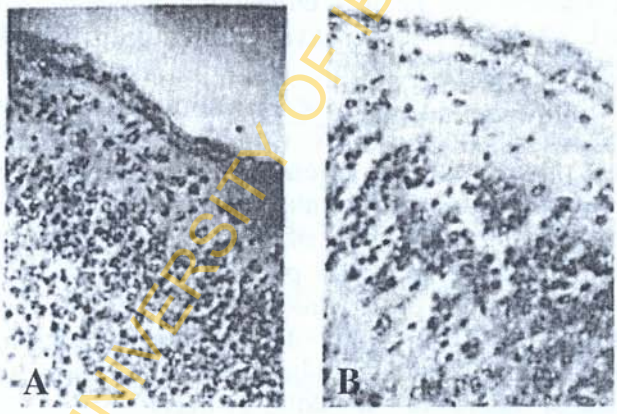

Fig. 11: Section of the cerebral cortex of an 18-day mouse embryo cleared in (a) *Adi-agbon* (b) xylene.

Note the relative looseness of the neuropil and the prominence of the extra cellular space in (b). x1600.

Of the tissues examined, the brain appeared to be most susceptible to this effect of *adi-agbon*. However, *adi-agbon* did not shrink adult soft tissues (Caxton-Martins *et al.* 1987). In spite of these minor short-comings, the use of *adi-agbon* is still encouraged because it is available locally and it could serve as a useful substitute for the other relatively expensive clearing agents—especially for routine histological work—where tissue identification is simply all that is required.

Improved Immunocytochemical Protocol that Employs Polyester Wax

I was privileged to participate, in conjunction with Dr. Carlos A. Suarez-Quian at the Department of Cell Biology, Georgetown University School of Medicine, USA, in developing an improved protocol for immunocytochemistry of the testicular tissues. Immunocytochemistry is an ideal tool for determining both tissue and cellular distribution of proteins. In the testis, successful immunostaining has been obtained through use of either frozen or paraffin sections, although both techniques have their limitations. With freezing, tissue preservation is not optimal whereas with paraffin embedding, antigenicity is often destroyed. These limitations have led to numerous ambiguous results and diagnostic problems.

In order to overcome these limitations, we attempted to immunocytochemically localize various proteins in the testis. Our trials (Oke and Suarez-Quian 1993) combined perfusion fixation with Bouin's fluid embedding of tissue in polyester wax. The general morphology of the polyester wax-embedded testis demonstrated excellent preservation of tissue which compares very well with similar tissues embedded in paraffin wax (fig. 12). Seminiferous tubules cut in cross section appear to retain normal morphology when treated with polyester wax with no major fixation or embedding artifacts.

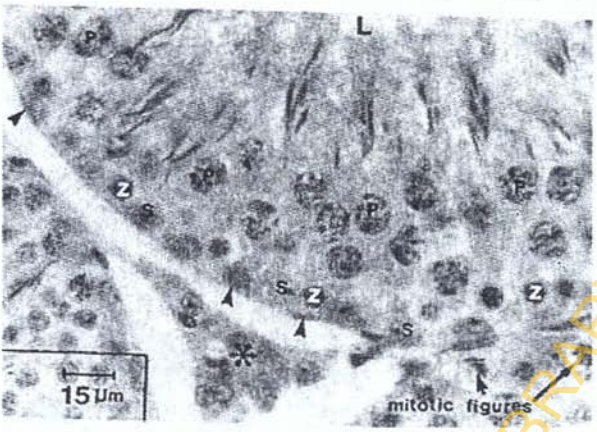

Fig. 12: General morphology of testis embedded in polyester wax. S = Sertoli cell nuclei, P = Pachytene spermatocyte, Z = Zygotene spermatocyte, * = interstitial cell. Arrows = mitotic figures, Arrowhead = peritubular cell x680.

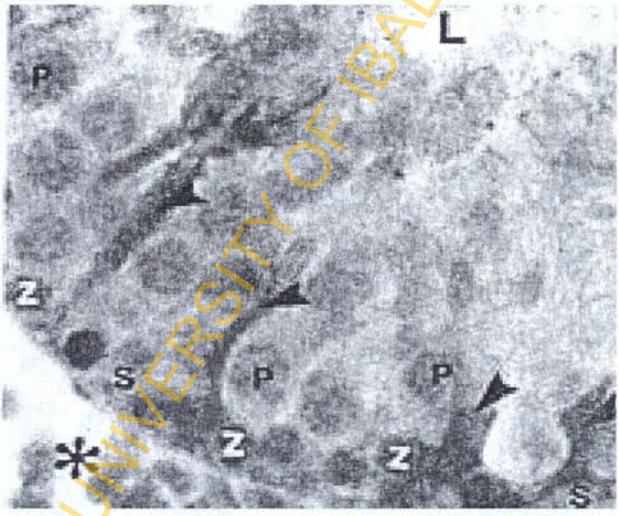

Fig. 13: Sertoli cell (S) immunostained for ABP (high power). Reaction product extends from base to lumen (L). Pachytene (P) and Zygotene (Z) spermatocytes not labeled. x625.

Using a specific antiserum probe for Sertoli cells (anti-ABP), a characteristic Sertoli cell immunostaining pattern was observed (fig. 13). The reaction product was observed to extend from the base of the tubule to the lumen. There is a general difficulty in obtaining good and consistent immunolocalization of ABP in Sertoli cells because of its bi-directional secretion (apical and basal) and its differential staining pattern as a function of the cycle of the seminiferous epithelium (Suarez-Quian and Niklinski 1990). However, we were able to show, through this method, that intense ABP immunostaining, spanning the height of the sertoli cell is present in all stages of the cycle of the seminiferous epithelium.

Specific immunostaining of populations of germ cells was also accomplished in the seminiferous epithelium using a monoclonal antibody that has been observed to immunostain nuclear lamins of certain cells *in vitro* (Suarez-Quian 1988). It specifically stained spermatogonia and pachytene spermatocytes (fig. 14).

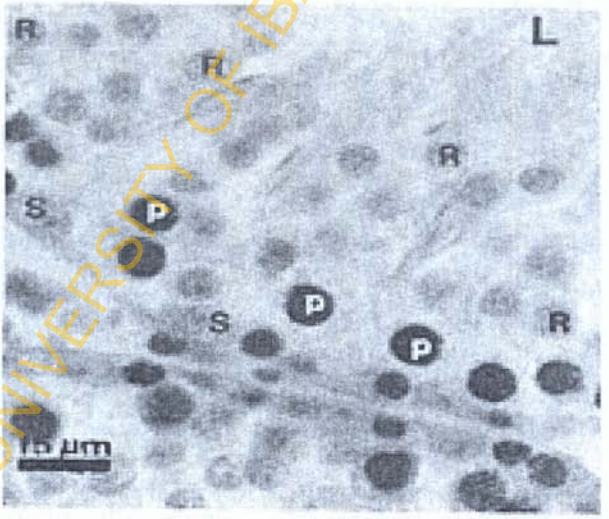

Fig. 14: Specific localization of reaction product only in the nuclei of pachytene spermatocyte. Sertoli cells (S) and Round spermtids (R) not immunostained. x625.

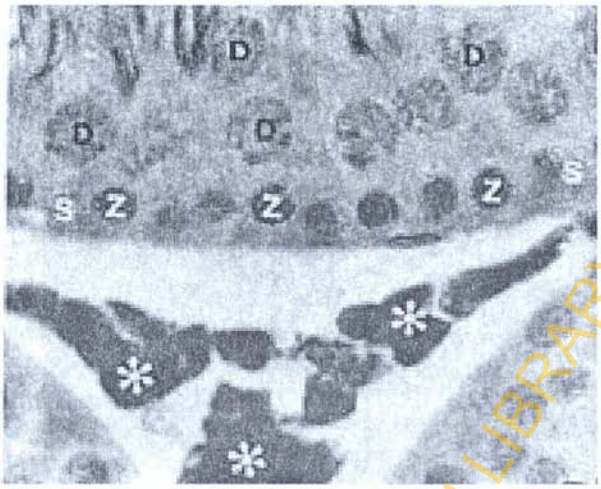

Fig. 15: Immunostaining for Leydig cells (*). Z and D = Zygotene and Diplotene spermatocytes. x625

The identity of the immunostained cells was ascertained readily because of the general preservation of the tissue made possible by this embedding protocol. This antibody probe has thus been suggested for identifying populations of these germ cells *in vitro*. Immunostaining of interstitial (Leydig) cells was also accomplished using the rabbit antiserum to the peripheral type benzodiazepam receptor (PBR) (fig. 15). Neither lymphatic nor capillary endothelial cells of the interstitium stained with this antiserum.

Immunolocalization of cytoskeletal proteins (vinculin, tubulin, and smooth muscle actin) also revealed the specific efficacy of this method. Vinculin was detected principally at ectoplasmic specializations between Sertoli cells and elongated spermatids, at junctional complexes between Sertoli cells and at kinetochores of spermatocytes at metaphase (fig. 16). Tubulin immunostaining was present in the cytoplasmic trunk of Sertoli cells and in the flagella of elongated spermatids (fig. 17).

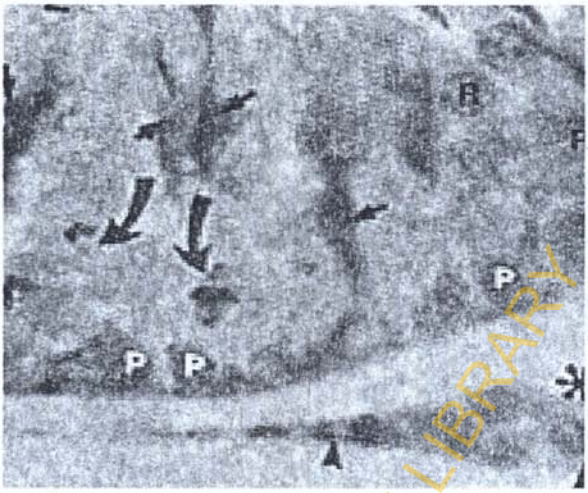

Fig. 16: Immunostaining for Vinculin in ectoplasmic specializations (short arrows) and at kinetochores of spermatocytes at metaphase (curved arrows) x625.

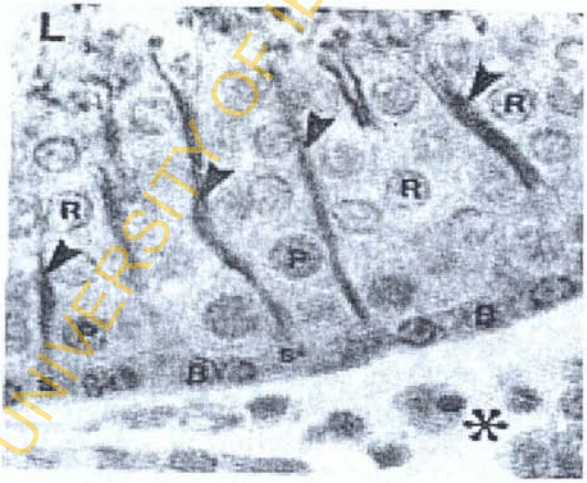

Fig. 17: Tubulin immunostaining in Sertoli cells apical cytoplasm (arrowhead) x605.

Thus, we have shown that antibodies to tubulin can serve as probes to indicate the presence of the supranuclear region, trunk region, and apical cytoplasm of Sertoli cells, which is consistent with previous reports on microtubules within Sertoli cell cytoplasm (Vogl 1988).

Generally therefore, this procedure for routine immunocytochemical localization of proteins in the testis has several advantages which are as follows:

(i) The low melting temperature (38^0 C) of polyester wax reduces loss of antigenicity.
(ii) Elimination of harmful solvents like xylene enhances antigen preservation.
(iii) The protocol does not require learning new techniques, is relatively quick and inexpensive.
(iv) Significantly, the excellent histological detail permits accurate detection of immunoreactivity.
(v) The results are permanent, allowing prolonged observations of even low immunostaining signals.

Gossypol Studies and Relevant Comments

Mr. Vice-Chancellor Sir, a number of our research activities have implications way beyond veterinary anatomy and, as a matter of fact, some of our research efforts can easily be extrapolated to the human population. A good example is our study on gossypol.

Gossypol is a polyphenolic substance that occurs naturally in cotton plants. It is a common constituent of livestock feeds. In addition, it has been evaluated extensively as a potential male contraceptive. It has been reported to reduce spermatogenesis in the male and to be a safe intravaginal contraceptive in the female. In addition to its antifertility effects, it exhibits antiproliferative, antimetastatic, and parasiticidal properties being very effective against trypanosomoses, a blood parasitosis of man and livestock, which causes debility and reduced productivity.

We sought to understand the antifertility effect of gossypol, its toxicity, and antitrypanosomal efficacy in a Third-World environment where several variables interplay simultaneously. Not fewer than three Ph.D theses have emanated from the series

of research hypotheses posed and for which answers have been provided for the 'knowledge bank'. One notable study that attracted unexpected international approbation and recognition was derived from the thesis of Dr. Benson Akingbemi (Akingbemi *et al.* 1996), in which we investigated reproductive parameters in gossypol-treated male rats that had been infected experimentally with *Trypanosoma brucei* and experimentally malnourished. This was with a view to generating information which may not only be useful to livestock farmers in trypanosome-endemic zones but, might also influence the design of clinical investigations concerning potential contraceptive agents, especially in the Third World where blood parasitoses, protein malnutrition, and rapid population growth are widespread.

The findings showed that most of the parameters studied (testicular size and diameter and length of seminiferous tubules) were lowest in the protein-malnourished—gossypol-treated—trypanosome-infected animals—when compared to those obtained from corresponding animals that were only gossypol-treated or trypanosome-infected, suggesting that reproductive capacity could be impaired in protein-malnourished, trypanosome-infected animals fed on gossypol-containing products even when there are no obvious clinical signs of disease. Therefore, we suggested screening for haemoparasitism prior to evaluation and/or use of gossypol in human subjects, especially in communities that are confronted with severe food shortages.

Mr. Vice-Chancellor Sir, the gossypol acetic acid used in this work was kindly donated by the WHO Special Programme of Research Development and Training in Human Reproduction, while the financial support came from University of Ibadan Senate Research Grant and University of Zimbabwe Research Board. Some parts of the work were done at the Institute of Veterinary Anatomy in Berlin, when Dr. Akingbemi was on a DAAD-funded study visit and at the University of Liverpool by Professor Aire while on a British Council-supported visit. It was eventually published by the International Journal of Andrology in 1996 and to our surprise, won the second prize of the European Academy of Andrology which was accompanied with

a cash award in 1997. Of great significance was an uncommon special editor's note, written at the end of the article as follows:

> Some readers may be puzzled by the paper by Akingbemi et al., and may consider that the journal should not be publishing studies in which so many experimental manipulations are inflicted in different combinations on laboratory rats. When so many variables are at play, how can we then learn anything of value? This was precisely my own reaction when I first received and read this paper. However, if you ignore your immediate response and read the rationale for the study design, you will appreciate that it is both logical and relevant to the evaluation and application of methods of fertility regulation in man and domestic animals in much of the world... .

He went further to query the use of gossypol in modern day scientific investigation, but quickly rationalized even this, based on the argument of the authors. The end-part of the comment reads:

> But most importantly, the quality of the scientific thought and practice in this paper is to be commended, not least because the circumstances under which this research was performed are probably a good deal worse than those which most of us take for granted.

In the light of these comments we must re-examine the current state of basic science research in our country and make concrete and determined efforts to advance scientific knowledge and improve the quality of life of both humans and animals.

Basic science research is pivotal in the development of new technologies and is the fastest route to a career in industry and technology. As today's high-tech industry is based on yesterday's basic research, so is our industrial and technological future based on the results of today's basic research. To attain sustainable development in science therefore, we need high

quality education—especially at the university level and cutting-edge research in our institutes. In a world where basic research is now being carried out at the biotechnological, molecular biology, genomic, proteomic, and metabolomic levels, the current situation in our university (or universities) leaves much to be desired. The lack of requisite research equipment and facilities in our laboratories, incessant power outages, and poor water supply militate greatly against rational and intelligent reasoning, which are keys to high productivity in basic research.

The dearth of research grants, poor funding, and non-availability of personnel (trained in biotechnology and molecular biology techniques) are also serious obstacles. Mr. Vice-Chancellor, at this juncture, mention must be made of the efforts by the current university administration to reverse the trend and point our university to the path of excellence. This is evidenced by the visible transformation of the campus and the remarkable achievement fostered by the MacArthur Foundation Grants which have assisted us a lot in the areas of Information Communication Technology (ICT), staff-training overseas, and the provision of a central multi-disciplinary research laboratory. Indeed, when the history of this university is written, certain names would stand out clearly in gold.

However, to attain and maintain world-class status to which this institution aspires, a lot more needs to be accomplished. In order to keep up with the ever advancing frontiers of scientific research, our university's academic exchange programme should be strengthened. Short- and medium-term training in international laboratories should continue as has been done in the last three years or so, while staff should be encouraged and sponsored to attend conferences and workshops both locally and internationally and to be members of specialized learned societies. The University as a matter of urgency needs to train a new crop of technologists particularly in the basic sciences.

At the risk of being too insistent on this issue of electric power and water generation, I think the university should seek to explore other alternatives for the provision of these basic amenities. While all kinds of suggestions have been proffered, I continue to wonder if dredging of our own Awba dam and the streams that supply it would not help in generating power and good drinking water, and in the process increase our internally

generated revenue. This can be based on a public-private partnership for the establishment of an independent power project that will benefit not only the university community, but also the immediate or surrounding communities. Yes, the big and usual question is: "Where is the money?"

Summary

I have, in the last forty minutes or so, shown how structural studies have been utilized in forming useful opinions on the functions of cells, tissues, and organs and how these can be utilized to advantage.

1. Unlike previously held opinion that the African giant rat is a seasonal breeder, our results show that the male giant rat can breed throughout the year. Therefore, seasonal changes may not affect a successful breeding of the rat. We have been able to elucidate this through morphological means.
2. Prior to our studies, the cell types in the epididymis of the giant rat had not been described. Our efforts have led to a detailed description of structure which is correlated with their functions, among which are protein secretion, absorption, local antigenic defense, and sperm maturation.
3. Our pioneering efforts in the studies of the female giant rat are bound to lead to a fuller understanding of the reproductive functions as evidenced by their structural peculiarities. It is our hope that these efforts will be translated to practical application at improving breeding in the giant rat especially with the aid of extension workers.
4. I have shown in this lecture that alternative materials and methods are available which can be exploited to replace expensive ones especially in testicular immunocytochemistry.
5. It has also been mentioned that feeding animals with gossypol-containing products especially when they are malnourished and trypanosome-infected could lead to impairment of reproduction even when there are no other signs of disease.

Acknowledgements

I wish to express sincere thanks to my parents: Papa, Simeon Emiola Oke and Mama, Irene Titilola Oke (nee Olukoga) both of blessed memory. Working together, they ensured that we, their children, all got quality education. Indeed, when I was yet a little boy, Papa was fond of calling me 'professor' because, according to him, I like reading all available literature, and my diction was way beyond my age. They both died before I could attain this position and to them, I dedicate this precious moment of my career.

I remember very vividly the very strong contribution of my principal at the Ijebu-Ode Grammar School, Rev. N. E. Ade Osisanya. I cannot fail to mention my mentor, Professor Tom Aire. I thank the late Dr. Charles Oluseyi Osunfisan, former CVO, Director of Veterinary Services and later Permanent Secretary in the Ogun State Civil Service; Drs Martin Dym, Carlos Suarez-Quian, Vasilli Papadopoulos and Mr. William Vornberger (all of the Anatomy Department of Georgetown University Washington D.C., USA), and the Rockefeller Foundation.

I will also like to thank my colleagues in the Faculty, for their immense love and cooperation, during my stint as Dean of the Faculty. In this connection, I must mention the past Deans especially my predecessor, Professor Arowolo, who nudged me into contesting for that office when I was not even thinking of it. I also thank the current Dean, Professor G.A.T. Ogundipe and all members of the Faculty especially those who worked with me during our modest effort in the Dean's Office at that time. My department has always been, for me, another home. It is such a wonderful place with wonderful people. I thank everyone for the wonderful working relationship, and pray it continues.

I want to acknowledge all members of the Oke and Olukoga families as well as my in-laws, the Aderinokuns, for their love and fraternity. May God continue to raise you all up. Amen. I certainly will not forget to publicly thank my wife, Professor Gbemisola Oke, my three children, Damilola, Babajide, and Temitope for their loving care, devotion, and understanding all these years. Finally, I thank God for all He has been to me over

the years and for making it possible for me to be alive today to give this lecture.

Mr. Vice-Chancellor, ladies and gentlemen, I thank you for your attention.

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