THE STRUCTURE OF MALE REPRODUCTIVE ORGANS IN LAKE MAGADI TILAPIA (*Alcolapia grahami*)

A thesis submitted in partial fulfillment of the requirements for Master of Science degree (Veterinary Anatomy) of the University of Nairobi.

MICHAEL BABAK PAPAH, BVM, (UoN)

Department of Veterinary Anatomy and Physiology

University of Nairobi

DECLARATION

This thesis is my original work and has not been presented for a degree in any other University.

Michael Babak Papah, BVM (UoN)

Signature.....Date 09/08/2012

This thesis has been submitted for examination with our approval as University supervisors

1. Dr. Daniel W. Onyango, BVM, MSc, PhD

   Signature.....Date 09/08/2012

2. Prof. Seth M. Kisia, BVM, PhD

   Signature.....Date 9/8/2012

3. Dr. Rodi O. Ojoo, BVM, MSc, PhD

   Signature.....Date 9/8/2012

DEDICATION

This thesis is dedicated to my grand mother, Josephine Atiang', who instilled in me the value of hard work at a very early age.

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ABSTRACT

Alcolapia grahami (formerly *Oreochromis alcalicus grahami*) is a small cichlid fish inhabiting the highly alkaline (pH~10) and hot (temp 25-42°C) lagoons of Lake Magadi, Kenya. Despite the growing number of studies that are being conducted on this fish, there remains paucity of information on their reproductive structure, associated adaptations and functions. In this study, the morphology of the mature male reproductive organs was studied using macroscopic, histological and ultrastructural techniques. The aim was to establish the structural organization of the reproductive organs in this fish and their adaptive features, if any, in relation to their survival in the severe Lake Magadi habitat.

Twenty mature fish specimens, obtained using seine nets from the Fish Springs Lagoon of Lake Magadi, were sexed, weighed, dissected and testes fixed and processed for routine histological and transmission electron microscopy. The male reproductive system comprised creamy white bilobed testis, oriented craniocaudally within the coelomic cavity and caudally connected to the spermatic duct which exited through the genital papilla. The coelomic cavity was lined by a parietal peritoneum containing a thin pigmented membrane laden with a highly opaque substance on the medial aspect, thought to protect the testis against UV radiation. The testicular parenchyma was bound by a capsule and comprised the germinal and interstitial compartments. The two compartments were separated by a lamina propria formed primarily by myoid cells. The germinal compartment comprised seminiferous lobules ending blindly at the testicular capsule. Several spermatocysts containing germ cells at the same stage of development occupied each seminiferous lobule. The spermatids underwent three developmental stages exhibiting type-I

spermiogenesis and culminating in the formation of typical primitive spermatozoa (aquasperm). At the end of spermatogenesis, mature cysts opened to release spermatozoa into the lobule lumen. The organization of the germinal compartment, therefore, presented an unrestricted lobular testicular type exhibiting complete cystic spermatogenesis. Sertoli cells rested on the basal lamina, appearing generally flat and elongated although a few appeared cuboidal in shape. They contained numerous membrane bound vesicles, phagolysosomes and cellular debris; structural characteristics that seemed to suggest their involvement in resorption and phagocytosis of damaged cells and residual bodies during spermiogenesis. They produced cytoplasmic extensions that formed the walls of cysts found in the lobular testes.

The interstitial compartment contained Leydig cells, fibroblasts and blood vessels. Leydig cells were spheroidal in shape with abundant lipid droplets, smooth endoplasmic reticulum and mitochondria in their cytoplasm, indicating a role in testicular steroidogenesis. Fibroblasts occurred both in the interstitial and boundary tissues, and were considered to be the precursor of some of the connective tissue components. The interstitium between the efferent ducts in some fish exhibited nodular structures containing spheroidal cellular aggregates thought to arise following stressful conditions in the lake.

The excurrent duct system consisted of collecting, efferent as well as main spermatic ducts intratesticularly while the common spermatic duct and genital papilla were located extratesticularly. Spawning appeared to initiate increased folding and apocrine secretion by the epithelium of the common spermatic duct leading to increased luminal surface area and seminal

fluid production. These findings show that this fish exhibits regular spermatogenesis irrespective of the harsh environmental conditions in which they live. The role of other adaptive mechanisms employed by this fish in ensuring normal body functions including spermatogenesis in the face of the extreme environmental conditions in the lake cannot be overruled.

CHAPTER 1

1.0 INTRODUCTION

1.1 General description of Lake Magadi tilapia

Lake Magadi tilapia, *Alcolapia grahami* (formerly *Oreochromis alcalicus grahami*), is a tilapiine cichlid teleost endemic to Lake Magadi; a highly alkaline and saline lake in Kenya (Coe, 1966; Seegers and Tichy, 1999; Walsh *et al.*, 2001; Wood *et al.*, 2002a; Denson *et al.*, 2010). It is a small-sized fish weighing up to 54g and grows to a maximum length of 11.5 cm (Maina *et al.*, 1996; Seegers and Tichy, 1999). This fish lives in isolated lagoons supplied by highly alkaline geothermal springs around the lake. They primarily feed on blue green algae (cyanobacteria) and, to a small extent, crustacean and dipterous larvae (Coe, 1966; Narahara *et al.*, 1996; Seegers and Tichy, 1999). In its natural environment, *A. grahami* eats and drinks throughout the day although it appears to do more of the latter at night (Bergman *et al.*, 2003; Onyango and Kisia, 2008).

1.2 Taxonomy

Lake Magadi tilapia belongs to the kingdom *Animalia*, phylum *Chordata*, sub-phylum *Vertebrata,* infraphylum *Gnathostomata*, class *Teleostei*, sub-class *Actinopterygii* and infraclass *Actinopterii.* Further examination of the taxonomic tree indicates that Lake Magadi tilapia belongs to the largest superorder, *Acanthopterygii,* which consists of three main series: *Mugilomorpha, Atherinomorpha* and *Percomorpha.* This fish is grouped in the series *Percomorpha*, which is the largest of all consisting of orders: *Perciformes, Scorpaeniformes, Pleuronectiformes*, *Dactylopteriformes* and *Tetraodontiformes*. Lake Magadi tilapia belongs to

the order *Perciformes* which, in addition, contains about 10,000 species of fish in 160 families, comprising about a third of all the fishes (**Fig. 1**). This order is generally considered the largest in fish as well as vertebrate taxonomy. *Percifomes* are thought to have evolved during the early Cenozoic era (65.5million years ago), with most members evolving at Eocene geological time (55.8 to 33.9 million years ago), indicating a very rapid evolution and diversification over a period of about 20 million years (Gene *et al.*, 2009). The order *Perciformes* has about 15 sub-orders including the sub-order *Labroidei* to which this fish belongs. The suborder *Labroidei* contains about 2,274 fish species in 6 families: *Cichlidae, Embiotocidae, Pomacentridae, Labridae, Odacidae* and *Scaridae*. This fish belongs to the family *Cichlidae*, which forms the basal group of the sub-order. The family contains about 236 genera with 1,350 cichlid species. Within the family, Lake Magadi tilapia is grouped under the sub-family and tribe *Pseudocrenilabrinae* and *Tilapiini* respectively (**Fig. 1**). Lake Magadi tilapia belongs to the genus *Alcolapia* which has 4 subgenera namely; *A. ndalani*, *A. latilabris* and *A. alcalicus* that inhabit Lake Natron, Tanzania, and *A. grahami* endemic to Lake Magadi, Kenya (**Fig. 1**). *Alcolapia grahami* was formally referred to by other names: *Tilapia grahami*, *Alcolapia alcalicus grahami, Sarotherodon alcalicus grahami* (Trewavas, 1983) and *Oreochromis alcalicus grahami* (Seegers and Tichy, 1999).

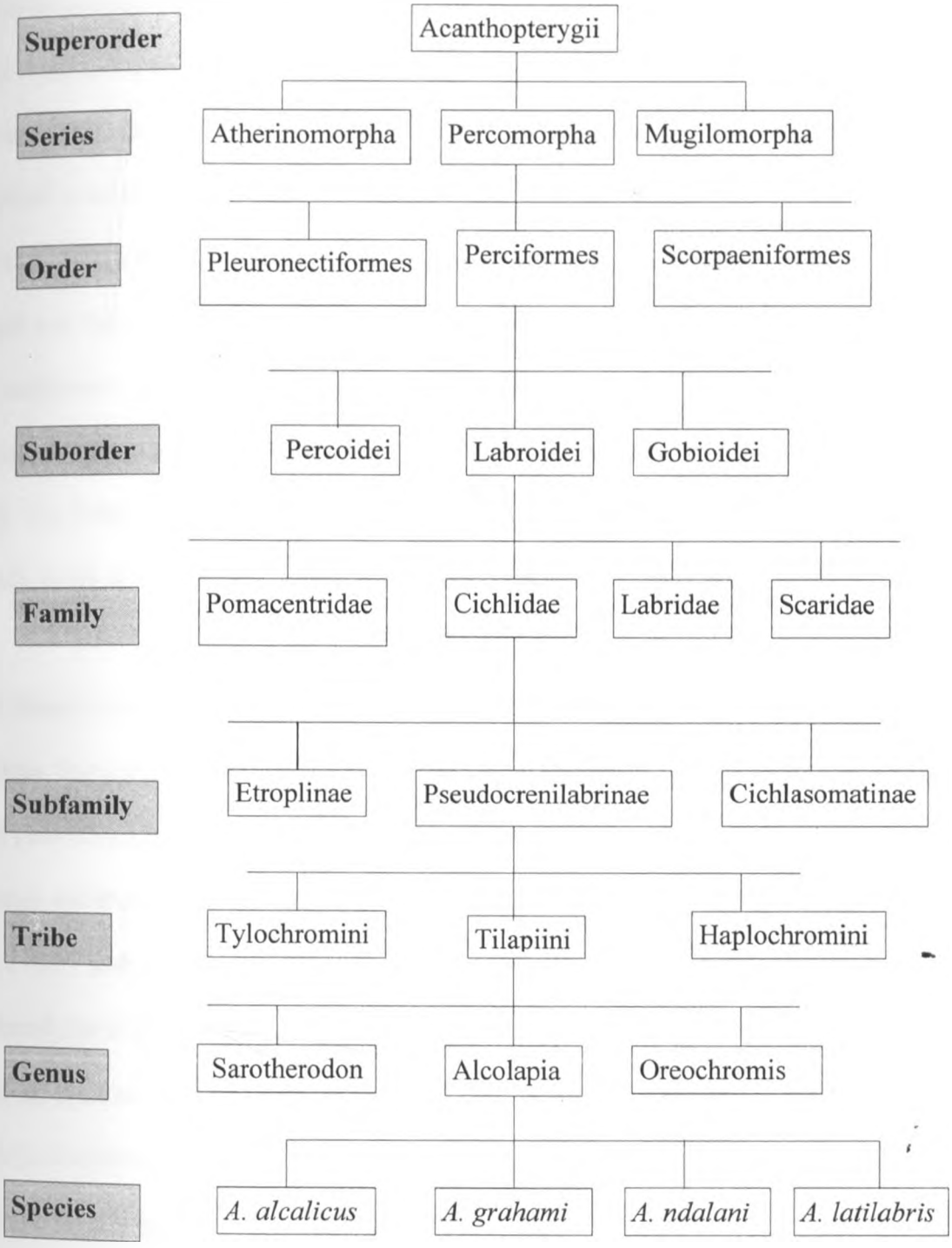


FIG. 1: Taxonomic tree of Lake Magadi fish

1.3 Habitat and distribution

Alcolapia grahami is the only vertebrate species that inhabits Lake Magadi. This lake is located on the floor of the Eastern arm of the Great Rift Valley of Kenya. It lies on latitude 1°43' to 2° 00'S and longitude 36° 13' to 36° 18'E and is the southernmost rift valley lake in Kenya. The lake is situated at an altitude of 600m above sea level and covers an area of about 100km^2. Although the lake is frequently considered as one, it actually exists as two separate lakes; the greater Magadi and the little Magadi. The former is regarded as the main lake with 90% covered by a thick solid crust of trona (about 15m thick) composed of sodium carbonate and sodium bicarbonate, commercially exploited by the Magadi Soda Company (Coe, 1966; Maina *et al.*, 1996). The little Magadi, on the other hand, is located 0.5km to the Northwest of the greater Magadi, lying at an altitude of about 620m above sea level (Wilson *et al.*, 2004).

Lake Magadi was once part of a continuous body of water with Lake Natron, called Lake Orolonga that had only one type of tilapia similar to the current Lake Magadi fish. The division of the paleolake Orolonga then began leading to the formation of the southern Lake Natron in Tanzania and the northern Lake Magadi in Kenya; a process that spanned over 7,000 to 10, 000 years (Tichy and Seegers, 1999; Seegers *et al.*, 1999; Wilson *et al.*, 2004). As separation continued, Lake Magadi water levels began to fall owing to shifting of the water source from humid environments to thermal hot springs; a process that occurred during the end of Pleistocene epoch (2.6 million to 11,700 years ago) (Wilson *et al.*, 2004). Selection pressures on the fish continued to pile up due to increased salinity, consequently, separation of the lake into lagoon system began. Intense selection pressures continued within the larger Lake Magadi resulting into

isolation of the lagoon environments. These lagoons were essentially separated by vast areas of trona overlying highly alkaline and anoxic water which could not support fish life (Wilson *et al.*, 2004). The lagoons therefore became the only habitable areas for the fish in the lake. The fish lagoons were strategically distributed at the periphery of the lake where hot alkaline springs discharged their water directly before it entered the main lake, providing a conducive environment for the survival of the fish and algae. There are six lagoons in the larger Lake Magadi basin offering a natural habitat for Magadi tilapia populations. These are: the Fish Springs Lagoon, Sports Club Lagoon, Southeast Lagoon, Southwest Lagoon, Wilson Springs Lagoon and West Lagoon. As at June 2010, the Sports club lagoon had dried up. The Northwestern Little Magadi, however, did not divide into lagoons as was the case in the main lake. It also provides another habitat for the Lake Magadi fish (Wilson *et al.*, 2004).

1.3.1 The aquatic conditions of the lake

Lake Magadi is almost entirely covered by a solid crust of trona ($Na_2CO_3/NaHCO_3$) underlain by anoxic water, hence, the fish occur only at the edges of the lake in shallow lagoons fed by underground hot springs (Coe, 1966). The water parameters of these lagoons present some of the most severe aquatic environments for the survival of any teleost in the world. Generally, the water chemistry in the Lake Magadi lagoons and little Magadi present very high pHs, alkalinity, salinity, temperatures, osmolality, Na^+ and Cl^- levels. The Fish Springs Lagoon is the most studied site whose water has been referred to as the "ideal Magadi water". The water parameters here include: osmolality close to 600mOsmL^{-1}(about 50% that of sea water), alkalinity of 380mmolL^{-1}, pH ~ 10, Na^+ ~350 mmolL^{-1}, Cl^- ~110 mmolL^{-1} and very low carbon dioxide

content reaching a high of 200mmolL^{-1}(Narahara *et al.*, 1996; Onyango and Kisia, 2007). There is diurnal fluctuation of temperature, ranging from less than 25°C at night to 40°C, sometimes even rising as high as 46°C at mid-day and a corresponding diurnal fluctuation of dissolved O_2 levels, ranging from virtual anoxia at night to extreme hyperoxia during the day. This phenomenon occurs as a result of respiratory processes of cyanobacteria in the lake at night followed by photosynthetic activities during the day (Johansen *et al.,* 1975; Wood *et al.,* 1989; Johnston *et al.*, 1994; Maina *et al.*, 1996; Narahara *et al.*, 1996; Kisia and Onyango, 2007). However, there are distinct differences in water parameters among the lagoons in Lake Magadi. Those with the most severe parameters are the little Lake Magadi and Sports Club Lagoon where the osmolality was 1,682 mOsmkg^{-1} and 1,465mOsmkg^{-1} and, alkalinity of 1,251 mEqL^{-1} and 1,625 mEqL^{-1} respectively. On the other hand, the lagoon with the least severe conditions was Wilson Springs with 278 mOsmkg^{-1} and 184 mEqL^{-1} as osmolality and alkalinity levels respectively (Wilson *et al.,* 2004). Fish in these lagoons are often subjected to intense ultra-violet solar radiation and predation by birds (Narahara *et al.*, 1996; Wood *et al.*, 2012).

1.3.2 Adaptations of *A. grahami* to the lake environment

A. grahami is the only known vertebrate inhabiting this lake and, perhaps, because of severe environmental pressures, they grow to a relatively small size (Maina *et al.*, 1996; Onyango and Kisia, 2007). Various physiological and structural adaptations of *A. grahami* designed to help it cope with the harsh environmental pressures in both the greater Lake Magadi lagoons and the little Magadi have been reported. The most important and unique adaptation of this fish, unlike other teleosts, is the excretion of all its nitrogenous waste as urea instead of ammonia (Randall *et*

al., 1989; Wood *et al.*, 1989; Wilson *et al.*, 2004). This adaptation is very critical in overcoming the difficulty of excreting ammonia into the highly alkaline and buffered environment (Wilkie and Wood, 1996). The conversion of nitrogenous waste into urea has been shown to occur via the Ornithine urea cycle throughout the white muscle mass (Lindley *et al.*, 1999) as well as the liver (Randall *et al.*, 1989; Wood *et al.*, 1994). Urea excretion, which is obligatory in this fish (Wood *et al.*, 2002a), occurs across the gills which are many times more permeable to urea compared to those of other teleosts (Walsh *et al.*, 2001). Urea transport is facilitated by a urea transporter found in pavement cells of the gill epithelium (Walsh *et al.*, 2001; Wilkie, 2002). This feature makes this fish wholly ureotelic which is unique among teleosts, rendering the species of special evolutionary importance (Wood *et al.*, 1989; Randall *et al.*, 1989; Wilson *et al.*, 2000, 2004; Onyango and Kisia, 2007).

The respiratory adaptations include high blood O_2 affinity (Narahara *et al.*, 1996) and a large gill surface area. The gill filaments have relatively large numbers of mitochondria-rich cells exhibiting abundant intracytoplasmic microtubular network. These features, together with a thin diffusion distance of the gills, ensure a high diffusing capacity for O_2 (Maina, 1990, 1991; Laurent *et al.*, 1995; Maina *et al.*, 1996; Onyango and Kisia, 2007). In addition, under hypoxic conditions, this fish often resorts to air breathing (Franklin *et al.*, 1995; Narahara *et al.*, 1996; Maina, 2000). This is enabled by the engagement of its physostomous and well vascularized swim-bladder to supplement gaseous exchange through the gills (Maina *et al.*, 1995, 1996; Maina, 2000; Kisia and Onyango, 2007).

Regarding the adaptation of the digestive system, these cichlids have evolved a unique pyloric bypass system which involves the formation of a temporary connection of the oesophagus and the pylorus when the stomach is empty. This system, therefore allows shunting of the highly alkaline lake water from the oesophagus directly into the duodenum without interfering with the acidic stomach (Narahara, 2000; Bergman *et al.*, 2003; Onyango and Kisia, 2007). Regarding osmoregulation, they drink water at a relatively high rate (Wood *et al.*, 2002b; Onyango and Kisia, 2007), hence, circumventing hyperosmolality through absorption of Na^+ and HCO_3^- using the respective co-transport systems found in the gut wall. As a result, water is also absorbed due to concentration gradient already created by the mono-ions (Bergman *et al.*, 2003). Besides the gut, the gills also participate in osmoregulation by allowing the excretion of excess ions (Eddy and Maloiy, 1984; Wright *et al.*, 1990) via a typical marine chloride cell system found on the branchial epithelium (Maina, 1990, 1991; Laurent *et al.*, 1995; Onyango and Kisia, 2007). Furthermore, the gills of Magadi tilapia have unusually low permeability to bicarbonate ions but high electrogenic bicarbonate extrusion capacity enabling them to survive in this environment of very high alkalinity (Wood *et al.*, 2012).

Other physiological adaptations noted include: stability of haemoglobin despite the high environmental temperature (Franklin *et al.*, 1994), maintenance of extremely high pH levels in blood and tissues (Wood *et al.*, 1994), absence of the Bohr Effect in blood and a marked depression of metabolic demand when temperature falls at night (Narahara *et al.*, 1996).

1.4 Reproduction and breeding

Very little information is available regarding reproduction and breeding in this fish. It is known that both sexes exhibit different behaviors during the breeding period. For example, mature breeding males, which usually display brilliant blue colour with white lips, construct pits at the edge of lagoons to attract sexually active females to spawn. Breeding females, usually display brownish grey colour and are mouth brooders. The fertilized eggs in the mouth generally take 12 to 16 days to hatch (Coe, 1966; Seegers and Tichy, 1999). Sexual dimorphism is not obvious in this fish although colour changes in females are indicative of the onset of spawning (Coe, 1966).

Therefore, this study sought to critically examine the structure of the male reproductive system of Lake Magadi tilapia using both macroscopic and microscopic techniques, with the aim of filling in gaps in the knowledge of reproduction in this fish. This study also sought to scrutinize the process of spermatogenesis in this fish with a view to determining existence of any unique feature suggestive of reproductive adaptation in the harsh environment of the lake.

CHAPTER 2

2.0 LITERATURE REVIEW

Fish remains the most numerous and diverse group of vertebrates adapted to life in a great variety of aquatic habitats (Lacerda *et al.*, 2006; Alvarenga and França, 2009). This diversity is demonstrated in their reproductive strategies that range from external to internal fertilization (Grier, 1981), modes of parental care, behavior, social structures (Fishelson, 2003), gonadal structure (Parenti and Grier, 2004), spermiogenesis and structure of spermatozoa (Mattei, 1991; Jamieson, 1991).

2.1 The male reproductive system

The male reproductive system of fish, as in other vertebrates, consists of the gonads (testes) and genital tracts that provide passage of mature germ cells to the exterior. The primary roles of this system in teleosts, therefore, are to generate the male germ cells and, production and controlled release of male sex steroids into the body (Matta *et al.*, 2002; Weltzien *et al.*, 2004). Studies on the male reproductive system of fish have continued to generate a lot of interest owing to their extreme diversity, hence, the information available is enormous.

2.1.1 The testes

There has been interest in the testicular structure of various teleostean fish from as early as 1890 (Fishelson, 2003) to the present times (Alvarenga and França, 2009; Schulz, *et al.*, 2010). In this group of fish, the testis is an elongated whitish organ stretching through the entire length of the

body cavity and connected to the dorsal body wall via the mesorchium. It is connected to a sperm duct that terminates at the urogenital papilla, caudal to the anal pore (Weltzien *et al.*, 2004). In most teleosts, the testes are paired organs, except in poeciliids where single sac testes have been observed (Nagahama, 1983). In Nile tilapia, *Oreochromis niloticus,* a fresh water fish and a close relative of *A. grahami,* the testes are paired organs, whitish in colour and located in the coelomic cavity, dorsal to the intestines, ventral to the kidneys and ventro-lateral to the swim bladder (Lacerda *et al.*, 2006). In general, the testis in fish is surrounded by a capsule (tunica albuginea) comprising the outer peritoneal layer and an inner myoid cell layer. Found between these layers are collagen fibers, small blood vessels, phagocytes, fibrocytes and mesenchymal-like cells (Koulish *et al.*, 2002; Lopes *et al.*, 2004; Fishelson *et al.*, 2006). Structurally, as in other vertebrates, the teleost testis is organized into two major compartments; the germinal and interstitial compartments separated by the basement membrane.

2.1.1.1 The germinal compartment

The germinal compartment is made up of seminiferous lobules/tubules containing spermatocysts at various stages of development. A spermatocyst is a unit consisting of an individual or group of germ cells at a defined stage of spermatogenesis enclosed by the cytoplasmic processes of Sertoli cells (Nóbrega and Quagio-Grassiotto, 2007).

The structural design of the germinal compartment within the testis of a teleost is a fixed characteristic among different fish taxa, hence, useful in defining the testicular type of a particular fish species in relation to its taxonomic grouping (Grier, 1993; Parenti and Grier, 2004). In this regard, two main testicular architectural types have been recognized in teleosts

namely; the anastomosing tubular testis type possessing the tubular germinal compartment found in the primitive osteichthyans (lower teleosts) and the lobular testis type which possesses lobular germinal compartment found in the atherinomorphs and higher teleosts/neoteleosts (Grier, 1993; Weltzien *et al.*, 2002; Parenti and Grier, 2004).

In the anastomosing tubular type, the germinal compartment form highly branched and anastomosing loops or tubules similar to those in mammals. This testicular type has been described in the orders Characiformes, Salmoniformes and Cypriniformes (Parenti and Grier, 2004). On the other hand, the lobules, separated by the interlobular boundary tissue in the lobular type, extend to the periphery of the testis terminating blindly beneath the tunica albuginea (Grier, 1993; Weltzien *et al.*, 2002; Parenti and Grier, 2004).

Moreover, the lobular testis type can further be divided into restricted and unrestricted types based on the arrangement and distribution of the primary spermatogonia within the testis. In the restricted type, primary spermatogonia are permanently confined to the terminal blind endings of the lobules found beneath the tunica albuginea, as exhibited in the orders Atheriniformes, Cyprinodontiformes and Beloniformes (Parenti and Grier, 2004). In this case, therefore, spermatogenesis entails migration of spermatocysts with developing germ cells down the lobule to eventually release mature spermatozoa into the efferent duct system at the end of spermiogenesis (Grier, 1993; Loir *et al.*, 1995; Parenti and Grier, 2004). Conversely, the unrestricted testis type presents spermatogonia distributed along the entire length of the germinal compartment throughout the testis. In this regard, spermatogonial cysts do not migrate or get displaced during development, but rather, grow and mature at the same spot throughout the process of spermatogenesis where ripe cysts eventually open to release mature spermatozoa into

the lumen (Schulz *et al.*, 2010). This type of testis is seen in the orders Percopsiformes, Ophidiiformes and Lophiiformes (Parenti and Grier, 2004). These two major types (restricted and unrestricted) of the lobular testis notwithstanding, an intermediate type exists where the undifferentiated type A spermatogonia show preferential but not exclusive location close to the tunica albuginea. The latter testicular type is found in a number of orders, for example, Perciformes, *Oreochromis niloticus* (Vilela *et al.*, 2003), Pleuronectiformes, *Solea senegalensis* (García-López *et al.*, 2005) and Gadiformes, *Gadus morhua* (Almeida *et al.*, 2008). Tubular testis type has also been shown to manifest unrestricted distribution of type A spermatogonia in the seminiferous tubules as evidenced in Zebra fish (Leal *et al.*, 2009).

In teleosts, spermatocysts open at specific stages during spermatogenesis. This phenomenon has been used to determine the type of spermatogenesis in fish. Consequently, two types of spermatogenesis have been distinguished: the cystic and semi-cystic types. The cystic type is the commonest, found in most teleosts (García-López *et al.*, 2005), where the entire process of spermatogenesis occur within the spermatocyst. At the end of the process, the cysts burst open to release mature spermatozoa into the lobular or tubular lumen (Mattei *et al.*, 1993). The semi-cystic type, on the other hand, involves release of germ cells from the cysts at some point during spermatogenesis for the process to be completed in the lumen. Semi-cystic spermatogenesis has been described in some fish species, for example, *Ophidion sp.* (Mattei *et al.*, 1993), *Opisthognathus whitehursti* (Manni and Rasotto, 1997), *Solea senegalensis* (Garcia-Lopez *et al.*, 2005), *Malapterurus electricus* (Shahin, 2006) and *Corydoras flaveolus* (Spadella *et al.*, 2007).

2.1.1.1.1 Cellular components of the germinal epithelium

2.1.1.1.1.1 Sertoli cells

The functional spermatogenic unit in fish, the spermatocyst (cyst), is formed when a single type A spermatogonium is completely enveloped by the cytoplasmic processes of Sertoli cell/s (Loir *et al.*, 1995; Schulz *et al.*, 2005). The non-differentiated spermatogonium within this unit then develops into highly differentiated spermatozoa (Koulish *et al.*, 2002). Generally, the developing germ cells within a particular cyst are derived from a single primary spermatogonium. This germ cell undergoes several synchronous mitotic and/or meiotic divisions, giving rise to a clone of isogenic germ cells that, at any one given time, are at the same stage of development. Therefore, Sertoli cells in anamniotic vertebrates (fish and amphibians) enclose a single germ cell clone throughout the spermatogenic process. This phenomenon is in contrast to the non-cystic spermatogenesis exhibited in reptiles, birds and mammals where Sertoli cells contact different germ cell clones at the same time (Weltzien *et al.*, 2004; Nóbrega *et al.*, 2009; Schulz *et al.*, 2010). Unlike mammalian species, where Sertoli cells show no proliferation after puberty (Sharpe, *et al.*, 2003), in adult fish, these cells retain their capacity to proliferate, ceasing only when the blood-testis barrier is formed. This feature has been associated with the increase in testis size and sperm production in teleosts (Schulz *et al.*, 2005). Furthermore, Sertoli cells in teleosts possess a higher efficiency (capacity to support germ cells) in comparison to mammalian ones (Vilela *et al.*, 2003; Schulz *et al.*, 2005; Nóbrega *et al.*, 2009).

Sertoli cells play an important role in the creation and provision of specific micro-environment necessary for the development of germ cells (Schulz and Miura, 2002; Schulz, 2003). In addition, they have been shown to play a key role in the phagocytosis of residual bodies, apoptotic as well as degenerating germ cells (Chung, 2008) besides harboring receptors for both follicle stimulating hormone and androgens (Weltzien *et al.*, 2004). They are also thought to aid in; the secretion of fluids into the tubular lumen, nutrition of germ cells, production of androgen binding proteins and the orthologue of antimullerian hormone as shown in the eel and flounder (Grier, 1981; Billard, 1986; Schulz and Miura, 2002; Schulz, 2003; Nóbrega *et al.*, 2009; Schulz *et al.*, 2010). Furthermore, Sertoli cell interactions, for example inter-Sertoli cell and Sertoli-germ cell interactions, serve to provide structural and physiological support to the developing germ cells during spermatogenesis in fish (Loir *et al.*, 1995; Nóbrega *et al.*, 2009). The inter-Sertoli cell interactions of importance involve junctional complexes which include intermediate adhering junctions (desmosomes), complex inter-digitations at the apex of Sertoli cells (Abraham, 1991; Loir *et al.*, 1995; Batlouni *et al.*, 2005) and gap junctions (Marina *et al.*, 2002; Batlouni *et al.*, 2005, 2009; de Montgolfier *et al.*, 2007). Once proliferation of Sertoli cells cease, which corresponds to the time when the cysts reach their maximum volume (usually at the pachytene stage of meiosis), occluding (tight) junctions appear resulting in the formation of the blood-testis barrier (Abraham, 1991; Loir *et al.*, 1995; Schulz *et al.*, 2005; Batlouni *et al.*, 2009). Subsequently, this barrier restricts molecules from the vascular and immune systems from interacting with the haploid germ cells in the cysts (Loir *et al.*, 1995; Leal *et al.*, 2009; Schulz, *et al.*, 2010). Unlike mammalian vertebrates where there is continuous destruction and regeneration of the Sertoli cell barrier to permit movement of developing germ cells from the basal to the

adluminal compartment, in fish, this phenomenon does not occur since all the germ cells in a given cyst develop synchronously (Abraham *et al.*, 1980; Batlouni *et al.*, 2009).

Structurally, Sertoli cells in teleosts are more-or-less squamous with elongated nuclei oriented in the direction of tunica propria (Koulish *et al.*, 2002) in contrast to the columnar type observed in mammals (Russell *et al.*, 1990). Their cytoplasm contain smooth and rough endoplasmic reticulae with the former having well developed vesicular and tubular elements. They also have longitudinally oriented actin and intermediate filaments often associated with desmosomes (Koulish *et al.*, 2002; Fishelson, 2003).

2.1.1.1.1.2 Spermatogenic cells

Spermatogenic cysts consist of a variety of germ cells at different stages of development occurring in several generations within the seminiferous tubule or lobule. The cysts are named according to the germ cells they contain and include spermatogonial, spermatocytic and spermatid cysts. Identification of spermatogenic cells is based on the nuclear and cytoplasmic changes including the density and distribution of chromatin (Grier, 1981).

Spermatogonial germ cells in fish exist as type A (primary spermatogonia) and type B (secondary spermatogonia). Type A spermatogonia is further divided into type A immature (undifferentiated) and type A mature (differentiated) (Schulz *et al.*, 2005, 2010; Nóbrega *et al.*, 2009). Type A undifferentiated spermatogonium occur individually and are neither connected with each other via cytoplasmic bridges nor lie directly on the basement membrane as commonly encountered in

mammals. They are always enclosed by Sertoli cells and are found closest to the tunica albuginea (Matta *et al.*, 2002; Schulz *et al.*, 2005; Nóbrega *et al.*, 2009). The type A differentiated spermatogonia, on the other hand, is smaller in size compared to its predecessor. They arise from the mitotic division of a single primary spermatogonium resulting into cells connected by cytoplasmic bridges due to incomplete cytokinesis (Loir *et al.*, 1995; Schulz *et al.*, 2005, 2010). The existence of cytoplasmic bridges allows synchronized germ cell division and development in the entire process of spermatogenesis, forming an isogenic clone of germ cells within a cyst. The bridges eventually break at the terminal stages of spermiogenesis to free the germ cells (Schulz *et al.*, 2005; Nóbrega *et al.*, 2009). Type B spermatogonia results from successive mitotic divisions of mature type A spermatogonia (Loir *et al.*, 1995; García-López *et al.*, 2005). They occur in several generations whose number is dependent on the fish species (Ando *et al.*, 2000); for example 6 in rainbow trout (Loir, 1999), 9 in zebrafish (Leal *et al.*, 2009) and 7 in Nile tilapia (Schulz *et al.*, 2005). After the final mitosis, the most mature type B spermatogonia then differentiate into primary spermatocytes which enter the first and second meiotic divisions to yield secondary spermatocytes and round haploid spermatids respectively (Fishelson *et al.*, 2006; Nóbrega *et al.*, 2009). Spermatocytes exist in various forms identifiable mainly on the basis of their nuclear characteristics such as size, chromosome condensation and presence or absence of synaptonemal complexes, and presence of meiotic figures (Fishelson *et al.*, 2006; Chung, 2008; Leal *et al.*, 2009; Chung *et al.*, 2010; Schulz *et al.*, 2010).

The process of spermatogenesis is concluded by the events of spermiogenesis where the round haploid spermatids are transformed to elongated spermatozoa. The morphological

transformations that occur on spermatids during spermiogenesis include nuclear condensation, elimination of excess cytoplasm and organelles and formation of flagellae (Jamieson, 1991; Fishelson *et al.*, 2006; Shahin, 2007; Schulz *et al.*, 2010). The process of spermiogenesis, therefore, presents three histomorphological stages of spermatids in teleosts, whose identification is based on homogeneity and/or condensation of chromatin material within the nucleus and inter-spermatid space. The stages are the early, intermediate and late spermatids (Schulz *et al.*, 2005; Leal *et al.*, 2009). Furthermore, ultrastructural studies on spermiogenesis in teleosts have revealed presence of three distinct types: types I, II, and III spermiogenesis. These types have been identified on the basis of either the presence or absence of nuclear rotation and orientation of the flagellum to the nucleus (Shahin, 2006; Quagio-Grassiotto and Oliveira, 2008; Schulz *et al.*, 2010). Type I spermiogenesis involves nuclear rotation with the flagellar axis oriented parallel to the nucleus in the early spermatids and perpendicular to the same nucleus in mature spermatozoa. Type II spermiogenesis shows no nuclear rotation, hence, the flagellum remains in a parallel or lateral position to the nucleus (Lahnsteiner and Patzner, 1997; Quagio-Grassiotto and Oliveira, 2008). Similar to type II spermiogenesis, type III spermiogenesis exhibits no nuclear rotation but results into a perpendicularly oriented flagellum without a cytoplasmic channel (Jamieson, 1991; Shahin, 2006, 2007; Quagio-Grassiotto and Oliveira, 2008). Apart from these three spermiogenic types, some fish species, for example, the cichlid *Oreochromis niloticus* (Lou and Takahashi, 1989), cyprinid *Cyprinus carpio* (Billard, 1986) and characid *Paracheirodon innesi* (Jamieson, 1991) demonstrate incomplete or partial nuclear rotation, hence, the flagellar axis is eccentrically oriented with respect to the nucleus.

Ultimately, the final products of spermiogenesis in teleosts, the spermatozoa, are presented as two types; aquasperm and introsperm, based on the mode of reproduction of the fish. Most teleosts exhibit external fertilization and therefore have retained the simple primitive spermatozoa known as the aquasperm, while the internally fertilizing ones have advanced type of spermatozoa referred to as introsperm (Jamieson, 1991; Mattei, 1991).

Morphologically, aquasperms are characterized by round to oval heads and short midpieces with few mitochondria while introsperms have elongated heads and long symmetrical midpieces with numerous mitochondria (Spadella *et al.*, 2006). Both types lack an acrosome or an acrosomal vesicle (Jamieson, 1991; Mattei, 1991); a phenomenon associated with lack of granulosa cells and presence of a micropyle on the post ovulatory oocyte to allow sperm entry (Patino and Sullivan, 2002). However, some fish species in the genera *Protopterus*, *Polypterus* and *Acipenser* possess a short acrosome in their spermatozoa (Mattei, 1991).

In general, a fish spermatozoon consists of a head, a midpiece and one or two flagellae. The head contains dense and homogeneous chromatin material, while the midpiece possesses varying numbers of mitochondria, with presence or absence of a cytoplasmic canal (Jamieson, 1991; Mattei, 1991; Schulz *et al.*, 2010). The number, shape and distribution of mitochondria around the midpiece determine the symmetry of the latter (Fishelson, 2003). Once spermatozoa within a cyst become mature enough, they break loose from the cytoplasmic bridges that connected them during spermatogenesis to become free and individualized (Fishelson *et al.*, 2006). The Sertoli cell cytoplasmic processes that constitute the cyst's wall degenerate, enabling the release of mature and free spermatozoa into the lobule lumen (Fishelson, 2003). This notwithstanding, some fish species show no individualization of spermatozoa during release, but rather form sperm

bundles which may either be in the form of spermatophores; the Sertoli cell secreted capsule containing spermatozoa (Grier, 1984) or spermatozeugmata which are Sertoli cell projections enclosing gametes against the luminal side of the cyst (Grier, 1993; Schulz *et al.*, 2010).

2.1.1.2 Lobular boundary tissue

2.1.1.2.1 Myoid cells

These are cells observed in teleost testes frequently bordering seminiferous lobules/tubules and, in some cases, occur between juxtaposed lobules, almost obliterating the entire space between them (Lo Nostro *et al.*, 2004). However, these cells do not always form complete circumferential layer around testicular lobules as is the case in mammals (Grier, 1981).

The structural and ultrastructural picture of the teleostean myoid cell, by and large, compares to the mammalian one (Dym and Fawcett, 1970; Grier, 1981). They are generally fusiform in shape, usually oriented in one direction along their longitudinal axis parallel to the lobule and connected to each other by desmosomes (Loir *et al.*, 1995; Cinquetti and Dramis, 2003). In teleosts, myoid cells have been shown to aid in transportation of spermatozoa within the testes (Grier *et al.*, 1989; Loir *et al.*, 1995). Besides this function, *in vitro* and *in vivo* studies in mammals have shown that myoid cells are also thought to be involved in the synthesis of the basement membrane, control and maintenance of Sertoli cell functions and spermatogenesis (Skinner, 1991; Verhoeven *et al.*, 2000), growth of seminiferous cords (Konrad *et al.*, 1998) and protection of the testis against viruses (Dejucq *et al.*, 1998).

2.1.1.3 The interstitial compartment

This compartment comprises the tissue occupying the spaces between adjacent germinal epithelia including the tissue beneath the tunica albuginea but excluding the seminiferous lobules. It is composed mainly of Leydig (interstitial) cells, blood vessels, macrophages, plasma cells, mesenchymal-like cells, fibroblasts, nerve fibers and connective tissue fibers (Loir *et al.*, 1995; Koulish *et al.*, 2002; Nóbrega and Quagio-Grassiotto, 2007; Nóbrega *et al.*, 2009). It is often bound by myoid cells and collagen fibers which lie on a basement membrane they share with Sertoli cells (Koulish *et al.*, 2002).

2.1.1.3.1 Leydig cells

These are ovoid to spheroidal cells found in the interstitial compartment of the testis and whose presence is typical of teleosts as in the rest of the vertebrate lineage (Nagahama, 1983). They occur individually or in clusters, mostly close to a blood vessel within the compartment (Koulish *et al.*, 2002; Lo Nostro *et al.*, 2004; Nóbrega and Quagio-Grassioitto, 2007). They are the main androgen producing cells in fish testes (Schulz, 2003). These cells are thought to arise from interstitial fibroblast-like cells, as evidenced in trout where regressed testes and testes resuming spermatogenesis showed cells with characteristics intermediate between those of fibroblast and Leydig cells (Loir, 1990; Loir *et al.*, 1995).

In mature testis of many teleosts, Leydig cells often exhibit features that are associated with steroid production. These features include; well developed smooth endoplasmic reticulum with anastomosing tubules and vesicles, numerous mitochondria containing dense matrix and well

developed tubular cristae, several lipid droplets and a large spherical nuclei containing peripherally arranged heterochromatin material (Nóbrega and Quagio-Grassiotto, 2007; Chung, 2008; Chung *et al.*, 2010). These cells have further been shown to express a strong activity for 3β- hydroxysteroid dehydrogenase (HSD); a key enzyme involved in the biosynthesis of steroid hormones found in smooth endoplasmic reticulum (Haider, 2004; García-López *et al.*, 2005; Nóbrega and Quagio-Grassiotto, 2007). Leydig cells have been shown to exhibit cyclical changes during an annual reproductive cycle in seasonally breeding teleosts. During the breeding season, Leydig cells in teleosts possess strong activity for 3β- hydroxysteroid dehydrogenase (HSD) accompanied by ultrastructural features associated with steroid production. Conversely, these cells show reduced activity for 3β-HSD accompanied by degenerative changes at the end of the reproductive cycle (Lo Nostro *et al.*, 2004; Cinquetti and Dramis, 2003; Nóbrega and Quagio-Grassiotto, 2007).

Moreover, the ultrastructural picture and activity of Leydig cells have been shown to vary with the stage of germ cell development within the testis. In this regard, these cells appear fully differentiated during stages of meiotic division and spermiogenesis (Loir *et al.*, 1995; Chung, 2008; Chung *et al.*, 2010), poorly differentiated during mitotic stage, and exhibit signs of degeneration in regressing or regressed testes (Loir *et al.*, 1995). These changes in morphology are frequently accompanied by respective changes in steroid synthesis (Van den Hurk *et al.*, 1978; Loir *et al.*, 1995).

The functions of Leydig cells are, to a large extent, controlled by the gonadotropin, luteinizing hormone, through interaction with its receptors found on these cells in most vertebrates. In

teleosts, however, Leydig cells bear both LH and FSH receptors; a feature lacking in mammals, since in the latter, these receptors are highly specific and reside on Leydig cells and Sertoli cells respectively. Therefore, in teleosts, interactions of any or both of these receptors with respective gonadotropins initiate androgen release (García-López *et al.*, 2009); the major one being 11-ketotestosterone (Schulz, 2003). In addition, fish Leydig cells express androgen receptors, thus, permitting autocrine regulated androgen release; a feature similarly exhibited in mammals (Takeo and Yamashita, 2001). Besides hormonal control, Leydig cells in teleosts have also been shown to receive nervous input via cholinergic and adrenergic innervations, evidenced by the presence of synaptic nerve endings lining the surfaces of these cells as well as myoid cells (Loir *et al.*, 1995).

2.1.1.4 Testicular blood supply

Blood supply to the teleost testis comes from the paired segmental arteries of the dorsal aorta as genital arteries. The arteries and nerves enter the two lobes of the testis at the cranial attachments of the mesorchium, branching posteriorly as they course through the dorsal portion of the tunica albuginea of each lobe. The vessels give rise to smaller branches that penetrate the testicular parenchyma through interlobular spaces to reach the interstitial tissue compartment (Sullivan *et al.*, 1997). Owing to their close proximity to the peritubular myoid cells, lobular basement membrane and interstitial components, this vascular system, as in mammals therefore, is sufficient to continuously bathe the interstitial components bringing nutrients to the germinal epithelium. However, unlike mammals, teleost testes lack lymphatics but, instead, possess an extra-vascular space containing blood plasma which drains between blood vessel endothelial cells

(Grier, 1981). Vascular drainage of the testis is by satellite veins which form the genital vein eventually leading into the caudal vein (Sullivan *et al.*, 1997).

2.1.2 Excurrent duct system

These are a series of ducts through which spermatozoa from the testis and seminal fluids are conveyed to the exterior during ejaculation. They essentially link the seminiferous tubules or lobules to the genital pore exteriorly. The excurrent duct system has been studied on a variety of fish species including the fresh water *Salmonidae* (Lahnsteiner *et al.*, 1993a), *Esocidae* (Lahnsteiner *et al.*, 1993b), *Cyprinidae* (Lahnsteiner, *et al.*, 1994) and some marine fish species (Lahnsteiner and Patzner, 1990; Lahnsteiner, 2003a). In fish, these ducts are thought to be derived from the somatic cells of the gonads and/or the coelomic epithelium (Suzuki and Shibata, 2004). They can broadly be divided into two main ducts; the intratesticular and extratesticular ducts.

2.1.2.1 Intratesticular ducts

The intratesticular ducts comprise the spermatic ductules (efferent ducts) and testicular spermatic ducts. The spermatic ductules are preceded by a collecting system formed at the area where the open ends of the tubules or lobules converge delimiting where active spermatogenesis occur and spermatozoa collecting site (Leal *et al.*, 2009). The spermatic ductules then unite to form the main testicular spermatic duct within each lobe.

2.1.2.2 Extratesticular ducts

The extratesticular ducts consist of the common spermatic ducts and urogenital papilla. The two main testicular spermatic ducts (one from each side) fuse to form the common spermatic duct which then opens, together with the urethra, into the urogenital papilla at the urogenital pore located slightly caudal to the anal opening (Lahnsteiner, 2003a; Fishelson *et al.*, 2006; Lacerda *et al.*, 2006).

Apart from providing the passage for spermatozoa and seminal fluids, the excurrent duct system in teleosts has been shown to play a number of roles including storage of spermatozoa, synthesis of various macromolecules (monosaccharides and proteins) that nourish the spermatozoa, phagocytosis and reabsorption of residual, apoptotic or necrotic spermatozoa and formation of ionic gradient in the seminal plasma (Rasotto and Shapiro, 1998; Lahnsteiner, 2003a; Lahnsteiner *et al.*, 2004). Unlike mammals, teleosts lack accessory sex glands (Lahnsteiner, 2003a). However, some teleosts have been shown to possess paired seminal vesicles in their gonads that empty their secretions into the spermatic duct (Mansour *et al.*, 2004).

2.2 Aims of the study

The testicular structure of Lake Magadi fish and its adaptation to the harsh environmental conditions in this lake remains to be elucidated (Onyango and Kisia, 2008). Additionally, a study conducted by Coe (1966) on breeding provides very limited information regarding the reproductive biology of Lake Magadi fish. The purpose of this study was therefore two fold; i) to provide a concise structural description of the male reproductive organs of this fish upon which future studies could be based, and ii) to critically examine the process of spermatogenesis in the Lake Magadi tilapia with a view to determining any incidental/unique characteristic suggestive of reproductive adaptation in the environment of Lake Magadi.

2.3 Objectives

2.3.1 General objectives

To study the morphological structure of the male reproductive system in *A. grahami*

2.3.2 Specific objectives

1. To study the structural organization of the testis and the excurrent duct system in a mature *A. grahami* using macroscopic and microscopic evaluations.
2. To study the process of spermatogenesis in *A. grahami.*
3. To establish existence of any adaptive features allowing normal functioning of the testis of *A. grahami* in the extreme habitat of Lake Magadi.

2.4 Justification

The perpetuation, survival and sustenance of this fish species in the lake is solely dependent on, among other factors, the structural functionality and adaptation of the reproductive system to the harsh environment of the lake. Presently, knowledge on the basic reproductive biology of *A. grahami* and its respective adaptation in the Lake Magadi environment is scarce. Moreover, the restricted distribution of this fish within the lake has led to their classification as "vulnerable" or "endangered" by the International Union for Conservation of Nature (IUCN) (Bayona and Akinyi, 2006). Besides, the environment within Lake Magadi appears to be very fragile and particularly sensitive to the effects of climate change. Indeed, some lagoons, for example the Sports Club Lagoon, has since dried up (personal observation). This, therefore, spells doom for the future survival of this unique and precious fauna. Not only will this study contribute immensely towards the understanding of the reproductive biology and adaptation of the male *A. grahami* to the lake's environment, but also, provide a pillar onto which other conservation and preservation measures for this fish species may be based in future. Such measures could include testicular transplantation, spermatogonial and spermatozoal cryopreservation and transplantation and production of genetic stocks, as performed in other endangered or valuable fish species (Lacerda *et al.*, 2006; Nóbrega *et al.*, 2009).

CHAPTER 3

3.0 MATERIALS AND METHODS

3.1 Study area

The study was carried out at Lake Magadi which measures approximately 100 Km^2 in size and located approximately 120km Southwest of Nairobi city (Denson *et al.*, 2010).

3.2 Fish Sampling

Fish for this study were collected between the months of July and August 2010 from the Fish Springs Lagoon on the periphery of Lake Magadi (see Coe, 1966; Narahara *et al.*, 1996 for maps) by use of seine and cast nets. This lagoon provides a convenient collection site and has come to be regarded as a standard site for previous studies on this fish species (Maina *et al.*, 1996; Narahara *et al.*, 1996; Wilson *et al.*, 2004). Besides, its water is considered the standard reference water, hence, termed 100% Lake Magadi water. Fish were collected between 6:30 and 8:30 am, and quickly transferred to round 20 liter plastic buckets filled up to ¾ of its capacity with the lake water and then transported immediately (15 min) to an outdoor laboratory that was set up on a balcony of one of the buildings, generously provided by Magadi Soda Company. The fish were consequently subjected to aeration in the buckets for a maximum of 24 hours as observations and investigations were proceeding. A total of 24 fish samples were used in this study while survivors were taken back to the lake.

3.3 Dissection and fixation of the testicular samples

A batch of 2 to 3 live fish were removed from the holding buckets periodically and rapidly anaesthetized by transfer into ice-cold water at a temperature range of 0.5° to 1°C for 3 to 5 minutes. (see Pic, 1978; Wood *et al.*, 2012). Each fish was then blot dried, sexed based on the colour of the pelvic fins and presence of the genital papilla, and if male, the total length and weight measured. A ventromedian incision was then made using a pair of scissors to expose the abdomen and its contents and to further confirm the sex by identifying the gonads. The abdominal viscera in the vicinity were deflected to one side to reveal the deep-seated testis, that were photographed *in situ* using a Sony® DSC-HX5V digital camera before being dissected out. Upon removal from the coelomic cavity, the weight and length of each testis was taken and recorded. The testicular samples for histology were then immediately fixed by immersion into 5% formaldehyde or Bouin's solution for at least 24 hours, while those for electron microscopy were immersed wholly into 2.5% glutaraldehyde in 0.1M sodium cacodylate buffer at a pH of 7.2 to 7.4 for at least 10min, then removed and diced using razor blades into $1mm^3$ pieces on a solidified sheet of paraffin wax. The diced pieces were further immersed into the same fixative and stored at 4°C for at least 24hrs. The samples were then transported in the same form to Chiromo campus labs, University of Nairobi for further processing.

3.4 Tissue processing for light microscopy

The formaldehyde fixed testes samples were rinsed in running water overnight then dehydrated in ascending concentrations of ethanol (50%, 70%, 80%, 90%, 95% and 100%), while those fixed in Bouin's solution were directly introduced into ethanol beginning from 70% then processed as the

formaldehyde fixed tissues. Subsequently, the tissues were cleared using methyl benzoate, infiltrated and embedded in molten paraffin wax then left to solidify overnight. Blocking was then done by attaching the wax embedded samples onto wooden blocks. Tissue sections 5 to 7μm thick were then cut from the embedded blocks using a Leitz Weitzlar® rotary microtome, mounted on glass slides, deparaffinized using xylene, rehydrated by passing through decreasing concentrations of ethanol (100%, 90%, 70%, 50%) and finally into water. The rehydrated sections were then stained using either Haematoxylene-Eosin (H/E), Masson's trichrome or Periodic acid Schiff (PAS). The stained slides were finally examined and photographed using a Leica® DM 500 light microscope.

3.5 Tissue processing for transmission electron microscopy

Gonadal tissue blocks measuring $1mm^3$ previously fixed in glutaraldehyde and stored at 4°C were washed in 0.1M sodium cacodylate buffer, two changes of 1hour each, then post fixed in 2% aqueous osmium tetroxide (2%w/v osmium tetroxide and 98%w/v de-ionized distilled water) for 2 h, rinsed in distilled water, two changes of 5min each, before dehydrating in ascending concentrations of ethanol (50%, 70%, 90%, 95% and 100%), two changes of 15 min each. The tissue blocks were then cleared twice, 30min each, using propylene oxide and infiltrated in araldite resin mixture, first as one part of propylene oxide to one part of resin mixture for 1 h, then as pure araldite resin mixture overnight; all processes done at room temperature. The infiltrated tissue blocks were then embedded using freshly prepared araldite resin mixture with accelerator in plastic capsules and finally polymerized in the oven at 60° C for 48 hrs. The resulting tissue blocks were sectioned using glass knives mounted on a Reichert® ultra microtome

for semi-thin (1μm thick) and ultra-thin (70-100nm thick) sections. The semi-thin sections were floated on moist, clean glass slides and fixed by heat on a hot plate at 70°C for 15min. The fixed sections were then stained with toluidine blue, rinsed using distilled water, examined and photographed using a Leica® DM 500 light microscope. Semi-thin sections served the general purpose of screening the tissue to locate the required area for electron microscopy and also capturing images at light microscopy. Ultra-thin sections were then cut from the selected area of interest using Reichert® ultra microtome, picked with copper grids, stained with 5% uranyl acetate, rinsed in distilled water and then counterstained with 0.5% lead citrate. The stained sections were finally rinsed with distilled water, air dried and examined and photographed using Philips® CM12 Transmission Electron Microscope.

CHAPTER 4

4.0 RESULTS

4.1 Body and testicular measurements

The Lake Magadi tilapia harvested for this study were generally of good body condition, mature ones weighing on average 8.86 ± 0.40g (n = 20) and measuring 8.25 ± 1.19cm (n = 20) in length. The mean testicular weight and length for mature males was 0.06 ± 0.02g and 1.97 ± 0.15cm respectively, resulting into a gonado-somatic index of 0.71 ± 0.13% (**Table 1**).

4.2 Sexual dimorphism

Lake Magadi tilapia is gonochoristic in nature although sexing on the basis of external features was not obvious. Nonetheless, few external features, proved pertinent in determining the sex of the fish used in this study. Firstly, the skin colour of sexually immature males and all the females varied from light to dark grey while mature males exhibited a characteristically bluish hue and thick white lips **(Fig. 2)**. Secondly, mature males had prominent genital papillae located immediately caudal to the anal pore, while females exhibited only the genital pores **(Fig. 3)**. In each sex, the colouration of pelvic fins was very distinct with males exhibiting dark-grey pelvic fins; the colouration being generalized in the relatively large sized or mature individuals and restricted to the margins in small sized or immature ones, and females displaying entirely creamy-white pelvic fins irrespective of the age or size **(Figs. 2, 3, 4 and 5).**

4.3 Breeding and brooding behaviour

At the lagoon site, several breeding nests (pits) measuring approximately 5 to 10 cm in diameter were observed on the floor of the lake, about 2 to 3 meters from the edge of the lagoon, and occasionally some fish were observed entering and leaving these nests **(Fig. 6).** *Alcolapia grahami* is a mouth brooder where breeding females incubate fertilized eggs in their buccal cavities until hatching. In some instances, up to 30 eggs were observed in the mouth, while in others, live embryos with unresorbed yolk sacs (**Figs. 7 and 8**) or fish frys with full capacity to swim **(Fig. 9)** were observed. At the lagoon site, fish frys were frequently encountered in isolated small pools of about 20 to 30 cm in diameter situated on springlets about 2 to 3 m from the main lagoon.

Table 1

Mean ± SEM (n=20) body and testicular weights and lengths of mature fish harvested from Lake Magadi. The ratio of body weights and testicular weights give the gonadosomatic index.

	Whole Body	Testis	Mean Gonadosomatic index (%)
Weight (g)	8.86 ± 0.04	0.06 ± 0.02	
Length (cm)	8.25 ± 1.19	1.97 ± 0.15	0.70 ± 0.13

4.4 Male reproductive system of *A. grahami*

4.4.1 The testes

In this fish, the male reproductive system comprised the testes, spermatic duct and genital papillae; the latter being the only part of the system exposed externally **(Figs. 3 and 10).** The testes of a mature *A. grahami* were bilobed, creamy-white, elongated organs located in the coelomic cavity whose parietal peritoneum was lined entirely by a heavily pigmented black membrane **(Figs. 4 and 10).** The testicular lobes were anatomically related to the liver and stomach cranially; swim bladder, kidneys and urinary bladder dorsally and the gut ventrally. They were oriented craniocaudally, merging at the caudal aspect to form the common spermatic duct which coursed caudally, eventually terminating at the urogenital papilla **(Figs. 10 and 11).** The lobes were more-or-less symmetrical and, in most males, cylindrically shaped **(Fig. 11).** However, some fish had regressed testes presenting slender, greyish-white, flabby lobes **(Fig. 12).** In the immature fish, the testis appeared thin, translucent and cylindrical in shape.

Histologically, the testis of Lake Magadi tilapia was covered entirely by a fibrous connective tissue capsule, the tunica albuginea. The capsule comprised the visceral peritoneum lining the external surface and layers of connective tissue (with predominantly collagen fibers and fibroblasts) on the inner aspect **(Figs. 13 and 14).** This connective tissue layer was continuous with the interlobular tissue running into the testicular substance as isolated septae **(Fig. 14).** These septations virtually divided the testicular substance into lobules; the latter ending blindly beneath the capsule. The organization of the testis of Lake Magadi tilapia presented two main

regions on transverse section; the cortex and medulla (**Fig. 15**). The cortical region was parenchymatous, occupying most of the ventral areas of the testicular lobe while the medulla occupied the central and dorsal aspects, encompassing both the efferent ducts and main testicular spermatic ducts (**Figs. 13, 15 and 16**).

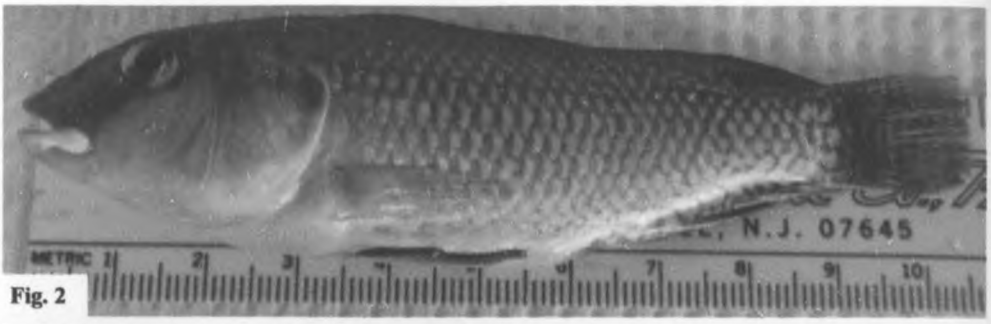


Fig. 2

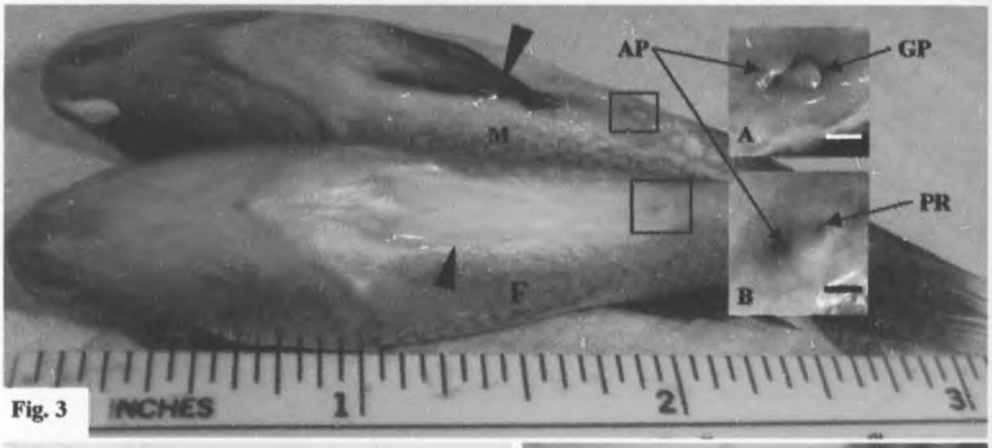


Fig. 3

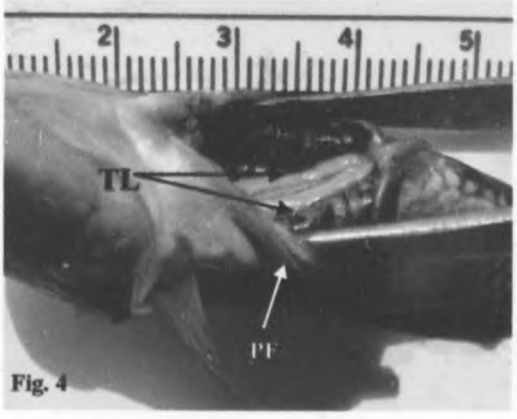


Fig. 4

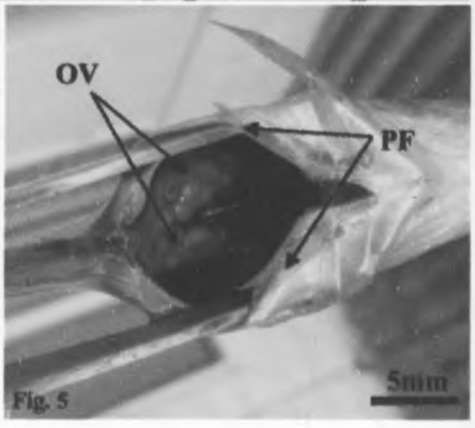


Fig. 5

FIG. 2: A photograph of a mature male Lake Magadi tilapia (*Alcolapia grahami*) measuring about 10.5cm in length. Notice the thick white lips, the bluish hue on the skin and the dark-grey coloured pelvic fins characteristic of mature males of this species. Scale in centimeters.

FIG. 3: A photograph of two mature Lake Magadi tilapia fish positioned side by side on dorsal recumbency to show the male (**M**) and female (**F**) external genitalia. Note that the colouration of pelvic fins (**arrowheads**) in the male and female are dark and creamy-white respectively.

Insets A: The male ano-genital area showing a prominent genital papilla (**GP**) situated immediately caudal to the anal pore (**AP**). Bar = 3mm

B: A similar region in the female showing the genital pore (**PR**) located caudal to the anal pore (**AP**). Bar = 3mm

FIG. 4: A photograph of male *A. grahami* demonstrating testicular lobes (**TL**) in the coelomic cavity and the dark pelvic fins (**PF**). Scale in centimeters.

FIG. 5: A female *A. grahami* showing ovaries (**OV**) containing eggs within the coelomic cavity. Notice the whitish pelvic fins (**PF**). Bar =5mm

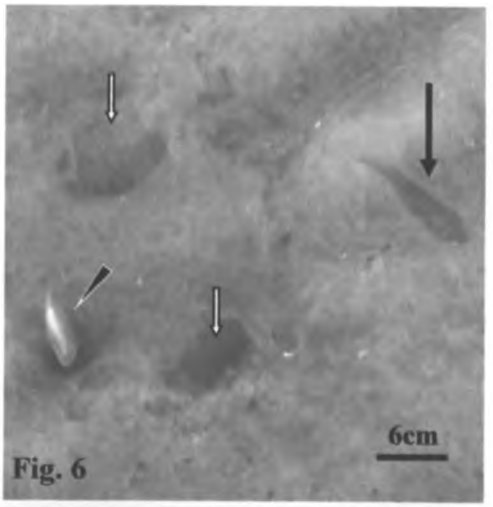


Fig. 6

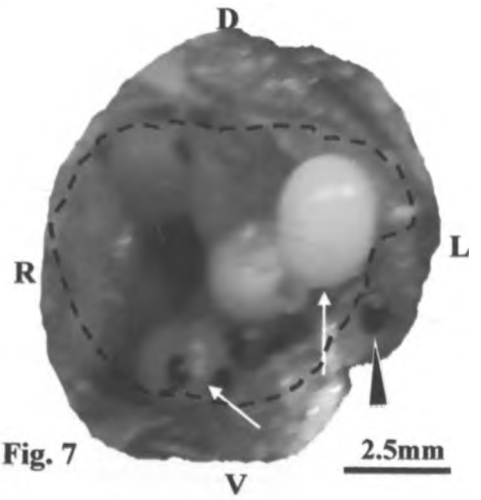


Fig. 7

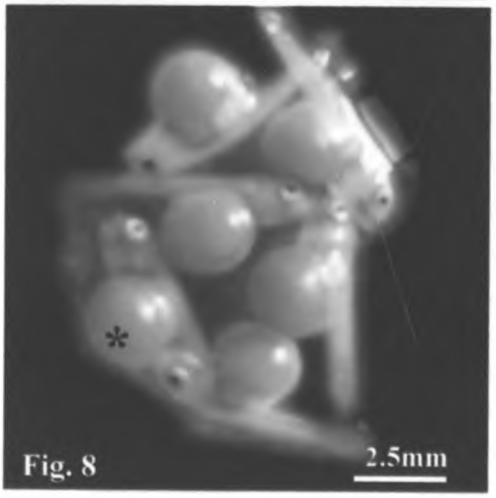


Fig. 8

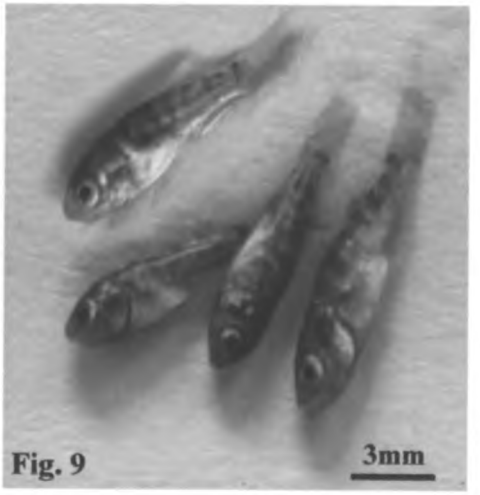


Fig. 9

FIG. 6: A photograph showing breeding nests on the floor of the lagoon (**open arrows**) created by fish during breeding. Notice one fish entering the nest (**arrowhead**) while free swimming fish are also observable nearby (**closed arrow**). Bar = 6cm

FIG. 7: A photograph showing embryos of *A. grahami* in the buccal cavity. The dotted lines show the edge of the mouth. Notice the eye of the mother fish (**arrowhead**). The dorsal (**D**), ventral (**V**) as well as left (**L**) and right (**R**) lateral sides; are shown. Bar = 2.5mm

FIG. 8: Embryos of *A. grahami* after removal from the mouth with their distended unresorbed yolk sacs (**asterisk**) on the ventral side. Bar = 2.5mm

FIG. 9: Frys of Lake Magadi tilapia found in the buccal cavity of some females upon capture. Similar frys were also found in the pools away from the main lagoon. Bar = 3mm

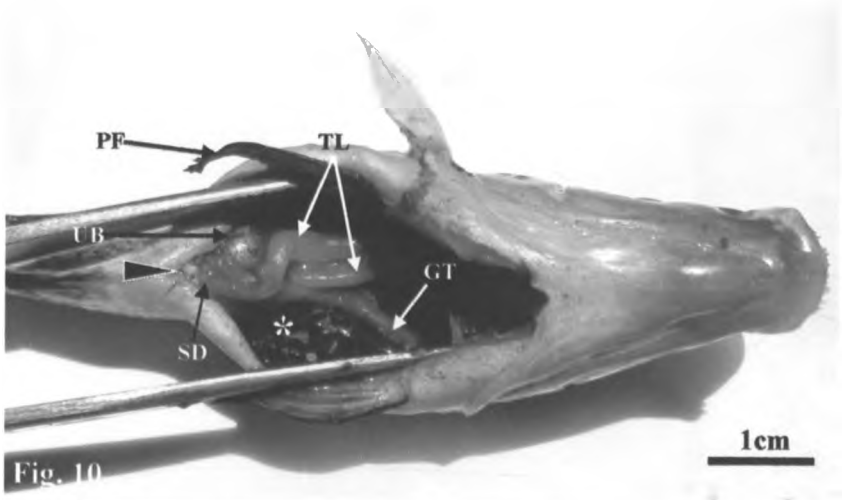


Fig. 10

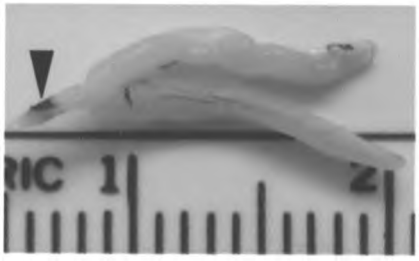


Fig. 11

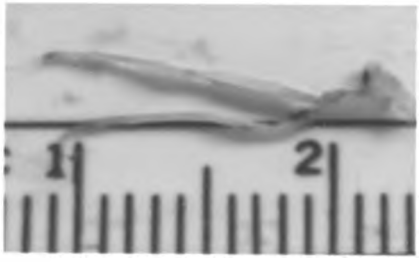


Fig. 12

Scale in centimeters

FIG. 10: Opened abdomen of *A. grahami* with part of the abdominal viscera removed to show the testicular lobes (**TL**) converging caudally to connect with the common spermatic ducts (**SD**). Note the dark coloured pelvic fin (**PF**) and parietal peritoneum (**asterisk**) covered by a black membrane. Part of the caudal gut (**GT**), urinary bladder (**UB**) and genital papilla (**arrowhead**) are visible. Bar = 1cm

FIG. 11: Isolated testis of *A. grahami* showing ripe lobes. The two lobes merge at the caudal aspect to form the common spermatic duct (**arrow head**).

FIG. 12: Regressed testicular lobes found in some fish. Notice its slender appearance compared to the ripe one in Fig. 11.

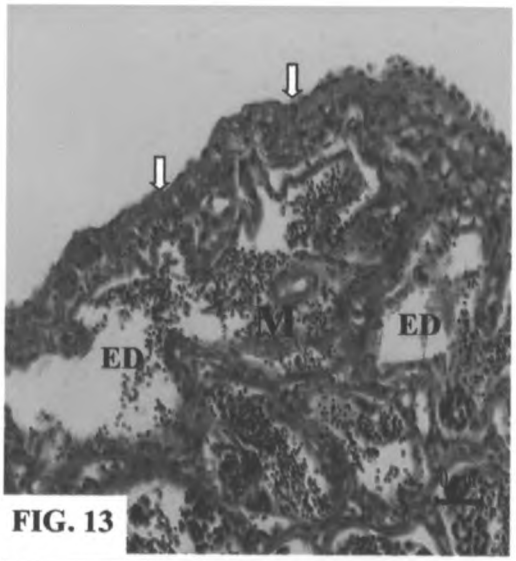


FIG. 13

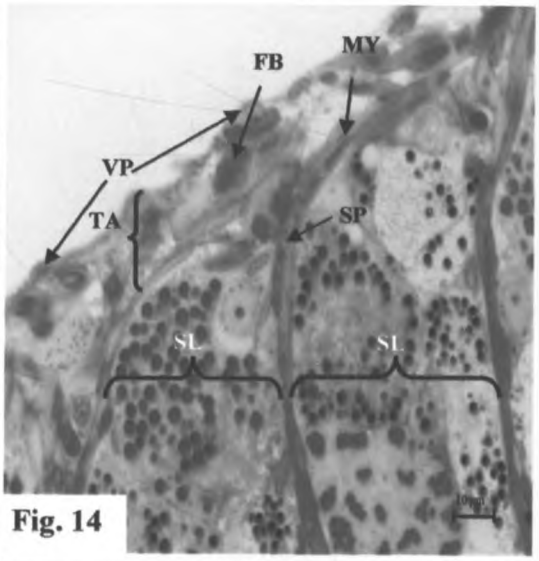


Fig. 14

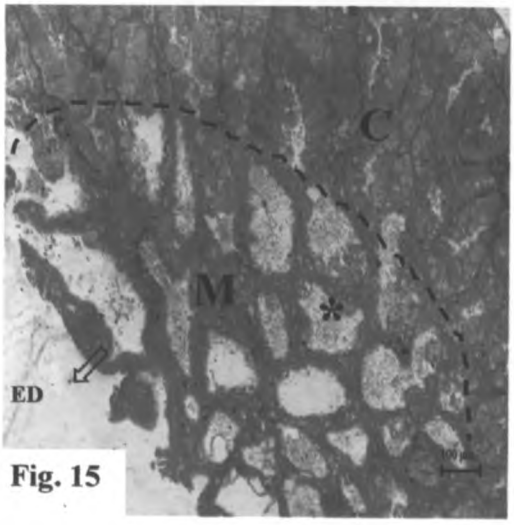


Fig. 15

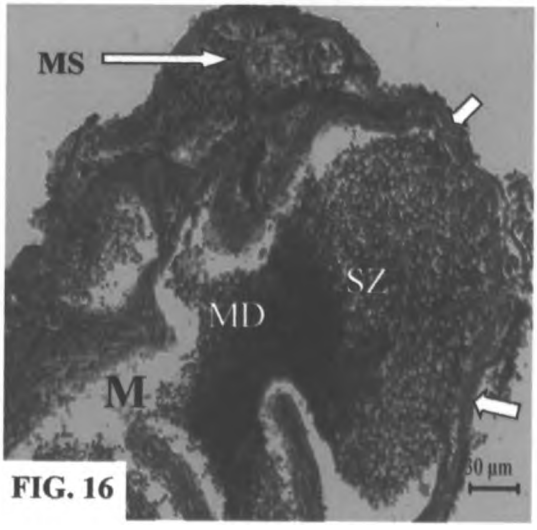


FIG. 16

FIG. 13: Transverse section of *A. grahami* testis showing the tunica albuginea (**open arrows**) consisting of collagen fibers (**blue staining areas**) and the medulla (**M**). Notice the presence of efferent ducts (**ED**) with walls formed by connective tissue (**pink staining areas**) in the medulla. Bar = 30μm. Masson's trichrome

FIG. 14: A semi-thin section of Lake Magadi tilapia testis showing the tunica albuginea (**TA**) and seminiferous lobules (**SL**) separated by septations (**SP**) arising from connective tissue layer of tunica albuginea. The capsular components; visceral peritoneum (**VP**), fibroblasts (**FB**) and myoid cells (**MY**) are present. Bar = 10μm. Toluidine blue

FIG. 15: A cross-section of *A. grahami* testis showing two distinct regions of the parenchyma; the cortex (**C**) and medulla (**M**). The collecting ducts (**asterisks**) and efferent spermatic duct (**ED**) are also present. Bar = 100μm. Toluidine blue

FIG. 16: A transverse section of *A. grahami* testis showing the medulla (**M**), encompassing the main testicular duct (**MD**) filled with spermatozoa (**SZ**). The collagen laden (**blue staining area**) testicular capsule (**open arrows**) encloses the testis. The mesorchium (**MS**) occupies the dorsal aspect of the testis. Bar = 30μm. Masson's trichrome

Two main compartments of the testicular parenchyma in mature *A. grahami* were identified; the germinal and interstitial compartments (**Fig. 17**).

4.4.1.1 The germinal compartment

This was the most conspicuous compartment of the testis formed by germ cells lining the seminiferous lobules. The lobules were bound by the tunica propria (**Fig. 17**) and supported by the basal lamina separating them from the interstitial compartment. The lobules exhibited a radial disposition running from the medulla to the peripheral cortex terminating blindly just beneath the tunica albuginea (see **Fig. 14**). They were lined by conspicuous germinal epithelium consisting of germ cells contained in spermatocysts that projected into the lumen of the lobule (see **Fig. 17**). These cystic units were circumscribed by cytoplasmic extensions of Sertoli cells forming a wall enclosing a clone of germ cells at the same stage of development (**Fig. 18**). At the end of the spermatogenic process, mature cysts opened up to release spermatozoa into the lumen (**Fig. 19**). The histological picture of the testis therefore presented a variety of spermatocysts of varying sizes with germ cells at specific stages of development.

4.4.1.1.1 Spermatogenic cells

4.4.1.1.1.1 Primary (type A) spermatogonia

These were the largest of all the spermatogenic cells present in the testis of *A. grahami.* Two types were distinguished; undifferentiated and differentiated type A spermatogonia (**Fig. 18**). These two cell types were identified on the basis of the cell size as well as the number of cells in a cyst.

4.4.1.1.1.1.1 Undifferentiated type A spermatogonia

Histologically, these were the largest spermatogenic cells in *A. grahami* fish. Most of these cells were found in close proximity to the tunica propria (**Fig. 20**) although some solitary cells were also found in the collecting duct walls in the medullary region (**Fig. 21**). Furthermore, some of these cells were found distributed among other spermatogenic cells in the epithelium (**Fig. 22**). Frequently, intact and dividing undifferentiated type A spermatogonia were encountered (**Fig. 23**). Each of these cells contained large, conspicuous, ovoid to spheroidal, pale staining nuclei frequently occupying the central region. They possessed a conspicuous, relatively pale cytoplasm surrounding the nuclei. Most of the nuclei contained single, spheroidal, dark staining, centrally located nucleolus. Most notable was the presence of relatively dense staining spots outlining the nuclear envelope (**Figs. 21 and 22**).

Ultrastructurally, primary spermatogonia were found located very close to the basal lamina, separated from the latter by Sertoli cell cytoplasmic processes. These cytoplasmic processes, produced by adjacent Sertoli cells, extended between neighbouring primary spermatogonia, hence, separating and encysting them as well (**Fig. 24**). The undifferentiated type A spermatogonia presented very scarce, electron-lucent euchromatin material, distributed uniformly in their large nuclei. The cytoplasm contained characteristic electron-dense substances positioned contiguous to a cluster of mitochondria. These inter-mitochondrial dense substances appeared to surround the nucleus and were frequently found adjacent to the nuclear envelope. Mitochondria were often observed in clusters within the cytoplasm and were fairly large sized and round in shape. In addition, the mitochondrial cristae were often not visible (**Fig. 24**). Besides the

mitochondria, Golgi apparatus was also apparent in the cytoplasm. On average, the nuclear diameter of undifferentiated type A spermatogonia measured 8.47± 0.24 μm (n = 20) (**Table 2**).

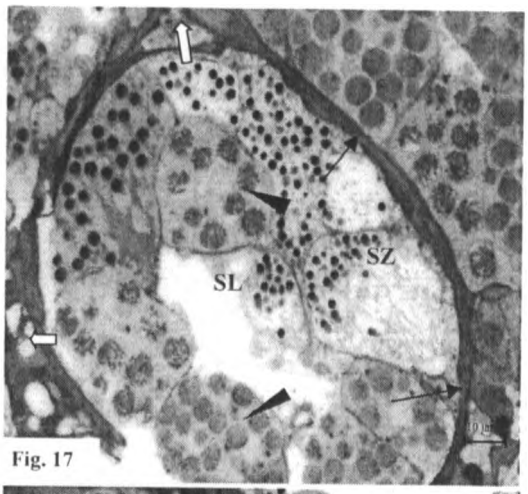


Fig. 17

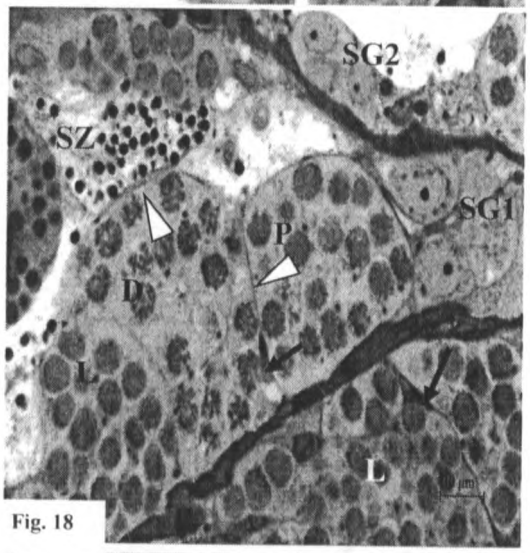


Fig. 18

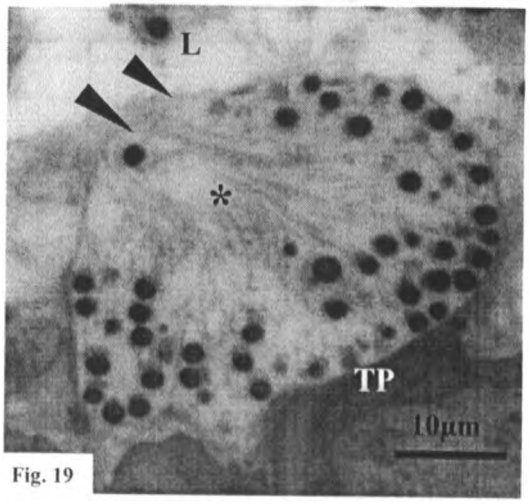


Fig. 19

FIG. 17: A semi-thin section of the seminiferous lobule (**SL**) showing different spermatocysts (**arrowheads**) and spermatozoa (**SZ**) within a cyst. The boundary tissue or tunica propria (**arrows**) and inter-tubular (interstitial) compartment (**open arrow**) are evident. Bar: A = 10μm. Toluidine blue

FIG. 18: A semi-thin section of the testis showing cysts containing primary spermatogonia (undifferentiated (**SG1**) and differentiated (**SG2**)) as well as primary spermatocytes (leptotene (**L**), pachytene (**P**) and diplotene (**D**). Spermatozoa (**SZ**) within the lumen and Sertoli cell nuclei adjacent to the cysts (**arrows**) with their respective cytoplasmic processes (**arrowheads**) are present. Bar = 10μm. Toluidine blue

FIG. 19: Shows an opening spermatocyst (**arrowheads**) containing mature spermatozoa that are yet to be released into the lumen (**L**). Notice the homogeneity and intense staining of the nuclei and the characteristic orientation of the spermatozoa heads towards the tunica propria (**TP**). The strand-like structures towards the opening (**asterisk**) are the tails of the spermatozoa. Bar = 10μm. Toluidine blue

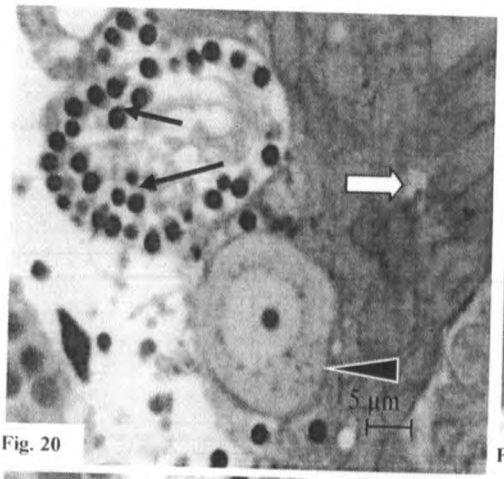


Fig. 20

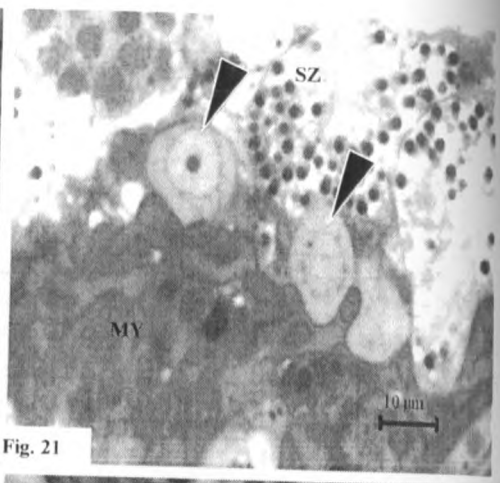


Fig. 21

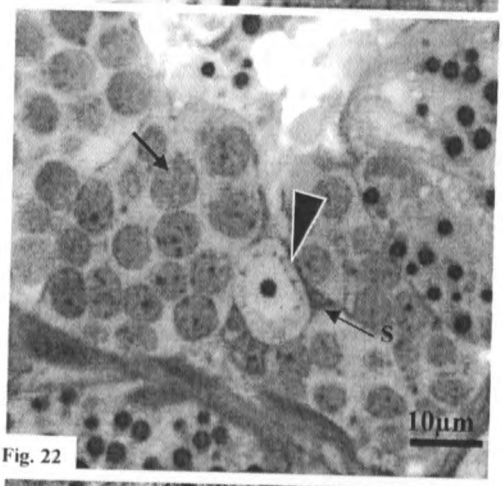


Fig. 22

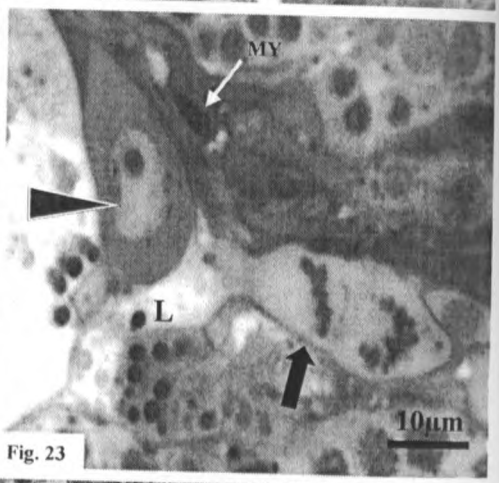


Fig. 23

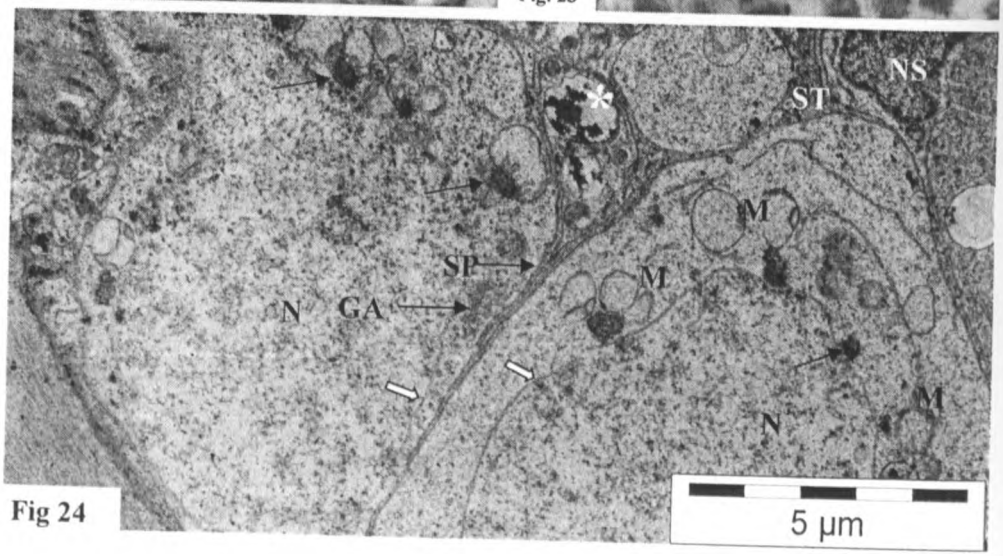


Fig 24

FIG. 20: Shows undifferentiated type A spermatogonia (**arrow head**) lying on the tunica propria (**open arrow**). Note the large spherical nucleus containing a central nucleolus. Late spermatids (**closed arrows**) are apparent. Bar = 5μm. Toluidine blue

FIG. 21: Semi-thin section showing undifferentiated type A spermatogonia (**arrowheads**) in the collecting duct wall. Notice the relatively dense staining spots delimiting the boundary of the nuclear envelope of these cells. Spermatozoa (**SZ**) and thick myoid cell layer (**MY**) are evident. Bar = 10μm. Toluidine blue

Fig. 22: A photomicrograph showing the undifferentiated type A spermatogonium (**arrowhead**) located in the germinal epithelium among other spermatocysts. Relatively dense staining spots delimit the boundary of the nuclear envelope. Early type B spermatogonia (**arrow**) and Sertoli cell nuclei (**S**) are evident. Bar = 10μm. Toluidine blue

FIG. 23: A Photomicrograph showing two sets of spermatocysts containing intact (**arrowhead**) and dividing (**closed arrow**) undifferentiated type A spermatogonia. Lumen (**L**) and myoid cell (**MY**) nucleus are evident. Bar = 10μm. Toluidine blue

FIG. 24: An electron micrograph showing two type A (primary) spermatogonia bordered by cytoplasmic processes (**SP**) of the Sertoli cell (**ST**). The nucleus (**N**), outlined by the nuclear envelope (**open arrows**), contains sparse electron-lucent chromatin material. In the cytoplasm are Golgi apparatus (**GA**) and electron-dense substances (**arrows**) associated with mitochondria (**M**) adjacent to the nuclear envelope. The Sertoli cell nucleus (**NS**) and a phagolysosome (**asterisk**) in its cytoplasm are evident. Bar = 5μm

4.4.1.1.1.2 Differentiated Type A spermatogonia

The undifferentiated type A spermatogonia underwent mitotic division (**see Fig. 23**) giving rise to cysts containing groups of 2 to 8 differentiated type A spermatogonia (**Figs. 18 and 25**). Histologically, differentiated type A spermatogonia appeared to be distributed along the seminiferous lobule close to the lamina propria with a high localization at the cortical region. They contained centrally located pale nuclei, outlined by small dark staining spots. Some cells contained nuclei with single centrally located nucleoli as in the undifferentiated spermatogonia (**Fig. 25**). Their nuclear diameters measured, on average, 7.32 ± 0.25 μm (n = 20) **(Table 2).**

4.4.1.1.1.2 Secondary (Type B) spermatogonia

Each differentiated type A spermatogonia further underwent progressive mitotic divisions to give rise to secondary spermatogonia. These latter groups of cells generally exhibited increased chromatin material in the nuclei with corresponding increase in density compared to their predecessors. Two types of type B spermatogonia were distinguished based on the staining intensity of chromatin material within the nuclei and the cell size within the cysts.

4.4.1.1.1.2.1 Early type B spermatogonia

These cells were spheroidal in shape with centrally located ovoid to spheroidal nuclei containing sparse, lightly condensed and irregularly distributed clumps of chromatin material (**Fig. 26**). The nucleolus was generally less apparent or poorly defined.

Ultrastructurally, early type B spermatogonia appeared ovoid to spheroidal in shape containing prominent ovoid to spheroidal nuclei surrounded by extensive cytoplasm (**Fig. 27**). These cells

contained increased density of chromatin material compared to the primary spermatogonia. The chromatin appeared as coarse electron-lucent material intermixed with foci of electron-dense areas throughout the nucleus. The cytoplasm also contained aggregates of ovoid mitochondria with ill-defined cristae, found in close association with inter-mitochondrial dense substances. These inter-mitochondrial dense complexes were found close to the nuclear envelope as in the primary spermatogonia. The amount of these dense substances in early type B spermatogonia was, however, less compared to those in primary spermatogonia. Other organelles found in the cytoplasm included small vesicles and Golgi apparatus (**Fig. 27**). These cells were smaller in size compared to spermatogonial cells with a nuclear diameter measuring, on average, 6.2 ± 0.17 μm (n = 21) (see **Table 2**). Subsequent mitotic divisions of the early type B spermatogonia resulted into intermediate type B spermatogonia (**Fig. 28**).

4.4.1.1.1.2.2 Late type B spermatogonia

These were derived from the intermediate generations of early type B spermatogonia. Histologically, they exhibited increased density of chromatin material compared to the earlier forms of spermatogonia. Their nuclei were more-or-less spheroidal with generalized distribution of chromatin material, appearing as a mixture of small dark granular spots against fine, diffuse and lightly condensed background (**Fig. 29**). Ultrastructurally, late type B spermatogonia exhibited more-or-less spheroidal nuclei, containing generally more electron-dense clumps of chromatin material compared to the early type B spermatogonia (**Fig. 30**). The coarse chromatin material occupied the entire nucleus albeit with foci of varying degrees of electron-density and, in some cells, nucleoli were evident. The cytoplasm contained centrioles and mitochondria, with the

latter organized as aggregates within the cytoplasm which, as in the primary spermatogonia, were frequently associated with the inter-mitochondrial dense substances. Cyst walls of these cells comprised thin cytoplasmic processes of Sertoli cells. These spermatogonial types were comparatively smaller in size than their predecessors, with a nuclear diameter of 5.61 ± 0.19 µm (n = 25) (**Table 2**).

Cell type	Nuclear diameter (μm)
Undifferentiated type A spermatogonia	8.47 ± 0.24 (n=20)
Differentiated type A spermatogonia	7.32 ± 0.25 (n=20)
Early type B spermatogonia	6.2 ± 0.17 (n=21)
Late type B spermatogonia	5.61 ± 0.19 (n=25)
Leptotene/ Zygotene spermatocytes	6.05 ± 0.2 (n=18)
Pachytene spermatocytes	6.33 ± 0.14 (n=25)
Diplotene spermatocytes	6.32 ± 0.19 (n=20)
Secondary spermatocytes	3.25 ± 0.18 (n=14)
Early spermatids	3.4 ± 0.18 (n=20)
Intermediate spermatids	3.4 ± 0.12 (n=20)
Late spermatids	2.75 ± 0.13 (n=20)
Spermatozoa	1.82 ± 0.08 (n=40)
Leydig cells	4.91 ± 0.15 (n=20)

TABLE 2: (Mean ± SEM) nuclear diameters of germ and somatic cells in the testis of *A. grahami*.

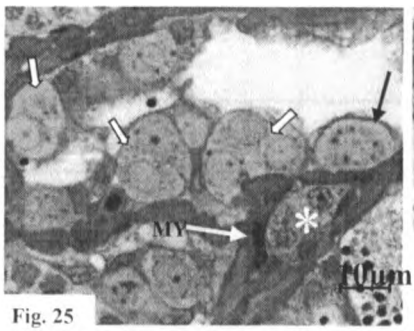


Fig. 25

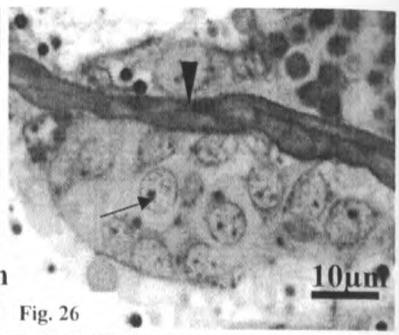


Fig. 26

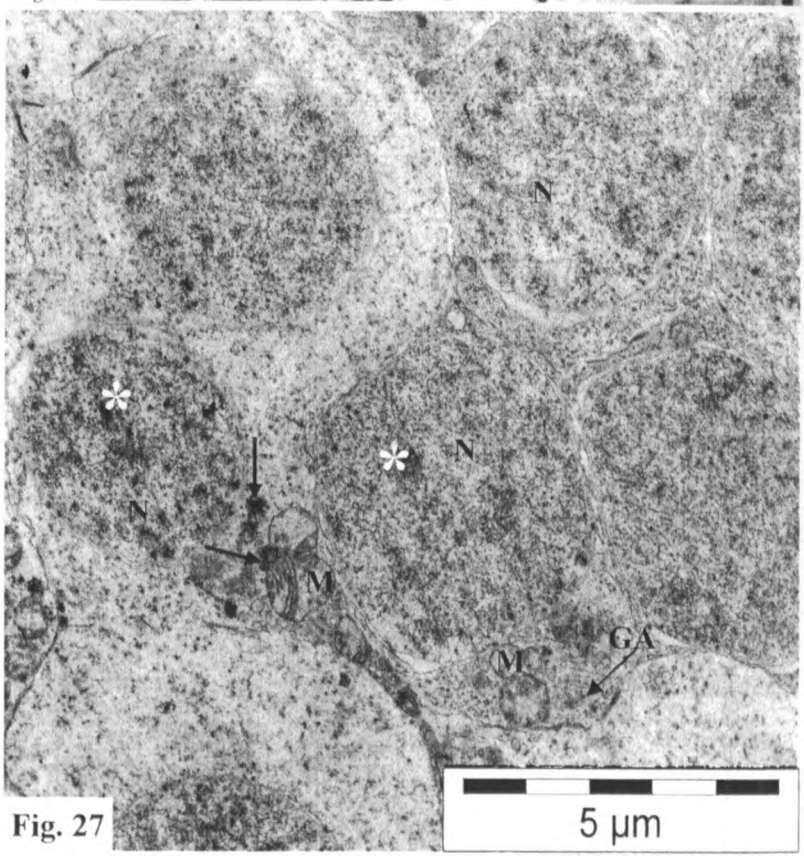


Fig. 27

FIG. 25: A photomicrograph showing three sets of spermatocysts containing 3 to 4 differentiated type A spermatogonia (**open arrows**). Notice the large nuclei, relatively dark staining spots along the nuclear envelope and the centrally located dark staining nucleoli in the differentiated type A spermatogonia. Undifferentiated type A spermatogonium (**closed arrow**) and myoid cell (**MY**) near interstitial tissue (**asterisk**) are also present. Bar = 10μm. Toluidine blue

FIG. 26: A photomicrograph showing a cyst with early type B spermatogonia (**arrow**) adjacent to the lamina propria containing myoid cells (**arrowhead**). Notice the spheroidally shaped nuclei and scarce irregularly distributed chromatin material. Bar = 10μm. Toluidine blue

FIG. 27: An electron micrograph showing early type B spermatogonia. The nucleus (**N**) is uniformly filled with coarse electron-lucent chromatin material intermixed with foci of electron-dense areas (**asterisks**). Ovoid mitochondria (**M**) in close association with inter-mitochondrial dense substances (**arrows**) and Golgi apparatus (**GA**) in the cytoplasm are evident. Bar = 5μm

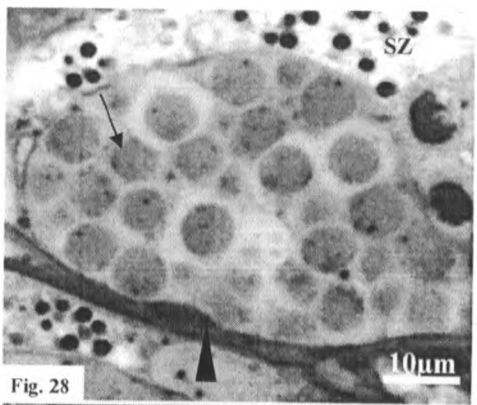


Fig. 28

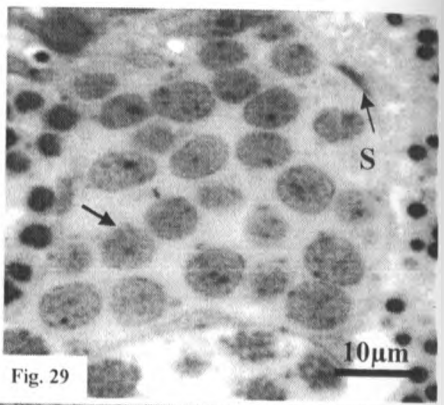


Fig. 29

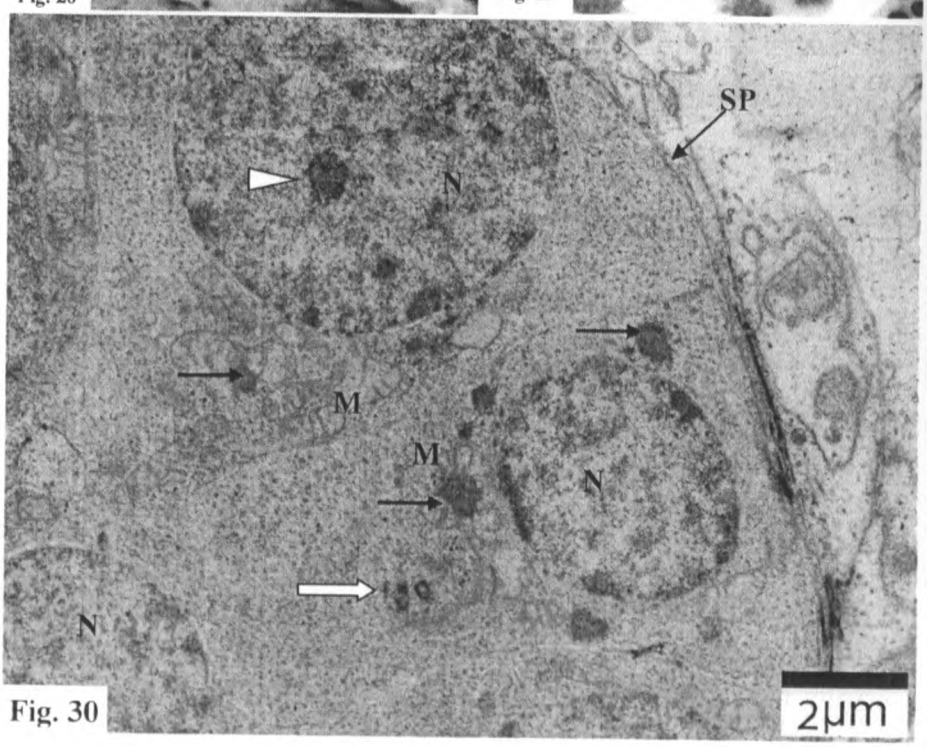


Fig. 30

FIG. 28: A photomicrograph of the intermediate type B spermatogonia (**arrow**) lying adjacent to the lamina propria (**arrowhead**). Notice the increased chromatin condensation compared to the early forms. Spermatozoa in the lumen (**SZ**) are also evident. Bar = 10μm. Toluidine blue

FIG. 29: A photomicrograph of the late type B spermatogonia (**arrow**). The nuclei contain chromatin material with increased staining density and clusters of small dark spots against lightly condensed background. Sertoli cell nucleus (**S**) on the border of the cyst is also noticeable. Bar = 10μm. Toluidine blue

FIG. 30: An electron micrograph showing a section of a cyst containing late type B spermatogonia. Their well defined spheroidal nuclei (**N**) contain clumps of electron-dense heterochromatin material with varying electron-density. A nucleolus in one of the cells (**open arrowhead**) is shown. Inter-mitochondrial dense substances (**closed arrows**), mitochondria (**M**) and centrioles (**open arrow**) are also present in the cytoplasm. Enclosing the cyst is a thin cytoplasmic process of Sertoli cells (**SP**). Bar = 2μm

Subsequent divisions of the most mature late type B spermatogonia gave rise to spermatocytes. Generally, the cytoplasm and plasma membrane of spermatocytes were hardly noticeable at light microscopy and appeared to be completely devoid of nucleoli (**Figs 31 and 32**). Two types of spermatocytes were observed in mature testis of *A. grahami*; primary and secondary spermatocytes.

4.4.1.1.1.3 Primary spermatocytes

These spermatogenic cells were represented in diverse histological forms and shapes in the testis of the Lake Magadi tilapia. The most frequently recognized primary spermatocytes were those in prophase I of meiosis. Their identification was based on the nuclear size, degree and characteristics of chromatin condensation and distribution and presence or absence of meiotic figures.

4.4.1.1.1.3.1 Leptotene/ Zygotene spermatocytes

These were spermatogenic cells mainly at the transitory phase between the leptotene and zygotene stages. At light microscopy, the nuclei appeared to contain relatively dense stained homogeneously chromatin material covering the entire nucleus (**Fig. 31**). Unlike most spermatocytic stages, this stage was often characterized by the presence of a noticeable 'halo' around the spherical nucleus representing the cytoplasm. They had a nuclear diameter of 6.05 ± 0.20 µm (n = 18) (**see Table 2**), being generally smaller than the type A and early type B spermatogonia but larger than late type B spermatogonia.

4.4.1.1.1.3.2 Pachytene spermatocytes

At light microscopy, these cells presented conspicuous nuclei with characteristically thickened chromatin clumps filling most of the nucleus albeit with irregular edges, hence, giving the nuclear envelope a serrated outline (**Fig. 32**). The nuclei measured 6.33 ± 0.14 μm (n = 25) in diameter (**see Table 2**), showing an increasing size compared to their predecessors. Ultrastructurally, these cells were enclosed by thin Sertoli cell cytoplasmic processes which, on the basal side, apposed the basal lamina. The pachytene spermatocytes appeared more-or-less spheroidal with prominent centrally located nuclei. The nuclei were clearly outlined by a typical nuclear envelope comprising the inner and outer layers (**Fig. 33**). These cells exhibited clumps of coarse chromatin material distributed as patches of relatively electron-dense areas within the nucleus. Most notable was the presence of several short synaptonemal complexes within the nucleus. The cytoplasm contained ovoid mitochondria with visible cristae, as well as centrioles and Golgi apparatus containing vesicles.

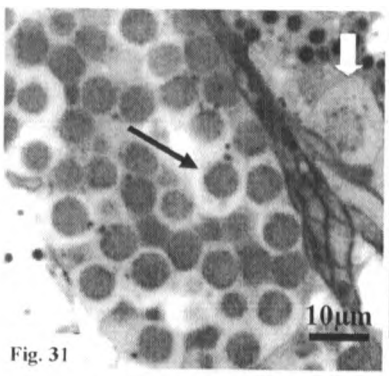


Fig. 31

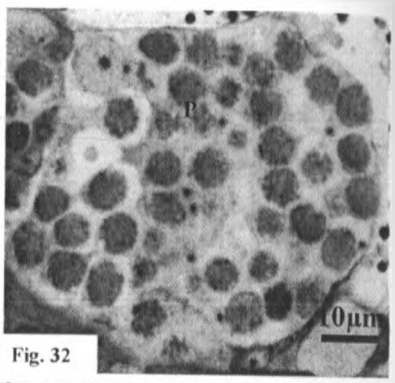


Fig. 32

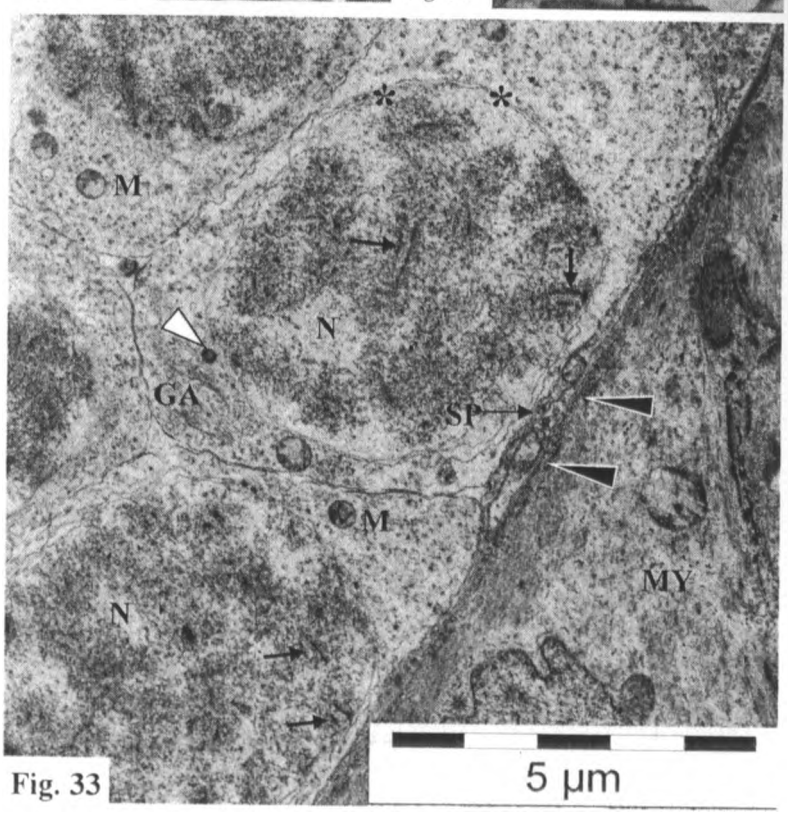


Fig. 33

FIG. 31: A semi-thin micrograph of primary spermatocytes at the transitory phase between leptotene and zygotene stages of prophase (**arrow**). Notice the relatively dense staining chromatin material and a 'halo' around the spherical nuclei. Undifferentiated type A spermatogonium (**open arrow**) in the adjacent lobule is evident. Bar = 10μm. Toluidine blue

FIG. 32: A micrograph of *A. grahami* testis showing primary spermatocytes at the pachytene (**P**) stage. Chromatin material appears as clumps within the nuclei. Notice the serrated edges of the nuclear envelope in these cells. Bar = 10μm. Toluidine blue.

FIG. 33: An electron micrograph showing a cyst containing primary spermatocytes at the pachytene stage. Their spheroidal nuclei (**N**) contain chromosomes forming synaptonemal complexes (**arrows**). In the cytoplasm are ovoid mitochondria (**M**), Golgi apparatus (**GA**) and centrioles (**open arrowhead**). The cells are confined by a thin Sertoli cell cytoplasmic extension (**SP**) lying on a basement membrane (**closed arrowheads**). The perilobular myoid cell (**MY**) and the inner and outer membranes of the nuclear envelop (**asterisks**) are evident. Bar = 5μm

4.4.1.1.1.3.3 Diplotene spermatocytes

Compared to pachytene spermatocytes, the nuclei of these cells contained slightly more condensed chromatin material at light microscopy (**Fig. 34**). The nuclear diameter, which on average measured 6.32 ± 0.19 µm (n = 20) **(see Table 2)**, showed a slight reduction in size compared to pachytene spermatocytes. The ultrastructure of these cells revealed more-or-less spheroidal nuclei enclosed by a double layered nuclear envelope. Chromatin material was organized into thick clumps of more electron-dense patches compared to pachytene spermatocytes. In addition, there were a few synaptonemal complexes within the nucleus (**Fig. 35**). The cytoplasm contained numerous ovoid mitochondria and vesicles. There was presence of cytoplasmic bridges contacting adjacent cells. Thin Sertoli cell cytoplasmic processes containing little cytoplasm formed the cyst wall enclosing the cells.

4.4.1.1.1.4 Secondary spermatocytes

These cells were rarely encountered due to their short lifespan before entering the second meiotic division. Nonetheless, some cysts containing a few intact secondary spermatocytes were encountered in some parts of the seminiferous lobule together with a number of metaphase figures of meiotic division (**Fig. 36).** Ultrastructurally, secondary spermatocytes exhibited electron-dense nuclei measuring 3.25 ± 0.18 µm (n = 14) **(see Table 2)**; an indication of a sharp decrease in size compared to the primary spermatocytes.

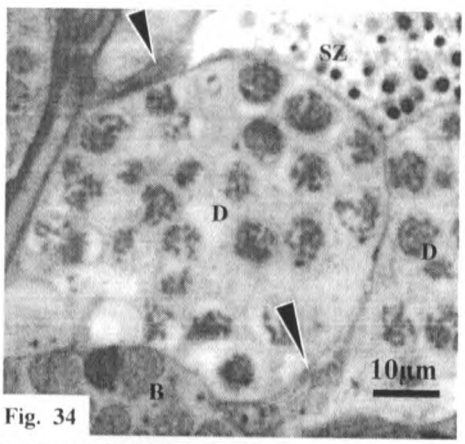


Fig. 34

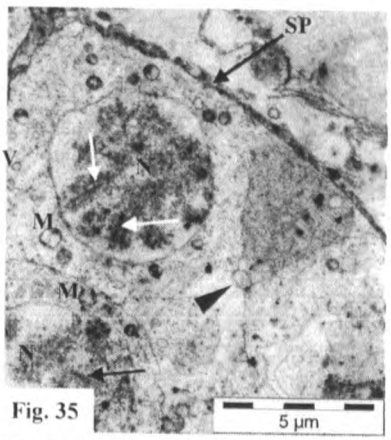


Fig. 35

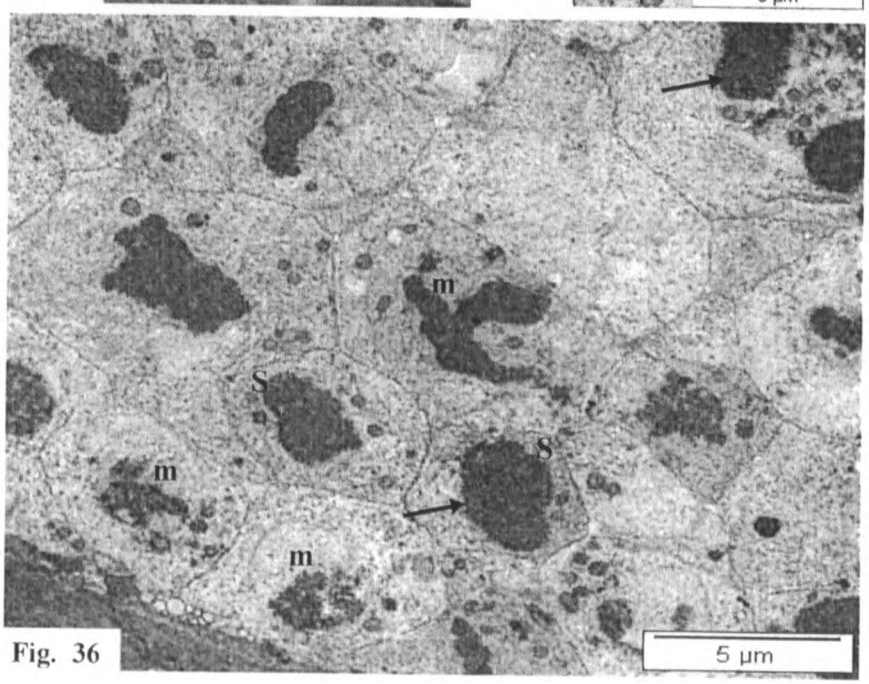


Fig. 36

FIG. 34: A photomicrograph of primary spermatocytes at the diplotene (**D**) stage showing thick short clumps of chromatin material within the nucleus. Sertoli cell cytoplasmic processes (**arrowheads**) form distinct outline of the cyst wall. Spermatozoa (**SZ**) and type B spermatogonia (**B**) are shown. Bar = 10μm. Toluidine blue

FIG. 35: An electron micrograph showing advanced primary spermatocytes at the diplotene stage enclosed by a thin Sertoli cell cytoplasmic extension (**SP**). The nucleus (**N**) contains synaptonemal complexes (**arrows**) with patches of thick electron-dense irregular clumps of chromatin material. The cytoplasm contains ovoid mitochondria (**M**) and numerous vesicles (**V**). An intercellular bridge (**arrow head**) is also apparent. Bar = 5μm

FIG. 36: An electron micrograph of a cyst containing secondary spermatocytes (**S**) with some undergoing meiotic division (**m**). Notice the highly electron-dense chromatin material in the nuclei (**arrows**). Bar = 5μm

4.4.1.1.1.5 Spermatids

These were the final group of spermatogenic cells found within the spermatocysts. Three spermiogenic stages recognized by their cell sizes, distribution and condensation of chromatin material within the nuclei and the inter-spermatid distance within the cysts were identified in the testes of mature *A. grahami.*

4.4.1.1.1.5.1 Early spermatid (spermatid 1)

The formation of these cells was preceded by the second meiotic division of secondary spermatocytes. Histologically, early spermatids presented unique shapes of chromatin material that initially assumed a 'C' then 'U'-shape before rounding up to appear ring-like in shape (**Fig. 37**). The ring-shaped chromatin in these cells circumscribed a translucent center typical of a fully formed early spermatid. Subsequently, the ring-like chromatin appeared to expand from both sides progressively approaching the center, hence, reducing and finally obliterating the translucent area altogether (**Fig. 38**). The chromatin density was moderate but uniform and their spheroidal nuclei, measuring 3.4 ± 0.18 μm (n = 20) in diameter (**see Table 2**), was slightly larger than the secondary spermatocytes. These cells appeared closely packed to each other. Soon after their formation following the second meiotic division of secondary spermatocytes, electron microscopy revealed them as ovoid to spheroidal shaped cells. They possessed corresponding ovoid to spheroidal nuclei containing heterogeneous chromatin material (**Fig. 39**). The nuclear envelope appeared either irregular or indistinct, indicating its re-formation immediately after the second meiotic division. Early spermatids possessed a more-or-less symmetrical cytoplasm

around the nucleus with a generalized distribution of ovoid mitochondria. Few developing flagellae were observed within the interstices of the early spermatids.

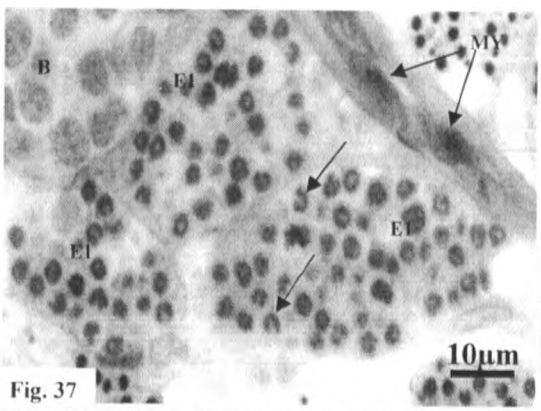


Fig. 37

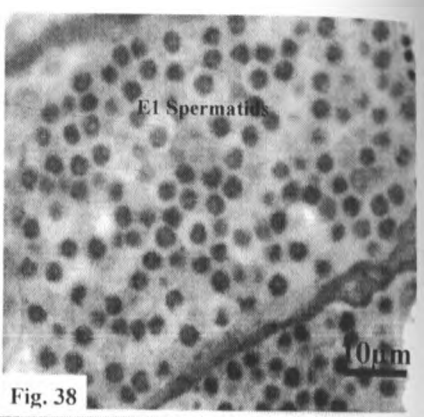


Fig. 38

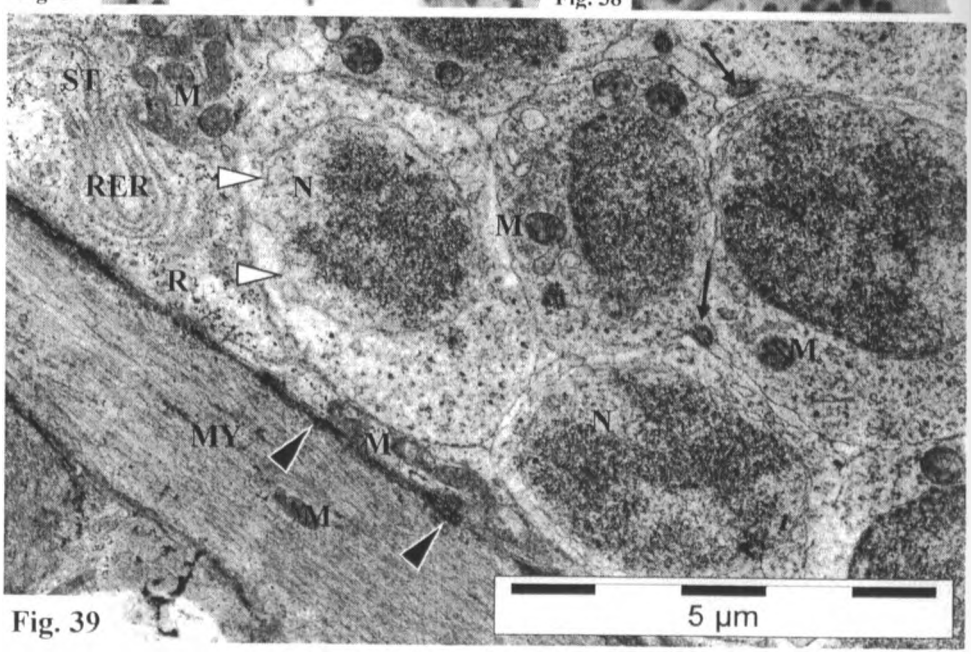


Fig. 39

FIG. 37: A photomicrograph of the Lake Magadi fish testis showing three cysts containing early spermatids (**E1**) at their initial stages of formation. Notice that some cells exhibit '**C**' shaped chromatin material (**arrows**) as they transit from the secondary spermatocytes, through the second meiotic division to form cells with translucent centers; a characteristic feature of the early spermatids. Tunica propria with myoid cells (**MY**) is evident. Bar = 10μm. Toluidine blue

FIG. 38: Advanced forms of early spermatids (**E1**) in *A. grahami* showing homogenously condensed chromatin material which progressively fills and obliterates the translucent centers in the nuclei. Bar = 10μm. Toluidine blue

FIG. 39: An electron micrograph showing a cyst containing ovoid to spheroidally shaped early spermatids immediately after differentiation from secondary spermatocytes. Their nuclei (**N**) contain irregularly distributed clumps of coarse heterochromatin. Notice the irregular outline of the nuclear envelope (**open arrowheads**) surrounded by symmetrical cytoplasm. At the interstices of the cells are developing flagellae (**arrows**). Sertoli cell (**ST**), Rough endoplasmic reticulum (**RER**), free ribosomes (**R**), mitochondria (**M**), basement membrane (**closed arrowheads**) and myoid cell (**MY**) are apparent. Bar = 5μm

4.4.1.1.1.5.2 Intermediate spermatids (spermatid II)

Histologically, these cells appeared almost the same size as the early forms, presenting a spheroidal nucleus with a nuclear diameter of 3.44 ± 0.12 µm (n = 20) (**see Table 2**). However, their chromatin condensation was comparatively advanced with an even distribution covering the entire nucleus including the central translucent region associated with early spermatids (**Fig. 40**). Ultrastructurally, intermediate spermatids assumed more-or-less spheroidal shapes, with prominent spheroidal nuclei. The nucleus, enclosed by an evident double layered nuclear envelope, contained fine granular and homogeneous chromatin material, occupying the entire nucleus. In contrast to the early spermatids, the cytoplasm was asymmetrically distributed towards one pole with respect to the nucleus. Consequently, the organelles within the cytoplasm appeared to have been displaced to one pole of the cytoplasm (**Fig. 41**). Among the organelles observed were Golgi complexes containing vesicles and spheroidal mitochondria with well defined cristae. The intercellular spaces contained increased numbers of developed axonemes compared to the early spermatids.

The process of flagellar development seemed to have been preceded by movement of the centriolar complex, comprising the proximal and distal centrioles, from its original site near the plasma membrane to the nuclear envelope. The point of attachment of plasma membrane to the distal centriole subsequently moved together with the centriolar complex towards the nucleus. Consequently, an invagination of the plasma membrane representing the future cytoplasmic canal was formed (**Fig. 42**). At this stage, the diplosome moved to lie tangential to the nucleus. An indentation into the nucleus was formed at the point in which the proximal centriole made contact

with the nuclear envelope. Concomitantly, a single flagellum developing from the basal body of the distal centriole continued to grow distally and tangential to the nucleus. The space created by the invaginating plasma membrane following the movement of the diplosome, continued to develop around the proximal aspect of the developing flagellum. At this time, the asymmetry of the cytoplasm was apparent with most of it displaced to the distal aspect of the cell around the base of the developing flagellum, representing the midpiece of the future spermatozoon. There were numerous vacuolar structures in the cytoplasm surrounding the developing flagellum (**Fig. 42**).

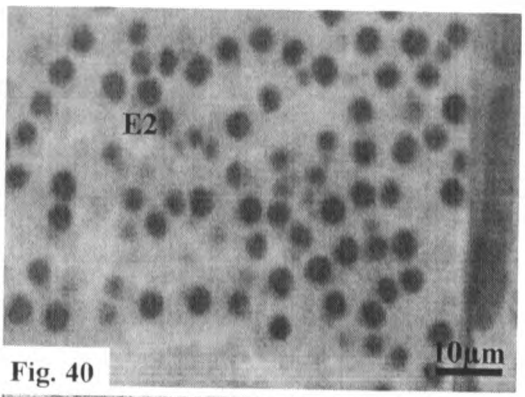


Fig. 40

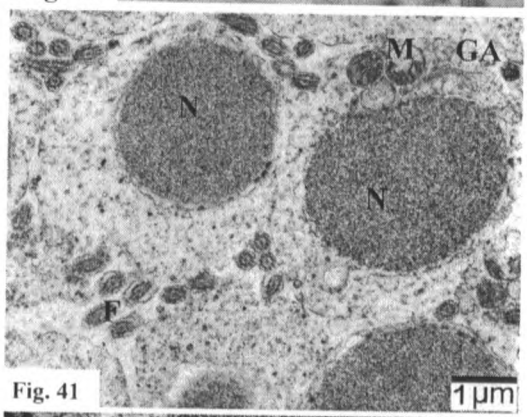


Fig. 41

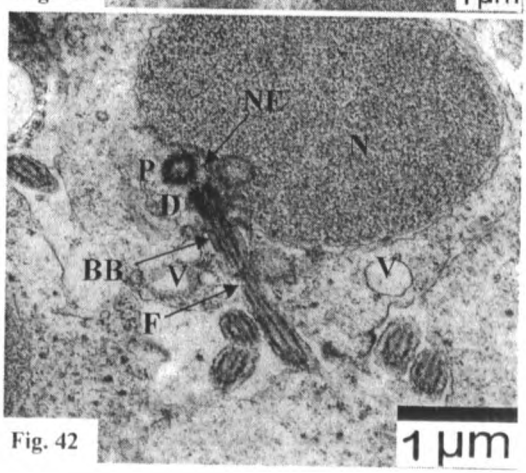


Fig. 42

FIG. 40: A photomicrograph of *A. grahami* seminiferous lobule showing intermediate spermatids (**E2**). The cell nuclei have fine, homogenous and densely stained chromatin material completely filling the nuclei leaving no noticeable clear translucent centers. Bar = 10μm. Toluidine blue

FIG. 41: An electron micrograph showing intermediate spermatids with spherical nuclei (**N**) containing fine, granular homogeneous chromatin material. Mitochondria (**M**), Golgi apparatus (**GA**) and axonemes (**F**) at cell interstices are evident. Bar = 1μm

FIG. 42: An electron micrograph of intermediate spermatid showing a flagellum (**F**) growing tangential to the nucleus (**N**). The proximal centriole (**P**), located at the indentation of the nuclear envelope (**NE**), distal centriole (**D**) with its basal body (**BB**), vacuolar structures (**V**) and axonemes are also evident. Bar = 1μm

4.4.1.1.1.5.3 Late spermatids (spermatid III)

These cells formed the final phase of spermiogenesis in the testis of Lake Magadi tilapia. At light microscopy, they seemed to be enclosed in cysts as other immature germ cells (**Fig. 43**). They were small sized and spheroidal in shape with a nuclear diameter of 2.75 ± 0.13 μm (n = 20) (**see Table 2**), showing a decrease in size compared to the intermediate spermatids. They had homogeneously dense stained nuclei with indistinct cytoplasm. The cells were spaced relatively far from each other, their heads arranged in small linear to curvilinear groupings directed towards the cyst wall, more often, adjacent to the tunica propria. This arrangement therefore left most of the cyst space behind the cells empty and amorphous.

Electron microscopy of the late spermatids revealed rounded cells with prominent spheroidal nuclei. The most notable process in this stage was the initiation of condensation and compaction of the chromatin material into small highly electron-dense globules. Initially, the fine, granular and generally homogeneous chromatin material associated with the intermediate spermatids began to condense into foci of highly electron-dense, homogeneous chromatin material, appearing as dark globules (**Fig. 44**). This process of condensation and compaction continued progressively as the fine granular chromatin material within the nucleus continued to be transformed into small spheroidal globules. Eventually, most of the nucleus was covered by the compacted chromatin material, distributed irregularly against an electron-lucent matrix (**Fig. 45**). Organelles within the cytoplasm were displaced to the distal end of the nucleus, leaving the cytoplasm proximal to the nucleus with lamellations. Most of the cytoplasm of the late

spermatids was shed off and phagocytosed by the thickened cytoplasmic processes of the Sertoli cell walling the cyst at this stage (**Fig. 46**).

As the process of condensation within the nucleus continued, the nuclear indentation initially formed in the intermediate spermatids continued to extend deep into the nucleus, forming the nuclear/implantation fossa. The entire diplosome, which initially was lateral to the nucleus in the intermediate spermatids, now became perpendicular to the nucleus and inside the nuclear fossa (**Fig. 47**).

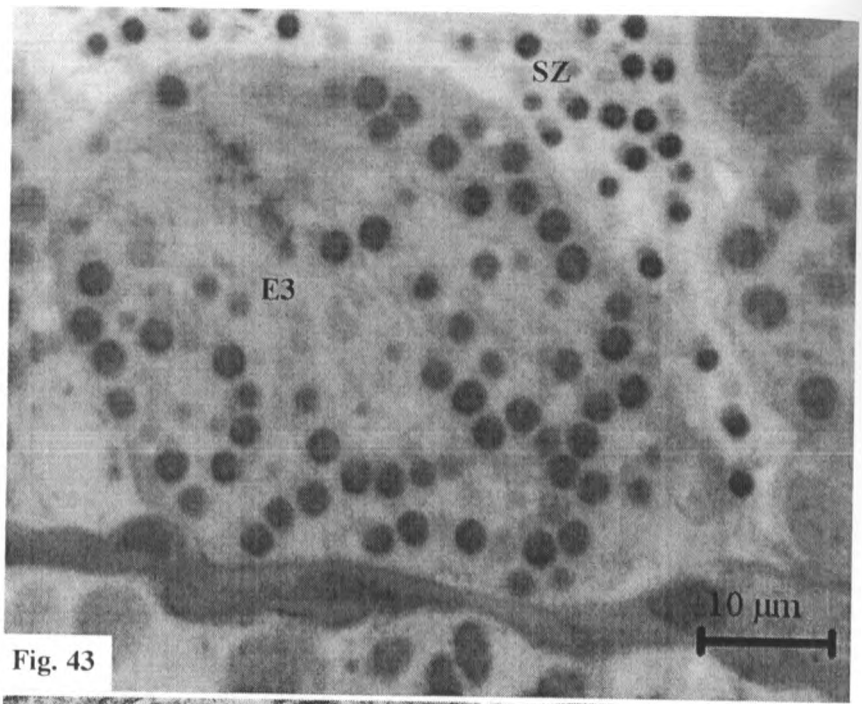


Fig. 43

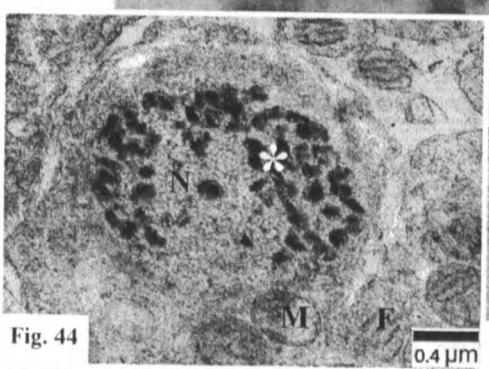


Fig. 44

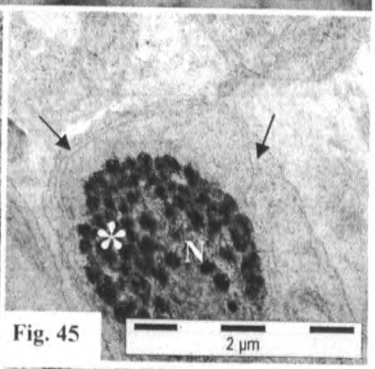


Fig. 45

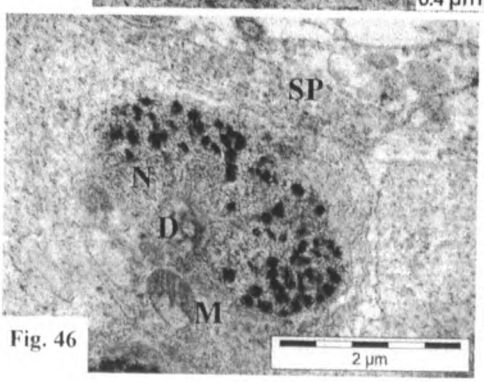


Fig. 46

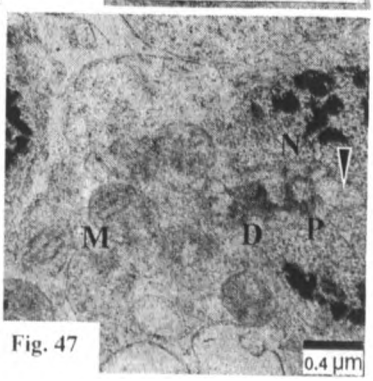


Fig. 47

FIG. 43: A semi-thin micrograph of late spermatid (**E3**) cyst showing spheroidally shaped nuclei and homogeneously dense staining chromatin material. More dense spermatozoa (**SZ**) within the lobule lumen are apparent. Bar = 10μm. Toluidine blue

FIG. 44: Head of the late spermatid exhibiting initial phase of chromatin condensation and compaction into highly electron-dense globules (**asterisk**) within the nucleus (**N**). Mitochondria (**M**) on the distal end of the cell and flagellae (**F**) are evident. Bar = 0.4μm.

FIG. 45: Head of the late spermatid showing the nucleus (**N**) under advanced phase of chromatin compaction evidenced by increased chromatin globules (**asterisk**). Lamellae (**arrows**) configurations in the cytoplasm, proximal to the nucleus, are present. Bar = 2μm

FIG. 46: Transverse section of the late spermatid at the proximal portion of the mid-piece. The distal centriole (**D**), mitochondria (**M**) and a portion of an enclosing cytoplasmic extension (**SP**) of the Sertoli cell are evident. Bar = 2μm

FIG. 47: Oblique section of the proximal portion of the mid-piece. The distal (**D**) and proximal (**P**) centrioles within the nuclear fossa (**closed arrowhead**), mitochondria (**M**) and part of the nucleus (**N**) are apparent. Bar = 0.4μm

4.4.1.1.1.6 Spermatozoa

These cells resulted from the final transformation of late spermatids. At light microscopy, mature sperm cells occurred either in cysts prior to opening (**see Fig. 19**) or freely in the lobule lumen (**Fig. 48**). Spermatozoa in the cysts and lumen exhibited dense staining spheroidal heads, oriented in the same direction generally towards the perilobular boundary tissue. Flagellae, often appearing histologically as a mass of thin faintly stained structures projecting behind the dark staining heads, were frequently observed in the lumen. These cells were also seen in the efferent duct system of mature fish (**see Fig. 21**). The nuclei, containing highly condensed chromatin material covering the entire nucleoplasm measured 1.82 ± 0.08 μm ($n = 40$) in diameter (**see Table 2**), therefore making them the smallest of all the germ cells.

Ultrastructurally, the mature spermatozoon of *A. grahami* comprised the head and a single flagellum, with the latter being surrounded by the midpiece proximally (**Figs. 49 and 50**). The head was generally round and largely composed of a large, conspicuous nucleus situated at the center (**Fig. 49**). The nucleus was packed with compacted, highly electron-dense chromatin material, organized as spheroidal masses closely attached to each other. The nuclear envelope appeared irregular and often indistinct owing to projections of the peripherally located chromatin globules. The cytoplasm surrounding the nucleus was thin, containing no noticeable organelle, especially the proximal and mid-portions of the head. Moreover, the cytoplasm showed several lamellae configurations running transversely from the irregular nuclear envelope to the plasma membrane (**Figs. 49 and 50**). Excess cytoplasm cast off by the developing spermatozoa as residual bodies occupied the intercellular spaces (**Fig. 49**). There was no evidence of an acrosome

or an acrosomal vesicle on the head. Distal to the head, the remaining cytoplasm initially displaced from the head region, surrounded the base and most of the proximal portion of the flagellum in a symmetrical manner. The cytoplasm and its contents surrounding the flagellum formed the midpiece of the mature spermatozoon of Lake Magadi tilapia. This midpiece was about 1.5µm in length. The cytoplasmic portion at the distal end of the midpiece was limited by a blind ending plasma membrane. Hence, the sagittal section of *A. grahami* spermatozoon depicted a midpiece made up of a bilobed structure separated centrally by the flagellum (**Fig. 50**). The most notable feature of the midpiece was the presence of several round mitochondria within the cytoplasm. These mitochondria were arranged in two transverse layers; the proximal and distal layers, both surrounding the centrally located axoneme (**Fig. 51**). The entire midpiece comprised about 10 mitochondria, distinctly isolated from each other by a thin cytoplasmic matrix (**Figs. 51 and 52**). At the distal end of the implantation fossa within the mature spermatozoon was the distal centriole typified by its nine microtubule triplets (9+3) pattern (**Fig. 52**). At this stage, the space between the plasma and flagellar membranes in the midpiece, the cytoplasmic canal, became more apparent and elongated compared to the same in spermatid stages (**Figs. 50, 51 and 53**). This cytoplasmic canal shared a common base with the flagellum proximally, and was open distally, facilitating a direct communication with the external environment. Still at the level of the midpiece, the axoneme was centrally positioned within the symmetrical, thin spaced cytoplasmic canal. Ultrastructurally, the axoneme of the flagellum consisted of 9 distinct microtubule peripheral doublets and a central pair enclosed by the flagellar membrane along its entire length (**Figs. 53 and 54**).

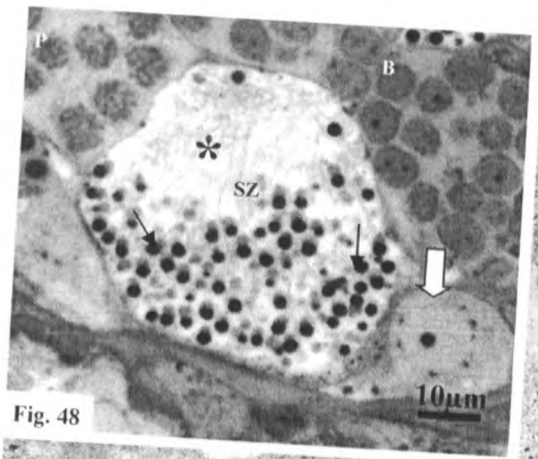


Fig. 48

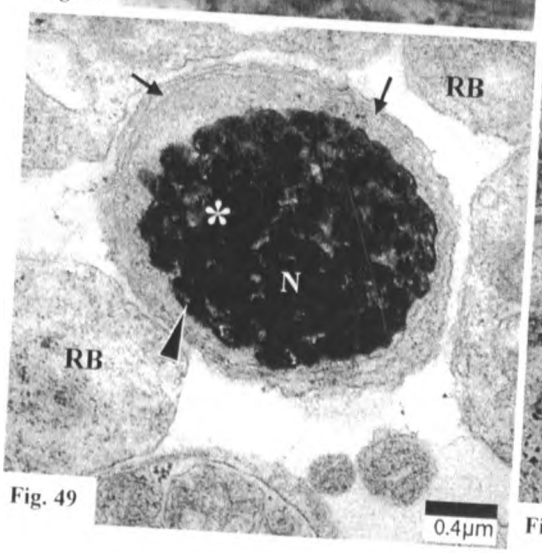


Fig. 49

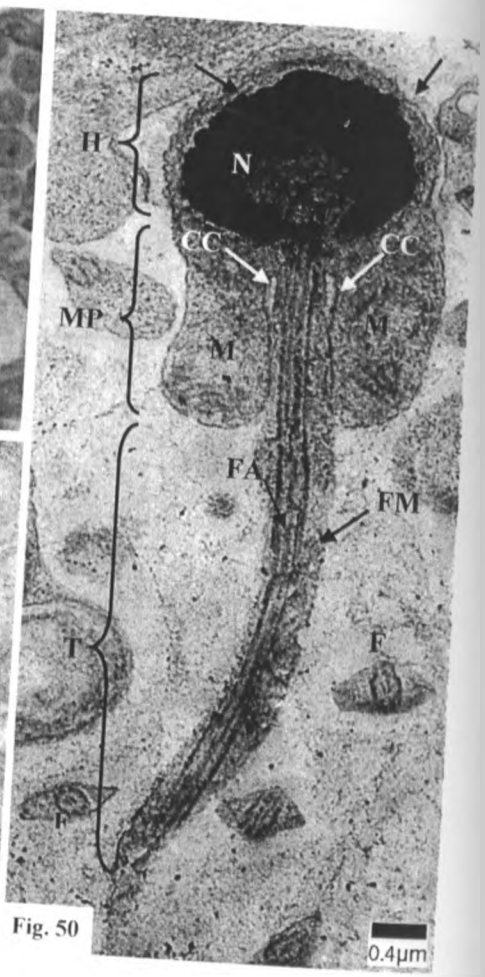


Fig. 50

FIG. 48: Photomicrograph of spermatozoa (**SZ**) showing dark staining heads (**arrows**) connected to faintly staining tails (**asterisk**) within the lobule lumen. Surrounding the lumen are cysts containing undifferentiated type A spermatogonia (**open arrow**), late type B spermatogonia (**B**) and pachytene spermatocytes (**P**). Bar = 10μm. Toluidine blue

FIG. 49: An electron micrograph showing a transverse section of a spermatozoon head of *A. grahami.* The nucleus (**N**) is packed with highly electron-dense globules of chromatin material (**asterisk**). Notice the lamellae configuration of the cytoplasm (**arrows**) and irregular outline of the nuclear envelope (**arrowhead**). Residual bodies (**RB**) are also present. Bar = 0.4μm

FIG. 50: A longitudinal section of a spermatozoon of *A. grahami* showing the major parts: head (**H**), midpiece (**MP**) and tail (**T**). The nucleus (**N**) is capped with a thin cytoplasm containing lamellations (**arrows**) and the midpiece contains mitochondria (**M**). The cytoplasmic channel (**CC**) and the flagellar axoneme (**FA**) covered by the flagellar membrane (**FM**) are evident. Notice the cross sections of flagellae (**F**) of other neighbouring spermatozoa. Bar = 0.4μm

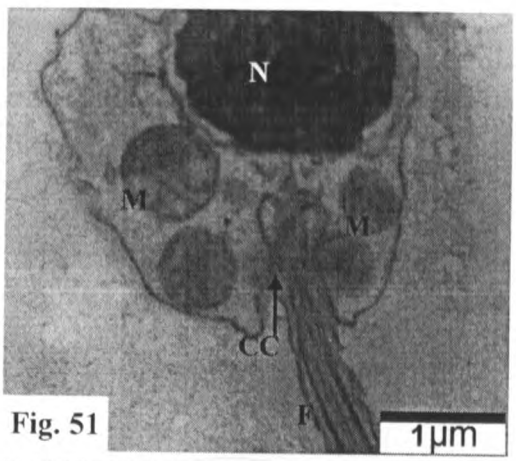


Fig. 51

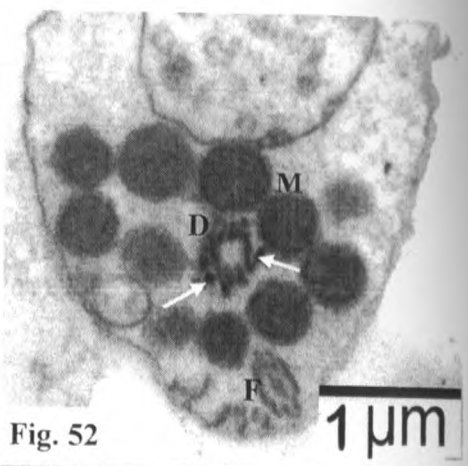


Fig. 52

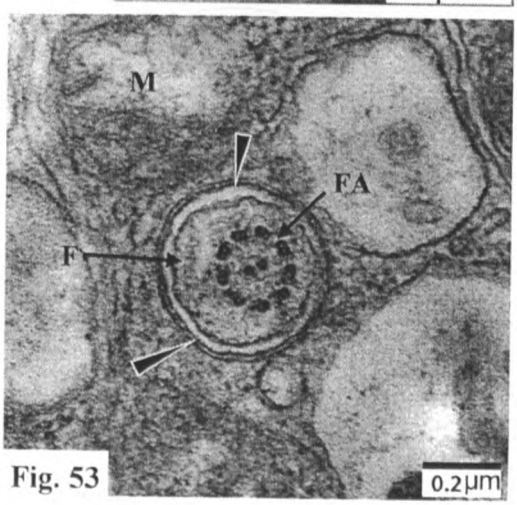


Fig. 53

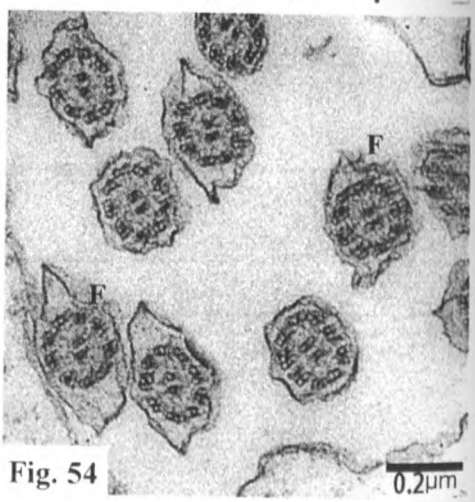


Fig. 54

FIG. 51: A longitudinal section of the spermatozoon at the midpiece region showing the two transverse layers of mitochondria (**M**) surrounding the proximal portion of the flagellum (**F**). The nucleus (**N**) and cytoplasmic channel (**CC**) are evident. Bar = 1μm

FIG. 52: An oblique-section of the proximal portion of the midpiece of a mature spermatozoon of *A. grahami* showing spheroidal mitochondria surrounding the distal centriole (**D**). Notice the nine microtubule triplets (9+3) of the distal centriole (**arrows**). The flagellum (**F**) projects distally. Bar = 1μm

FIG. 53: A cross-section of the distal portion of the midpiece revealing the central location of the flagellum (**F**) within the cytoplasmic channel/canal (**arrowheads**) and the axoneme (**FA**). Surrounding the canal are mitochondria (**M**). Bar = 0.2μm

FIG. 54: Shows cross-sections of the tails revealing the 9 peripheral doublets and a central pair of the microtubule pattern of the axoneme in the flagellae (**F**). Bar = 0.2μm

4.4.1.1.2 Sertoli cells

These were thin or flattened cells situated at the periphery of spermatocysts. Histological examination of these cells revealed presence of elongated nuclei in isolated cysts or triangular nuclei where two to three cysts approximated each other (**see Fig. 18**). They had thin cytoplasmic processes often forming a wall confining all the immature and developing germ cells to form respective spermatocysts. At the end of a spermatogenic process, Sertoli cell cytoplasmic processes opened thereby permitting release of mature spermatozoa from cysts into the lobular lumen (**see Fig. 19**).

Ultrastructurally, Sertoli cells were generally found lying on the basement membrane separating the lobular system and the perilobular boundary tissue. These cells presented highly irregular pleiomorphic nuclei containing coarse electron-lucent chromatin material distributed in a diffuse manner (**Fig. 55**). Heterochromatin was also present, limited mostly to the marginal areas of the nucleus. Within the cytoplasm, there was presence of numerous elongated mitochondria, rough endoplasmic reticulae, free ribosomes, phagolysosomes and membrane bound vesicles containing granular substances (**Figs. 55 and 56**). Sertoli cells were also found at the interstices of cysts, in which case, the nucleus was triangular in shape (**Fig. 56**). A single inter-cystic Sertoli cell was observed to produce cytoplasmic extensions that were in contact with early type B and intermediate spermatid cysts.

In particular, Sertoli cells presented specific cellular characteristics depending on the spermatogenic stage or the type of germ cells encysted or the region of the testis where they were

observed. Sertoli cells ensheathing and nursing primary spermatogonia were frequently found lying on the basement membrane with their nuclei closest to the latter (**see Fig. 24**). Such Sertoli cells essentially possessed a basal and adluminal surfaces. The basal surface was anchored onto the basement membrane common to the myoid cells, while the adluminal side associated with developing primary spermatogonia (**Figs. 24 and 57**). Moreover, it was at the adluminal side of Sertoli cells that thin cytoplasmic processes projected towards the lobule lumen, circumscribing a single undifferentiated spermatogonium, to form the new cyst (**see Fig. 24**). A substantial amount of Sertoli cell cytoplasm found between the basement membrane and primary spermatogonia contained membrane bound phagolysosomes, numerous round mitochondria, sparse rough endoplasmic reticulum and free ribosomes (**Fig. 57**). Sertoli cells ensheathing secondary/ type B spermatogonia and primary spermatocytes presented thin cytoplasmic processes containing few organelles and membrane bound vesicles (**see Figs. 30, 33 and 34**).

Sertoli cells found in the vicinity and enclosure of various spermatid stages appeared well developed, presenting thick cytoplasmic processes. In this regard, Sertoli cells found adjacent to the cyst with early spermatids, presented a cytoplasm packed with organelles similar to those found in the primary spermatogonia (**see Fig. 39**). Conversely, late spermatids were enclosed by thick Sertoli cell cytoplasmic processes equipped with numerous rod-like and ovoid mitochondria, lysosomes, sparse rough endoplasmic reticulum and free ribosomes (**Figs. 46 and 58**).

At the distal portion of the lobules, Sertoli cells lining the basement membrane produced extensive cytoplasmic processes directed luminally, and appeared to contact other Sertoli cell

processes from neighbouring cysts at the center of the lumen (**Fig. 59**. These Sertoli cells exhibited extensive and prominent cytoplasm with numerous lysosomes and phagolysosomes containing cellular debris and degenerating germ cells. Also observed was the presence of finger-like projections of the basement membrane on which the Sertoli cells were lying. These finger-like projections were directed towards the Sertoli cell cytoplasm (**Fig. 59**). At the proximal end of the collecting ducts, Sertoli cells appeared cuboidal in shape. At this point, these cells were observed connected to each other via occluding junctions at their apical termini and desmosomes at their mid-lateral regions (**Figs. 60 and 61**). They possessed conspicuous, centrally located, ovoid shaped and indented nuclei containing electron-lucent euchromatin material. The cytoplasm featured mitochondria, numerous lysosomes of varying sizes and phagolysosomes containing whole spermatozoa undergoing degradation or degeneration and other phagocytized material. Other organelles were not observed in the cytoplasm of Sertoli cells in this region (**Figs. 60 and 61**).

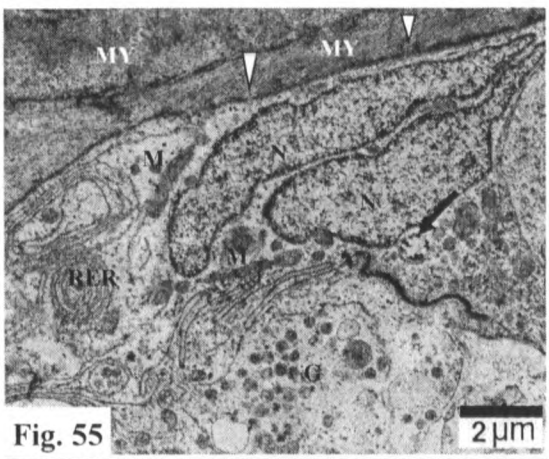


Fig. 55

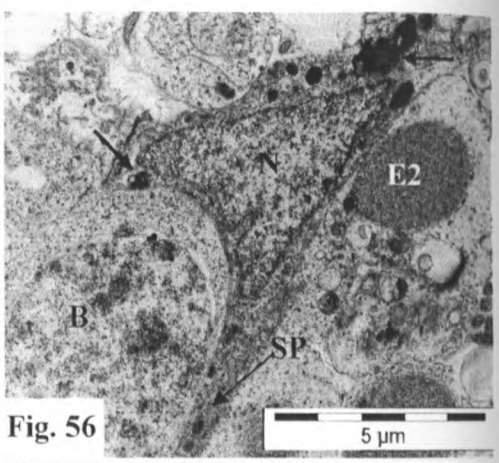


Fig. 56

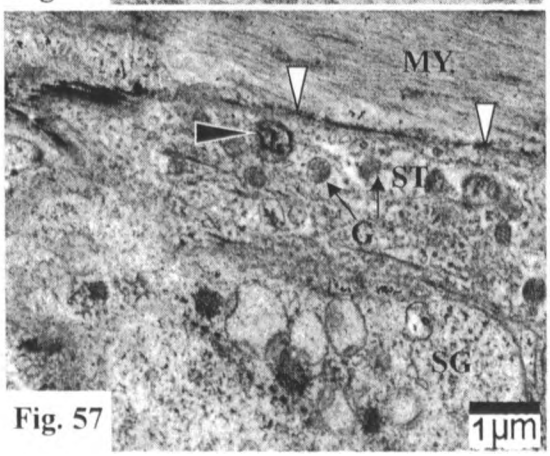


Fig. 57

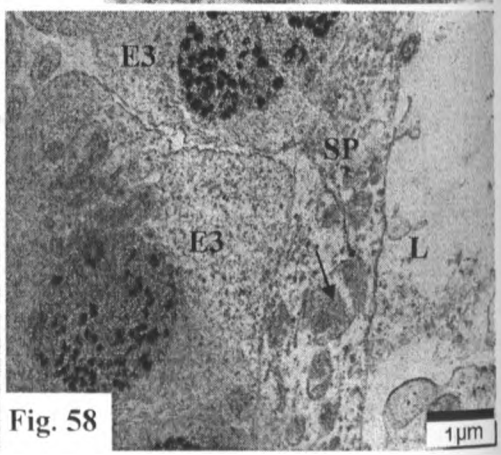


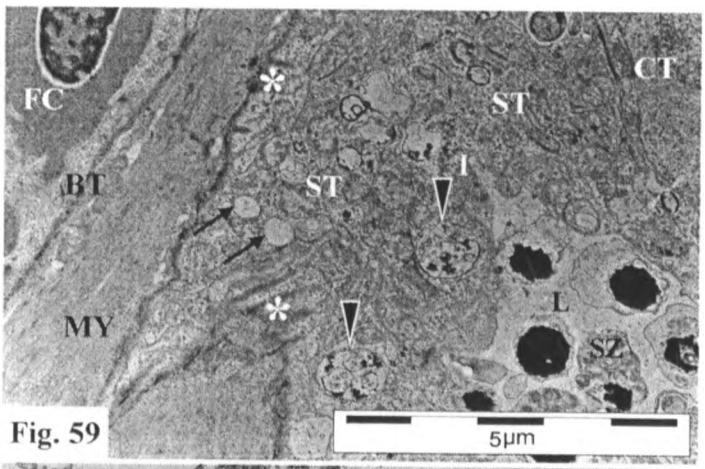
Fig. 58

FIG. 55: An electron micrograph showing a Sertoli cell lying on the basement membrane (**arrowheads**) common to myoid cells (**MY**). Irregular nucleus (**N**) containing electron-lucent heterochromatin material, elongated mitochondria (**M**), rough endoplasmic reticulum (**RER**), membrane bound vesicles with granular substances (**G**) and phagolysosome (**arrow**) are present. Bar = 2μm

FIG. 56: An electron micrograph showing an inter-cystic Sertoli cell. Notice the triangular shaped nucleus (**N**) in the interstice of early type B spermatogonia (**B**) and intermediate spermatid (**E2**). Cytoplasmic process (**SP**) and phagocytosed materials in the cytoplasm (**arrows**) are evident. Bar = 5μm

FIG. 57: An electron micrograph showing the association of primary spermatogonia (**SG**) and Sertoli cell (**ST**) lying on the basement membrane (**open arrowheads**) common to myoid cells (**MY**). Notice the membrane bound vesicles with granular substances (**G**) and phagolysosome (**closed arrowhead**) in the Sertoli cell. Bar = 1μm

FIG. 58: Shows a Sertoli cell cytoplasmic process (**SP**) enclosing late spermatids (**E3**). Vesicles (**arrow**) are also present in the cytoplasmic process. The lobule lumen (**L**) is evident. Bar = 1μm



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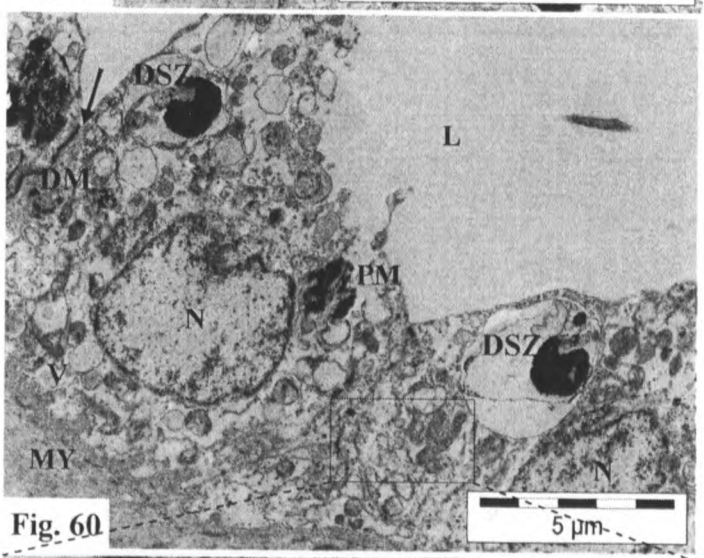

Fig. 60

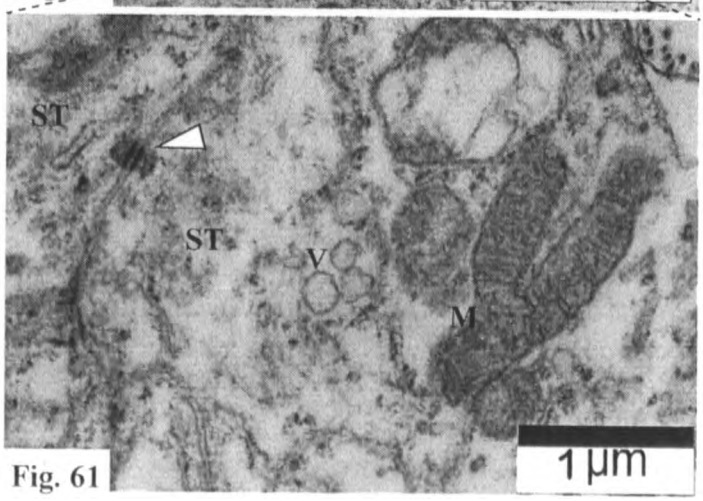

Fig. 61

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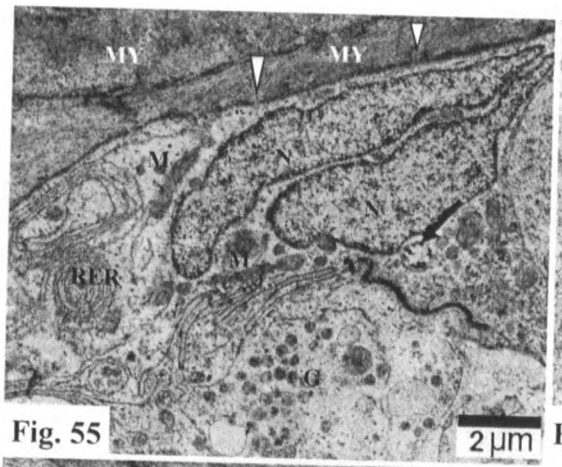
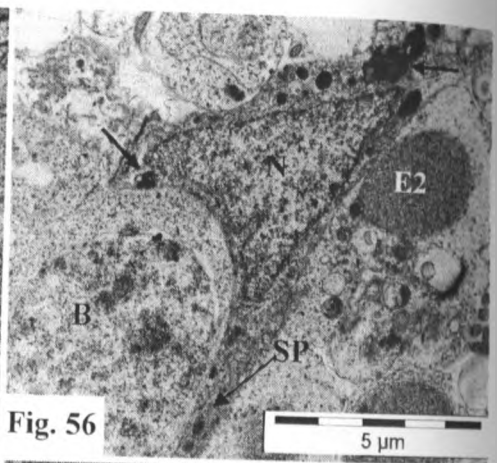


Fig. 55



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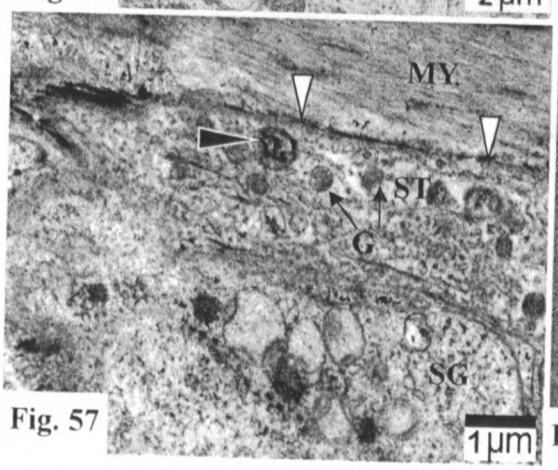

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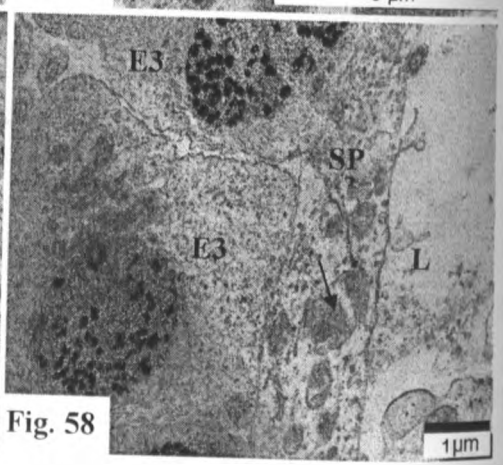
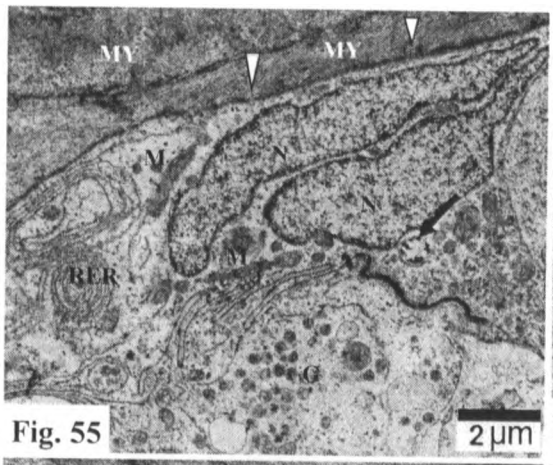


Fig. 58

processes from neighbouring cysts at the center of the lumen (**Fig. 59**. These Sertoli cells exhibited extensive and prominent cytoplasm with numerous lysosomes and phagolysosomes containing cellular debris and degenerating germ cells. Also observed was the presence of finger-like projections of the basement membrane on which the Sertoli cells were lying. These finger-like projections were directed towards the Sertoli cell cytoplasm (**Fig. 59**). At the proximal end of the collecting ducts, Sertoli cells appeared cuboidal in shape. At this point, these cells were observed connected to each other via occluding junctions at their apical termini and desmosomes at their mid-lateral regions (**Figs. 60 and 61**). They possessed conspicuous, centrally located, ovoid shaped and indented nuclei containing electron-lucent euchromatin material. The cytoplasm featured mitochondria, numerous lysosomes of varying sizes and phagolysosomes containing whole spermatozoa undergoing degradation or degeneration and other phagocytized material. Other organelles were not observed in the cytoplasm of Sertoli cells in this region (**Figs. 60 and 61**).



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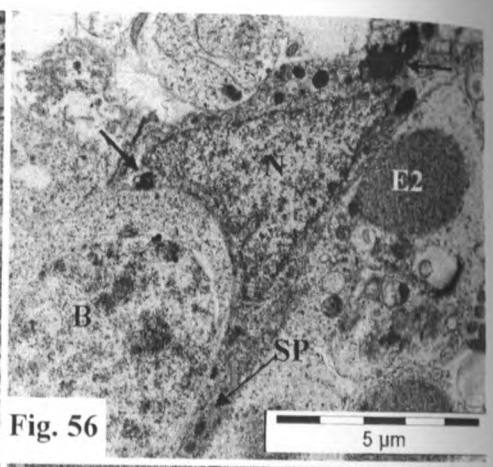

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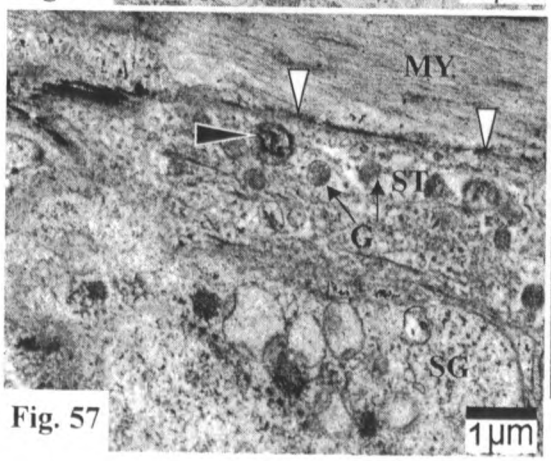

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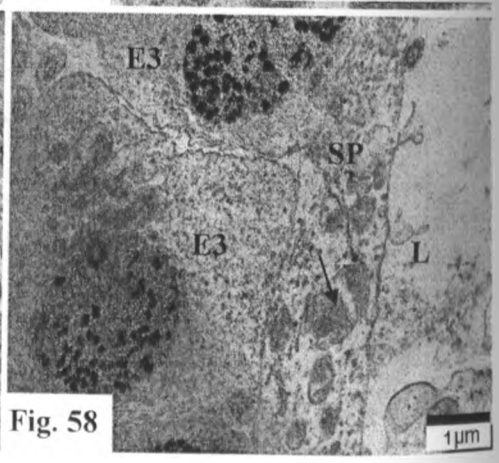

Fig. 58

FIG. 55: An electron micrograph showing a Sertoli cell lying on the basement membrane (**arrowheads**) common to myoid cells (**MY**). Irregular nucleus (**N**) containing electron-lucent heterochromatin material, elongated mitochondria (**M**), rough endoplasmic reticulum (**RER**), membrane bound vesicles with granular substances (**G**) and phagolysosome (**arrow**) are present. Bar = 2μm

FIG. 56: An electron micrograph showing an inter-cystic Sertoli cell. Notice the triangular shaped nucleus (**N**) in the interstice of early type B spermatogonia (**B**) and intermediate spermatid (**E2**). Cytoplasmic process (**SP**) and phagocytosed materials in the cytoplasm (**arrows**) are evident. Bar = 5μm

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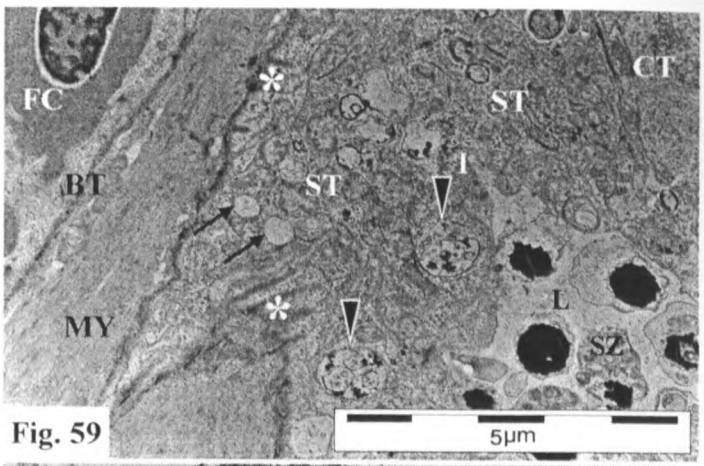

Fig. 59

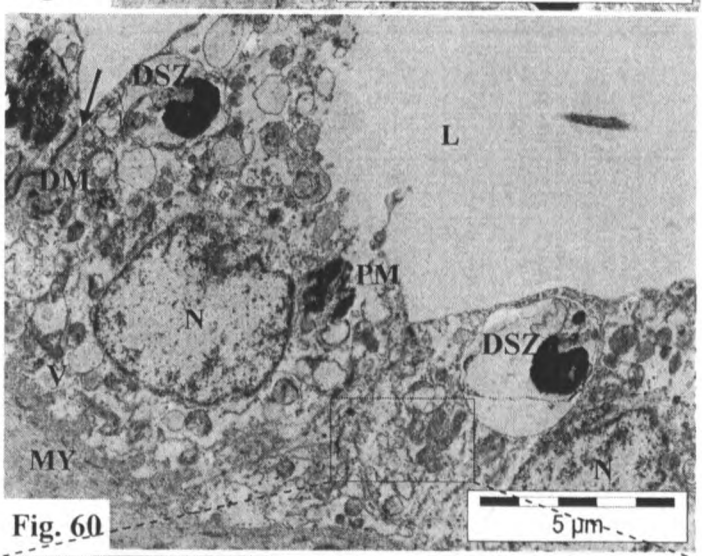

Fig. 60

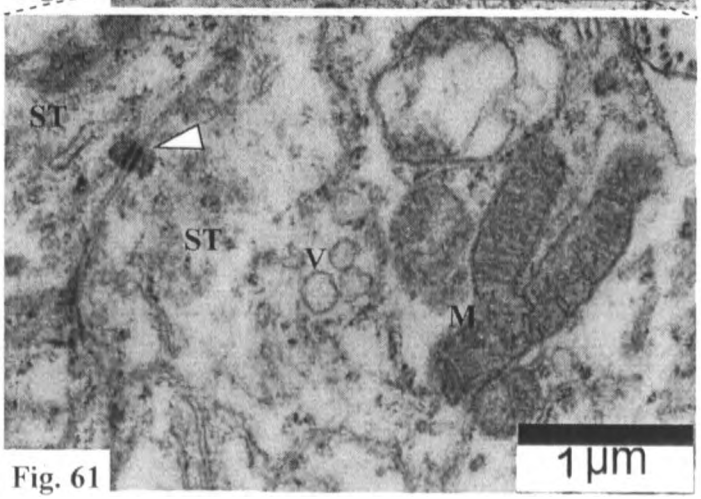

Fig. 61

FIG. 59: Shows Sertoli cell (**ST**) cytoplasmic extensions from the cyst (**CT**) and boundary tissue (**BT**) interconnecting (**I**) in the lobule lumen. Within the cytoplasm are vacuoles (**arrows**) and phagolysosomes (**arrowheads**). Notice the finger-like projections of the basement membrane into the Sertoli cell (**asterisks**). The lumen (**L**) containing spermatozoa (**SZ**) and the boundary tissue with a fibrocyte (**FC**) and myoid cells (**MY**) are present. Bar = 5μm

FIG. 60: Shows cuboidal Sertoli cells lining the proximal portion of the collecting duct. Notice the occluding junction at the apical terminus of the cell (**arrow**). The nuclei (**N**) contain electron-lucent euchromatin. In the cytoplasm are numerous vacuoles (**V**), degenerating mitochondria (**DM**), phagolysosomes with degenerating spermatozoa (**DSZ**) and phagocytized material (**PM**). The duct lumen (**L**) and a portion of the myoid cell (**MY**) are evident. Bar = 5μm.

FIG. 61: Shows a magnified portion of **Fig. 60** between two Sertoli cells (**ST**). Notice a desmosome (**arrowhead**) between the cells. Intact mitochondria (**M**) and vacuoles (**V**) are present. Bar = 1μm

4.4.1.2 Lobular boundary tissue

4.4.1.2.1 Myoid cells

Light microscopic examination of Lake Magadi tilapia testis revealed myoid cells as the main cellular component forming the boundary tissue/lamina propria separating contiguous seminiferous lobules (**see Fig. 26**). This tissue was also observed between the lobular system and interstitial tissue (**see Fig. 25**). Myoid cells also constituted part of the tissue components of the testicular wall, frequently occurring at the terminal blind endings of the seminiferous lobules (**see Fig. 14**). Histologically, they were fusiform or spindle shaped, arranged in a discontinuous pattern along the lamina propria. They often exhibited elongated nuclei which assumed the shape and orientation of the cell (**see Figs. 23 and 37**).

Ultrastructurally, myoid cells were observed to be the most abundant and conspicuous cells in the lamina propria. They were frequently arranged in 1 to 3 overlapping layers along the boundary tissue. Often, they were oriented longitudinally along the lamina propria (**Fig. 62 and 63**). Occasionally, desmosomes and hemidesmosomes were observed between adjacent myoid cells. Separating each contiguous and succeeding myoid cell within the boundary tissue was a thin layer of electron-dense collagen fibers, disposed more-or-less obliquely to the myoid cells. Myoid cells on either side of the collagen fiber layer were bound to the latter via hemidesmosomes (**Fig. 63**). Myoid cell layers disposed laterally within the boundary tissue contacted the basement membrane common to Sertoli cells. A thin layer of electron-dense collagen fibers was also present between the laterally placed myoid cells and the basement membrane. Where this collagen layer was present, hemidesmosomes appeared to form a junction

between the myoid cells and the collagen fiber layer in this region of the tunica propria (**Figs. 63 and 64**). The internal ultrastructure of myoid cells showed smooth muscle-like characteristics. They possessed prominent, centrally located, ovoid (**Fig. 62**) or indented and elongate (**Fig. 63**) nuclei containing relatively electron-dense chromatin material, mostly euchromatin, lying mainly along the nuclear envelope. The cytoplasm was almost completely occupied by the closely packed contractile fibers (microfilaments) oriented along the longitudinal axis of the cell. Other organelles were scarcely present except for a few ovoid mitochondria scattered within the cytoplasm together with numerous pinocytotic vesicles limited mostly along the margins of the plasma membranes (**Figs. 62, 63 and 64**). Occasionally, some myoid cells demonstrated presence of buds on their medial surfaces sprouting into the interlobular space, while others possessed branches which appeared to enter into other boundary tissues.

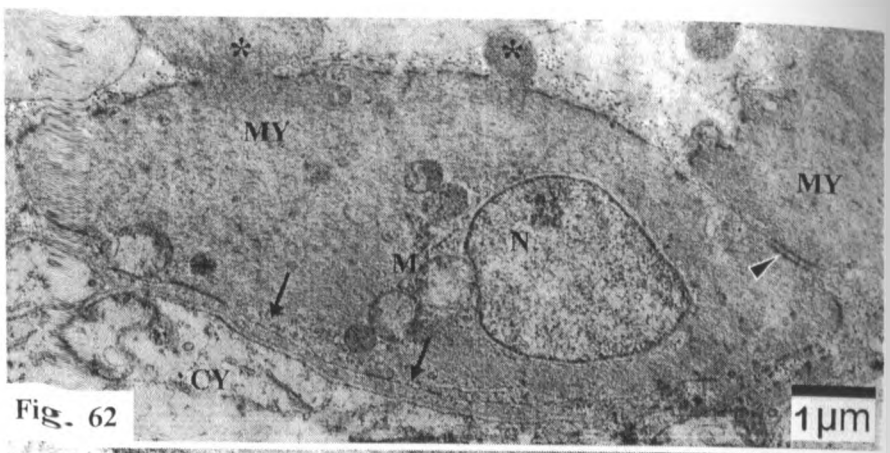

Fig. 62

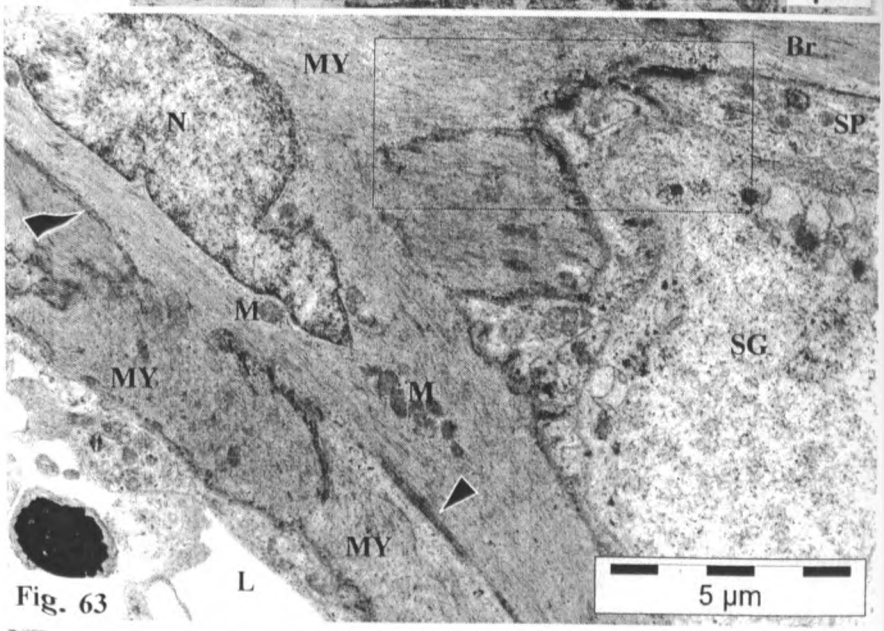

Fig. 63

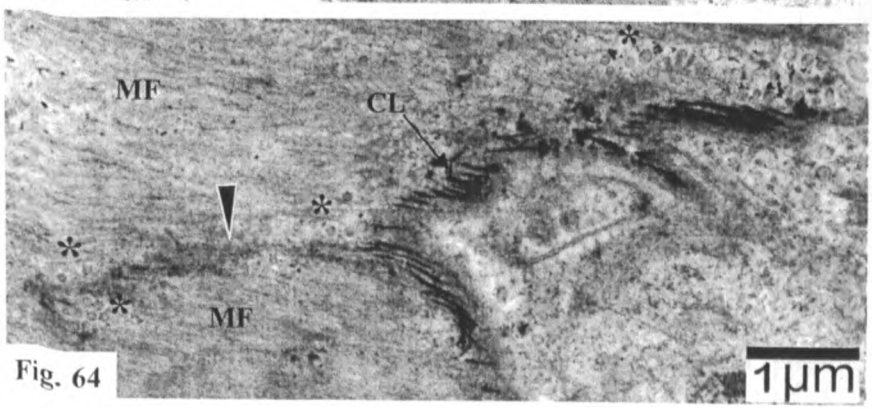

Fig. 64

FIG. 62: An electron micrograph showing a myoid cell with a prominent ovoid nucleus (**N**) and numerous spheroidal mitochondria (**M**). Notice myoid cell 'buds' (**asterisks**) and a desmosome junction (**arrowhead**) between two adjacent myoid cells (**MY**). Prominent basal lamina (**arrows**) and a cyst in the lobule lumen (**CY**) are present. Bar = 1 μm

FIG. 63: An electron micrograph of the boundary tissue showing the layers of myoid cells (**MY**) joined by hemidesmosomes (**arrowheads**). Notice an indented and elongated nucleus (**N**) and a branch of a myoid cell (**Br**) in another boundary tissue. Mitochondria (**M**), Sertoli cell process (**SP**), primary spermatogonia (**SG**) and the lobule lumen (**L**) are evident. Bar = 5 μm

FIG. 64: A magnified portion of **fig. 63** above (**see rectangle**) showing microfilaments (**MF**) occupying the cytoplasm and pinocytotic vesicles (**asterisks**) along the plasma membrane of myoid cells. Note the collagen fibers (**CL**) binding myoid cells to the basement membrane and also to each other. Hemidesmosome (**arrowhead**) between myoid cells and collagen fibers is evident. Bar = 1 μm

4.4.1.3 Interstitial tissue compartment

This compartment comprised mainly the tissue adjacent to the seminiferous lobules and also collecting and efferent ducts. This tissue was particularly very prominent in the interstices of three or more lobules (**Fig. 65**). Part of this compartment was also distributed below the testicular capsule in spaces circumscribed by the blind endings of seminiferous lobules, hence, referred to as subcapsular interstitial tissue (**Fig. 66**). The tissue components found in these areas were mainly Leydig cells, fibroblasts and blood vessels.

4.4.1.3.1 Leydig cells

These were roughly spheroidal cells exclusively observed both in the interlobular and subcapsular interstitial tissue, either singly or in small clusters (**Figs. 65 and 66**).

Under light microscopy, they exhibited prominent spheroidal nuclei measuring 4.91 ± 0.15 μm (n = 20) in diameter (**see Table 2**) and contained one or more nucleoli with varying amounts and density of chromatin material. Ultrastructurally, Leydig cells occurred within the interstitium bordered by myoid cells (**Figs. 67 and 68**). The cytoplasm of the Leydig cells contained an elaborate network of smooth endoplasmic reticulum comprising mainly of the vesicular components, lipid droplets and numerous well developed mitochondria exhibiting well defined cristae.

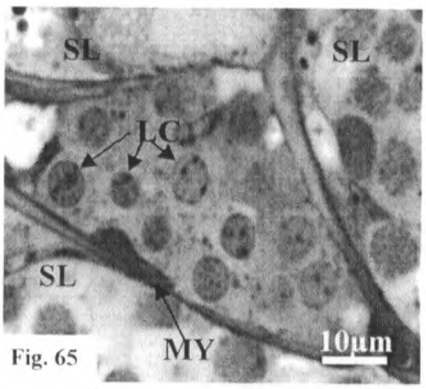

Fig. 65

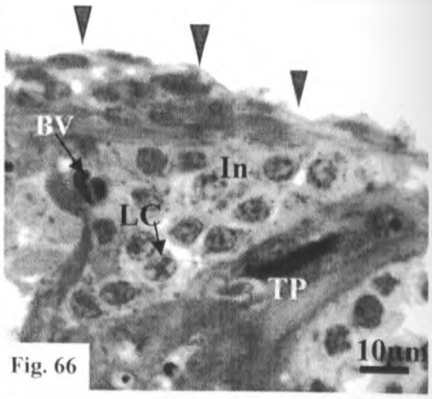

Fig. 66

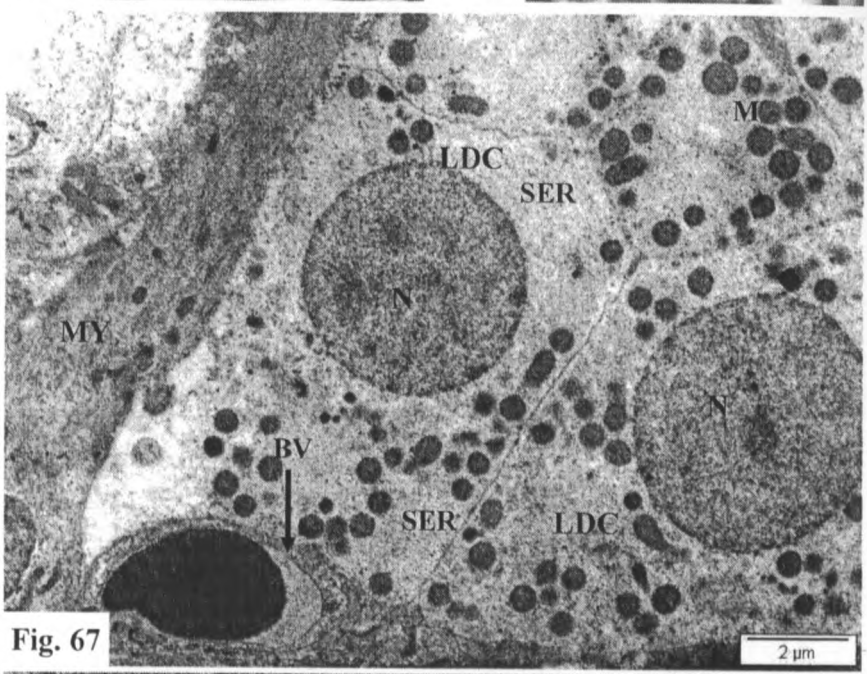

Fig. 67

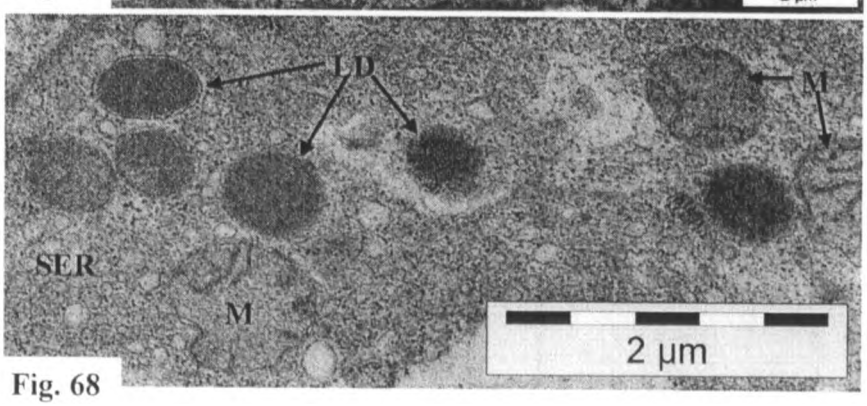

Fig. 68

FIG. 65: The interstitial tissue of *A. grahami* testis located in the interstices of three seminiferous lobules (**SL**). Several Leydig cells (**LC**) are distributed within the interstitial space. Notice the boundary tissue containing myoid cell (**MY**) demarcating the interstitial tissue from the seminiferous lobules. Bar = 10μm. Toluidine blue

FIG. 66: A photomicrograph of subcapsular interstitial tissue (**In**) beneath the tunica albuginea (**arrowheads**) and well bound on the inner border by the tunica propria (**TP**). Notice the small blood vessels (**BV**) and Leydig cells (**LC**). Bar = 10μm. Toluidine blue

FIG. 67: An electron micrograph of the interstitial tissue showing Leydig cells (**LDC**). Within the cytoplasm are mitochondria (**M**) and smooth endoplasmic reticulum (**SER**). The spheroidal nucleus (**N**), a blood vessel containing erythrocytes (**BV**) and bordering myoid cell (**MY**) are evident. Bar =2μm

FIG. 68 A magnified portion of the cytoplasm of Leydig cell. Smooth endoplasmic reticulum (**SER**), Lipid droplets (**LD**) and mitochondria (**M**) with clear cristae are evident. Bar =2μm

4.4.1.3.2 Collagen fibers and fibroblasts

Collagen fibers were the most abundant connective tissue network within the testis of *A. grahami*. They provided an elaborate stroma upon which the lobular and spermatic ducts were embedded. Collagen fibers were distributed within the interstitial tissue found between the seminiferous lobules and also in the testicular capsule continuous with the boundary tissue through the cortex to the medulla. In addition, fibroblasts also occurred in these areas disposed along the longitudinal axis of the boundary tissue **(see Figs. 14 and 69).**

4.4.1.3.3 Blood vessels

The testicular tissue was endowed with numerous small blood vessels in the interstitial tissue compartment. Some small blood vessels were found in the interlobular tissue at the cortical region (**Figs. 66 and 70**) while fairly large ones were limited to the interstitium of the medullary region separating the efferent ducts (**Fig. 71**). Clusters of Leydig cells were found adjacent to these blood vessels (**Figs. 70 and 71**).

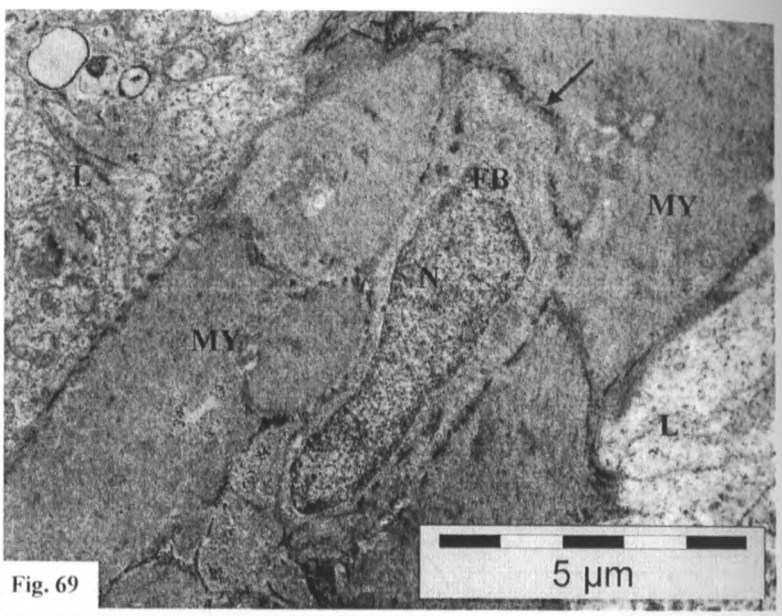

Fig. 69

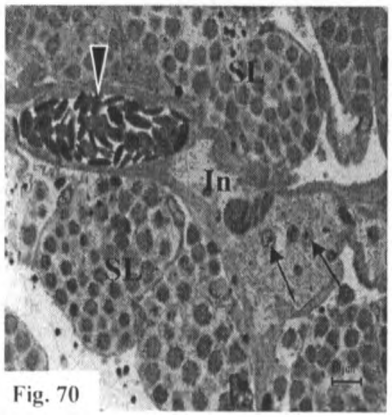

Fig. 70

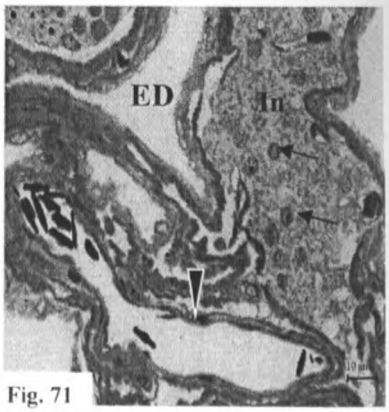

Fig. 71

FIG. 69: An electron micrograph showing a fibroblast (**FB**) containing an elongated nucleus (**N**) sandwiched between myoid cells. A layer of collagen fibers (**arrow**) separating the cells and lobule lumen (**L**) are evident. Bar 5µm

FIG. 70: A photomicrograph of the testicular parenchyma depicting a small blood vessel (**arrowhead**) laden with erythrocytes and Leydig cells (**arrows**) within the interstitial tissue (**In**). Bordering the interstitium are seminiferous lobules (**SL**) with various spermatocysts. Bar = 10µm. Toluidine blue

FIG. 71: A photomicrograph showing a large blood vessel (**arrowhead**) and Leydig cells (**arrows**) in the interstitial tissue (**In**) bordering the efferent ducts (**ED**). Bar = 10µm. Toluidine blue

4.4.2 Efferent duct system

The efferent duct system in the Lake Magadi tilapia appeared to originate from the medullary region of the testis where the lobular system opened into the collecting duct system (**see Fig. 15**). The collecting duct system consisted of a variety of ducts whose lumina were wider than those of the lobules. The germinal epithelium in the collecting ducts was scarce and discontinuous, with the undifferentiated spermatogonia being the prominent germ cells (**see Fig. 21**), and their lumina were often filled with spermatozoa (**Fig. 72**). Collecting ducts were separated by thick boundary tissue consisting of myoid cells and connective tissue arranged in several layers. These ducts subsequently merged to form the spermatic ductules (efferent ducts). Consequently, the density and thickness of the boundary tissue increased towards and at the level of the efferent ducts; the latter having walls whose inner linings were completely devoid of the germinal epithelium (**Figs 71, 73 and 74**). In some of the fish, nodular structures appeared within the interstitial tissue as an incidental finding, separating efferent ducts in the medullary region of the testis (**Figs. 74 and 75**). These structures were distinctly enclosed by several layers of myoid cells and connective tissue within the interstitium. Each of these nodular centers contained numerous cells with spheroidally shaped nuclei. Under light microscopy, cells in these centers had dark staining nucleoli, positioned more-or-less at the center. However, their cytoplasm and plasma membranes were rather indistinct (**Fig. 75**).

The efferent ducts then opened into the largest intratesticular duct; the main testicular spermatic duct at the dorsum of the testicular lobe located just beneath the mesorchium (**Figs. 16 and 76**). Each testicular spermatic duct running along the longitudinal axis of each testicular lobe contained spermatozoa collected from a number of efferent ducts within the lobe (**Fig. 76**).

Consequently, the two main spermatic ducts from each lobe converged to form the common spermatic duct. The common spermatic duct was located in close proximity and ventral to the urethral duct; the two being surrounded and separated from each other by a dense irregular connective tissue, consisting mostly of collagen fibers. The lumina of both ducts were thrown into folds, projecting into the luminal space (**Fig. 77**). Interestingly, the common spermatic duct from the non-spawning Lake Magadi fish presented different morphological features compared to that from the spawning fish. In this regard, the luminal wall of the common spermatic duct from a non-spawning fish was formed by relatively short folds lined by a simple cuboidal epithelium resting on a basement membrane. Beneath the basement membrane, was a network of loose connective tissue in which were embedded connective tissue cells and subepithelial blood vessels (**Fig. 78**). On the other hand, the common spermatic duct from the spawning fish contained spermatozoa intermixed with cellular secretory material (**Fig. 79**). In addition, the luminal wall was thrown into numerous deep folds forming crypts into which cellular secretory material, seemingly produced by apocrine secretion, were collected. The folds were highly pronounced, each penetrated by a core of collagen fibers at the center and lined by simple cuboidal epithelium.

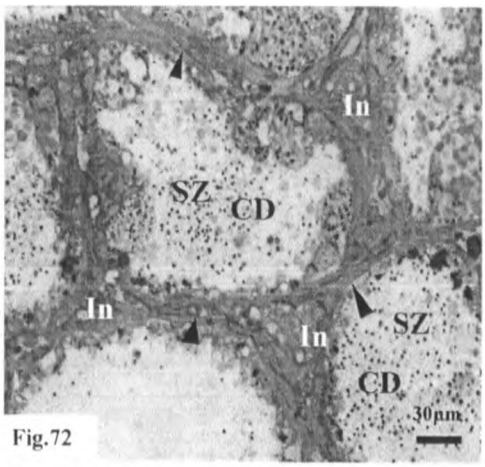

Fig.72

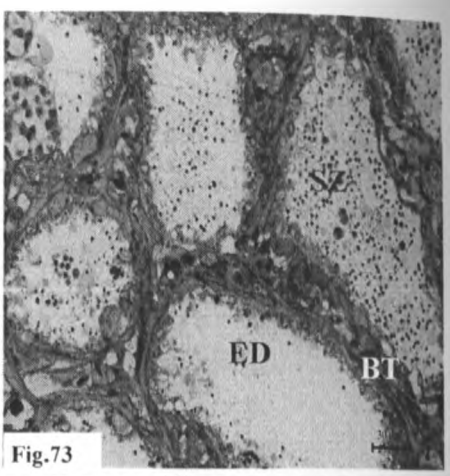

Fig.73

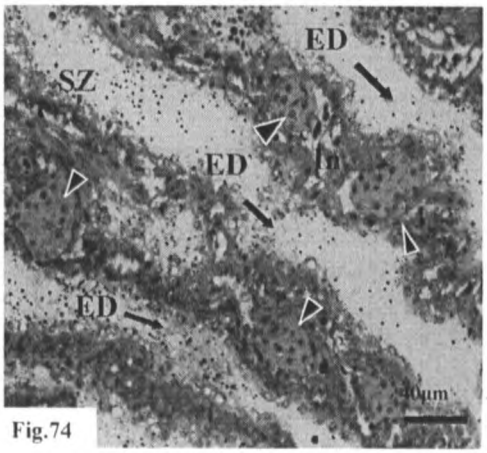

Fig.74

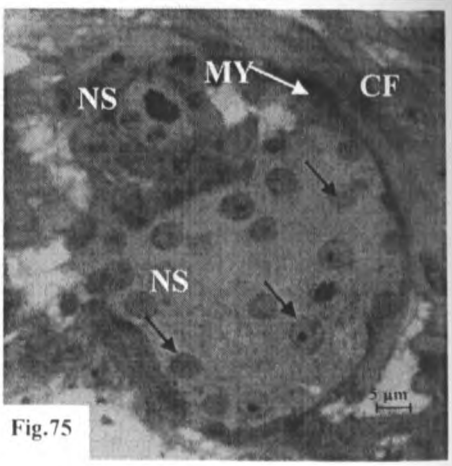

Fig.75

FIG. 72: Transverse section of the duct system of *A. grahami* showing the interstitium (**In**) and collecting ducts (**CD**). Spermatozoa (**SZ**) and prominent tunica propria (**arrowheads**) are shown. Notice that most of the duct wall is devoid of spermatocysts. Bar = 30μm. Toluidine blue

FIG. 73: Transverse section of Magadi tilapia testis showing efferent ducts (**ED**) in the medullary region containing spermatozoa (**SZ**) and separated by thick boundary tissue (**BT**). Notice the characteristic absence or discontinuity of the germinal epithelium in the inner lining of the efferent ducts. Bar = 30μm. Toluidine blue

FIG. 74: Longitudinal section of the efferent ducts (**ED**) containing spermatozoa (**SZ**). Notice the presence of nodular structures (**arrowheads**) distributed along the interstitium (**In**). Bar = 40μm. Toluidine blue

FIG. 75: A high power magnification of the nodular structure (**NS**) containing cells with ovoid to spheroidal nuclei (**arrows**). Notice the thick myoid cell (**MY**) and collagen fibers (**CF**) around the nodule. Bar = 5μm. Toluidine blue

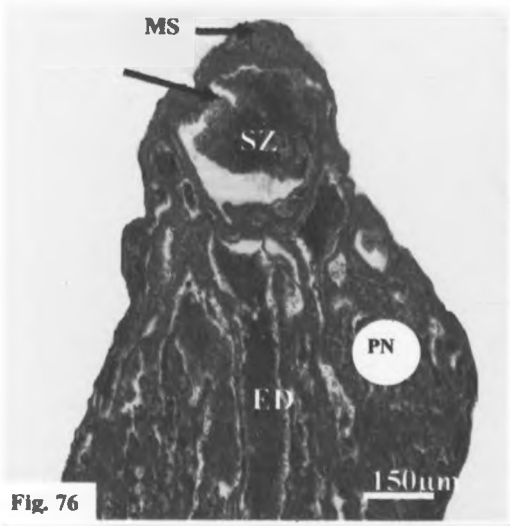

Fig. 76

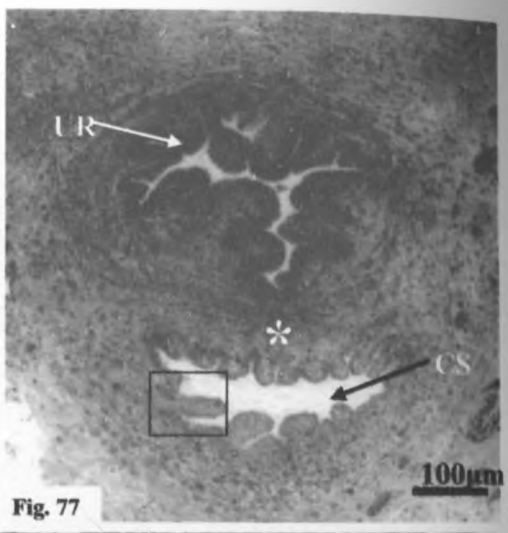

Fig. 77

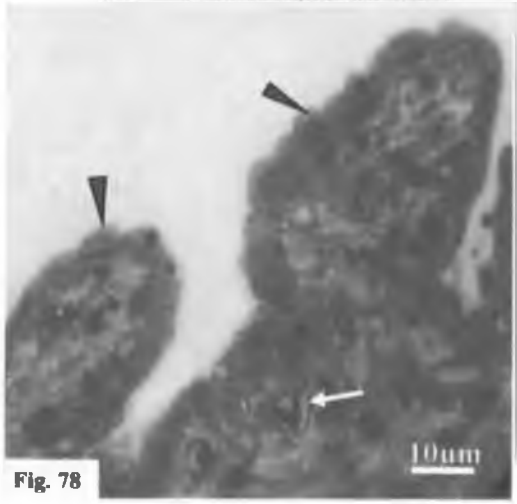

Fig. 78

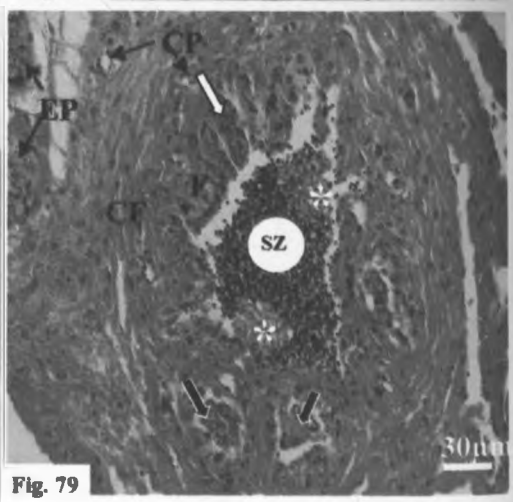

Fig. 79

FIG. 76: Transverse section of *A. grahami* testicular parenchyma (**PN**) showing efferent ducts (**ED**) and the main testicular spermatic duct (**arrow**) with spermatozoa (**SZ**). The mesorchium (**MS**) is evident. Bar = 150μm. H/E

FIG. 77: Transverse section of the extratesticular spermatic duct, the common spermatic duct (**CS**), from a non-spawning *A. grahami* separated from the urethral duct (**UR**) by thick connective tissue (**asterisk**). Notice that both ducts have their walls thrown into folds projecting into their respective lumina. Bar = 100μm. Toluidine blue

FIG. 78: A high power magnification of a portion of the common spermatic duct of a non-spawning Magadi fish (**see square in Fig. 77**) showing simple cuboidal epithelium (**arrowheads**). Notice the sub-epithelial blood vessels (**arrow**) in the lamina propria. Bar = 10μm. Toluidine blue

FIG. 79: Transverse section of the common spermatic duct of a spawning *A. grahami* fish containing spermatozoa (**SZ**) and cellular secretory material (**asterisks**) within the lumen. Notice the prominent folds (**F**) forming deep crypts (**closed arrows**) into the lumen. Sub-epithelial capillaries (**CP**), a portion of the urinary bladder epithelium (**EP**) and sub-epithelial collagen fibers (**CF**) with a portion of the latter extending into the folds (**open arrow**) are evident. Bar = 30μm. Masson's trichrome

CHAPTER 5

5.0 DISCUSSION

5.1 Breeding and brooding behaviour

Cichlids exhibit a wide spectrum of social and reproductive behaviors (Fishelson, 1983, 2003). The most common reproductive behaviour in cichlids is brooding, generally categorized as substrate and mouth brooding. These brooding behaviors have subsequently been used to classify various cichlid species, especially tilapiines. Under this classification, three genera of tilapiines have been established; *Sarotherodon*, the biparental and paternal mouth brooders (Trewavas, 1966 a, b), *Tilapias*, the substrate spawners (Trewavas, 1981, 1982, 1983) and *Oreochromis*, the maternal mouth brooders (Trewavas, 1983). The genus *Oreochromis* has subsequently been subdivided into five sub-genera: *Oreochromis*, *Nyasalapia*, *Vallicola*, *Alcolapia* and *Neotilapia* (Nagl *et al.*, 2001). *Alcolapia* as a subgenus has recently been moved into the genus level (Seegers *et al.*, 1999) with *A. grahami*, *A. ndalani*, *A. latilabris* and *A. alcalicus* being the main species. The breeding habits of *Alcolapia grahami* observed in this study confirm that it is indeed a maternal mouth brooder, as previously documented by Coe (1966) and Seegers and Tichy (1999). Generally, this is also in agreement with the brooding characteristics of the genus *Oreochromis* (Nagl *et al.*, 2001) exemplified by the Nile tilapia, *Oreochromis niloticus* (Fishelson, 2003); a close phylogenetic relative to the genus *Alcolapia*. In addition, the reproductive behavior of *Alcolapia grahami* regarding construction of breeding nests in the lagoon site ostensibly to attract spawning females, also appears to be a tilapiine trait (Fishelson, 2003). These breeding nests seem to be the site of fertilization in this fish following deposition

of female and male spawns (Coe, 1966; Seegers and Tichy, 1999). This, however, remarkably contrasts with what is obtained in other closely related tilapiines such as *O. macrochir*, *O. aureus* and *O. hornorum* where fertilization is reported to occur in the mouth cavity (Fishelson, 2003; Grier and Fishelson, 1995). In some cases, for example *Astatotilapia flaviijosefi*, females nuzzle at the males genital papillae during spawning in order to collect semen (Fishelson, 2003). Accordingly, mouth brooders incubate fertilized eggs in the mouth through the hatching process until complete metamorphosis is achieved before the juveniles are released into the surrounding water (Fishelson, 2003).

Apart from the eggs and embryos, free swimming frys were also observed in the mouth cavity of the female *A. grahami* in this study, upon capture. This phenomenon has also been observed in other tilapiines and haplochromines (Fishelson, 2003) and is considered a protective mechanism provided by the parents to their juveniles. Generally, mouth brooders release free swimming juveniles into the water but continue to guard them, occasionally taking them back into the mouth in cases of imminent danger or at night time, until they are 2 to 4 weeks of age. Therefore, this explains the presence of the free swimming frys in the buccal cavity of the female Lake Magadi fish as they were possibly being protected from the dangers posed when fishing exercise was underway. In the lagoon site, the clustering of frys in small shallow pools (20 to 30cm in diameter) situated 2 to 3 m from the main lagoon, may also be considered an additional protective strategy, since keeping frys in these pools ensures protection from constant predation by other mature fish in the main lagoon. In addition, numerous crevices present around these pools offer the frys good hiding sites in cases of any impending danger. Besides the provision of defense,

these pools were also directly supplied by water from springlets which was free from contamination frequently encountered in the main lagoon. The main lagoon is frequently associated with adverse and drastic diurnal changes with regard to environmental conditions, notably oxygen and temperature changes. The shallowness of the pools and the apparent absence of algae render them less likely to experience drastic oxygen changes often experienced in the main lagoon. These pools therefore became the best alternative growing grounds for the fragile frys of Lake Magadi tilapia.

5.2 Male reproductive system of Lake Magadi tilapia

The male reproductive system in *A. grahami* comprised the testes, excurrent ducts and the genital papilla; features which are common to most teleosts. The testes have a major role in production of germ cells and testicular androgens (Matta *et al.,* 2002; Weltzien *et al.,* 2004).

5.2.1 The testes

The anatomical location of *A. grahami* testes in the coelomic cavity, stretching longitudinally and suspended dorsally in the body wall by the mesorchium is a presentation common to many teleosts (Fishelson *et al.*, 2006; Almeida *et al.,* 2008). In the mature male and female *A. grahami*, the entire coelomic cavity was covered by a parietal peritoneum laden with a black pigment contained in a thin membrane on its medial surface. This black membrane was also reflected on the mesorchium suspending the testis. Practically, all the visceral organs in the peritoneal cavity, including the gonads were enclosed by this pigmented peritoneum. Ultra-violet radiation within Lake Magadi environment are reportedly high (Narahara *et al..*, 1996; Wood *et al.*, 2012),

therefore, the presence of this pigmented peritoneal membrane may be presumed to provide protection to the gonads of *A. grahami* against the UV radiation. A similar strategy has been reported in some species of lizards where pigmented parietal peritoneum provided protection against UV radiation and infra-red absorption while those without this pigmented layer depended, to a large extent, on the deep dermal layer of melanocytes for protection (Bauer, 1997).

The Gonadosomatic index (GSI) of *A. grahami* (0.71%) in this study is relatively higher compared to that of Nile tilapia (0.29%), (Matta, *et al.*, 2002). Increase of the GSI in fish has, to some extent, been associated with high water temperatures. According to Alvarenga and França (2009), temperatures between 30-35°C triggers rapid germ cell proliferation in the testis. Consequently, the high temperatures recorded in Lake Magadi (35-40°C), may be presumed to contribute to the high GSI observed in *A. grahami*.

The gross morphological picture of *A. grahami* testes consisting of a paired, elongated, bilobed organ, exhibiting a whitish/creamish colouration, is similar to what has been reported in most teleosts (Fishelson, 2003; García-López *et al.*, 2005; Fishelson *et al.*, 2006). The elongated testicular morphology is the most common among teleosts, as shown in the cichlid; Nile tilapia (Lacerda *et al.*, 2006), apogonid; cardinal fish (Fishelson *et al.*, 2006) and cyprinid; zebrafish (Leal *et al.*, 2009). Siluriforms possess longitudinal testes but, in addition, have characteristic digitiform projections or fringes of varying sizes and lengths depending on the species, disposed laterally along the entire length of the testes (Lopes *et al.*, 2004; Barros *et al.*, 2007; Santos *et al.*, 2010). There is, however, a deviation from the general longitudinal morphology of the testes

among some groups of fish. The Gadiforme (Atlantic cod), for example, possess testes with several lobes arranged around a central collecting duct (Almeida *et al.*, 2008) while Pleuronectiforms (*Solea senegalensis* and *Solea solea*) have rounded testes (Baynes *et al.*, 1994; Bromley, 2003; García-López *et al.*, 2005). Among teleosts with elongated testes, the lobes may be partially or completely fused. In *A. grahami*, the testes lobes were partially fused with a connective tissue septum loosely apposing them. This phenomenon has also been observed in other fish species, for example, Perch (Billard, 1986) and Nile tilapia (Lacerda *et al.*, 2006). Guppies, on the contrary, have completely fused lobes (Billard, 1986). The two lobes join caudally to connect with the common spermatic duct, eventually opening via a urogenital papilla, located just caudal to the anal pore.

The histological organization of the testes of Lake Magadi tilapia is, in many ways, similar to what is found in many teleosts (Koulish *et al.*, 2002; Lopes *et al.*, 2004; Fishelson *et al.*, 2006). It is covered by an outer wall (tunica albuginea) consisting of the visceral epithelium, fibroblasts, collagen fibers and myoid cells. The amount of collagen fibers appeared to increase dorsally towards the mesorchium which, in this study, was presumed to offer strong anchorage of the testicular lobes onto the dorsal body wall. Often, septae from the tunica albuginea penetrate the substance of the testis forming the boundary tissue (Koulish *et al.*, 2002; Fishelson *et al.*, 2006) and, in effect, carry with them blood vessels that supply the testicular parenchyma. The testicular parenchyma in Lake Magadi tilapia comprised the cortex, where active spermatogenesis occurred and, medulla, mainly containing the excurrent ducts. This regional characterization bears resemblance to what has been observed in flatfish *Solea senegalensis* (García-López *et al.*, 2005).

These two regions, as in other teleosts (Grier, 1981; García-López *et al.*, 2005; Nóbrega *et al.*, 2009), and mammals (Russell *et al.*, 1990), further comprised the germinal and interstitial compartments separated by a basement membrane.

5.2.1.1 The germinal compartment

The disposition of the germinal compartment among different groups of teleosts has necessitated the classification of the testicular architecture in this group of vertebrates into two major categories; the lobular and anastomosing tubular types (Grier, 1981, 1993; Weltzien *et al.*, 2002; Parenti and Grier, 2004). The Magadi tilapia possesses a germinal compartment organized into lobules, running from the medulla to the peripheral cortex, terminating blindly just beneath the testicular wall. This disposition of the germinal compartment conforms to the previously described lobular testis type (Grier, 1993; Parenti and Grier, 2004). Lobular testicular structure is considered a synapomorphy of the Neoteleostei (Parenti and Grier, 2004), therefore, suggesting that Lake Magadi tilapia is a member of the higher teleosts/ Neoteleosts.

The distribution of primary spermatogonia in the germinal epithelium is also a classifying feature of the teleostean testis. In this study, the primary spermatogonia (undifferentiated and differentiated) were distributed throughout the seminiferous lobule, including the areas adjacent to the collecting ducts of the testis. This form of distribution of primary spermatogonia closely approximates the previously described unrestricted spermatogonial type (Parenti and Grier, 2004). Consequently, the testis type of Lake Magadi tilapia may, therefore, be appropriately described as unrestricted lobular testis type. This type of testis is frequently encountered in a

number of cichlids, for example, *Cichlasoma nigrofasciatum*, *Labeotropheus trewavasae*, *Pterophyllum scalare* and *Sarotherodon aurea* (Grier *et al.*, 1980) and *Oreochromis sp* (Grier, 1993; Vilela *et al.*, 2003). Moreover, it is also demonstrated in other members of the orders Perciformes (Grier, *et al.*, 1980; Taylor *et al.*, 1998; Cinquetti and Dramis, 2003; Thacker and Grier, 2005), Synbranchiformes (Lo Nostro *et al.*, 2003), Gasterosteiformes, (Parenti and Grier, 2004), Pleuronectiformes, (Weltzien *et al.*, 2002; García-López *et al.*, 2005) and Gadiformes, (Almeida *et al.*, 2008).

The germinal compartment of *A. grahami* comprised numerous spermatocysts of varying sizes and developmental stages lining the seminiferous lobules. A spermatocyst is the basic functional unit of the spermatogenic epithelium in fish (Schulz *et al.*, 2010) and it is generally made up of germ cells at the same stage of development enclosed by Sertoli cell cytoplasmic processes. It has been suggested that the development of a spermatocyst starts with the complete enclosure of a primary spermatogonium by the Sertoli cell cytoplasmic processes (Loir *et al.*, 1995; Nóbrega *et al.*, 2009; Schulz *et al.*, 2010). As spermatogenesis progresses, germ cells within each cyst divide and grow synchronously resulting in a clone of cells with a common origin (Grier *et al.*, 1980; Loir *et al.*, 1995; Schulz *et al.*, 2005; Fishelson *et al.*, 2006). In this study, the germinal cells in each cyst were generally of the same size and exhibited similar morphological features, suggesting that they were at the same stage of development and hence, developed synchronously presumably from a common origin; the primary spermatogonium. Whereas these results are in agreement with similar studies undertaken on a number of teleosts, for example, Cichlids (Vilela *et al.*, 2003; Lacerda *et al.*, 2006), Cyprinid (Leal *et al.*, 2009) and Gadiforme (Almeida *et al.*,

2008), in some teleosts, however, spermatocysts have been shown to contain heterogeneous germ cells (Billard, 1986; Selman and Wallace, 1986; Quagio-Grassiotto and Carvalho, 1999; Fishelson *et al.*, 2006). In this latter case, branches of Sertoli cells enclose more than one primary spermatogonium which, upon division, results into germ cells at varying stages of development. Consequently, germ cells within a cyst develop without synchrony, hence, show no clones in the entire process of spermatogenesis and, ultimately, mature cysts liberate both spermatids and spermatozoa (Fishelson, 2003; Fishelson *et al.*, 2006).

5.2.1.1.1 Spermatogenic cells

In the testis of *A. grahami*, several cellular stages were demonstrated; primary and secondary spermatogonia, primary and secondary spermatocytes, spermatids and spermatozoa. All the cellular stages, except spermatozoa, were found in cysts suggesting that each cyst functions as an independent spermatogenic unit where germ cells undergo the entire spermatogenic process culminating in the liberation of spermatozoa into the lobule lumen. Taken together, these observations signify that *A. grahami* undergo complete cystic spermatogenesis as previously reported in tilapia species (Hyder, 1969; Schulz *et al.*, 2005, 2010), mudskipper (Chung, 2008), zebrafish (Leal *et al.*, 2009) and silver pomfret (Chung *et al.*, 2010). In contrast, however, some teleosts, for example, Senegalese sole (García-López *et al.*, 2005) and Nile electric catfish (Shahin, 2006) exhibit semi-cystic spermatogenesis.

Unlike mammals (de Rooij and Russell, 2000), the primary (type A) spermatogonia in Lake Magadi tilapia were distinguished as undifferentiated and differentiated. Undifferentiated primary

spermatogonia were the largest, in terms of the nuclear size, compared to their differentiated counterparts. This distinction has also been reported in the Nile tilapia (Schulz *et al.*, 2005, 2010; Alvarenga and França, 2009), Atlantic cod (Almeida *et al.*, 2008) and zebrafish (Leal *et al.*, 2009; Schulz *et al.*, 2010). The Magadi tilapia, however, differs from zebrafish and Nile tilapia in having only one type A undifferentiated spermatogonia while the latter group has two (Leal *et al.*, 2009; Schulz *et al.*, 2010). In this context, it is type A undifferentiated spermatogonia that show a great deal of similarity with those of *Tilapia leucosticta (*Hyder, 1969), *Oreochromis karongae* (Msiska, 2002) and the *Gadus morhua* (Almeida *et al.*, 2008). Type A undifferentiated spermatogonium in teleosts has been regarded as the spermatogenic stem cell (Schulz *et al.*, 2005, 2010; Nóbrega *et al.*, 2009). Stem cells are the most undifferentiated cells of specific lineage and have an inherent capacity for self renewal (Li and Xie, 2005; Hofmann, 2008). It seems reasonable, therefore, to state that in the testis of *A. grahami,* type A undifferentiated spermatogonia are the stem cells that give rise to all the other spermatogenic cells. Consequently, the Sertoli cell cytoplasmic processes and the adjacent extracellular basement membrane may be considered to play key roles in provision of specific and unique micro-environment required to maintain the 'stemness' of these cells.

The morphological appearance of the undifferentiated spermatogonia in Lake Magadi tilapia which includes, among other things, large, single and centrally located nuclei with or without a single, spheroidal, dark staining, centrally located nucleolus, are features shared by most other studied fish species (Quagio-Grassiotto and Carvalho, 1999; Koulish *et al.*, 2002; Fishelson *et al.*, 2006; Chung, 2008; Chung *et al.*, 2010). Particularly conspicuous in the type A undifferentiated

spermatogonia of this fish was the dark staining spots surrounding and outlining the nuclear envelope. Ultrastructurally, these spots appeared to be inter-mitochondrial dense substances closely associated with clusters of relatively large and rounded mitochondria in the cytoplasm located adjacent to the nuclear envelope. These inter-mitochondrial dense substances have previously been observed in the primary spermatogonia of a number of teleosts where they have been variously referred to as 'nuages', 'inter-mitochondrial cement', 'lamellae annulata', 'dense nuclear bodies' or 'dense germinal bodies' (Billard, 1984; Stoumboudi and Abraham, 1996; Muñoz *et al.*, 2002; Fishelson, 2003; Fishelson *et al.*, 2006; Jun *et al.*, 2006; Chung, 2008; Chung *et al.*, 2010). These substances are electron-dense cytoplasmic materials without membranes occurring in different shapes and sizes and, are present during the whole life cycle of the earliest fish male germ cells (Quagio-grassiotto and Carvalho, 1999). In some reports, these 'nuages' have been described as units of RNA detached from the nucleus and actively crossed the nuclear membrane into the cytoplasm (Eddy, 1975). They are regarded as the initial indicators of spermatogonia formation, but disappear in later stages of germ cell development (Koulish *et al.*, 2002; Fishelson *et al.*, 2006). In addition, these dense substances have further been observed at very early stages in primordial germ cells of *Barbus conchonius*, within 24 hrs after fertilization (Timmermans and Taverne, 1989; Gevers *et al.*, 1992 a, b). The major components of these 'nuages' are RNA and proteins, with the latter destined to the mitochondria. It has therefore been postulated that 'nuages' play a key role in propagation and clustering of mitochondria during the early developmental stages of germ cells (Chung, 2008; Chung *et al.*, 2010). Moreover, it has been suggested that the presence of rRNA in these inter-mitochondrial dense substances and their

closeness to mitochondria is an indication that they aid in eliciting the initial energy-dependent, enzymatic processes in the cell (Fishelson *et al.*, 2006).

In Lake Magadi tilapia, type A undifferentiated spermatogonia gave rise to type A differentiated upon undergoing the first mitotic division, similar to other teleosts (Schulz *et al.*, 2005, 2010; Nóbrega *et al.*, 2009). Spatial and structural picture of differentiated spermatogonia in this fish appeared more-or-less like the undifferentiated type A spermatogonia. Indeed, it has been suggested in the past that these cells may have some potential for 'stemness' in teleosts (Schulz *et al.*, 2010). However, the main distinguishing features of the differentiated spermatogonia from their predecessors was their general small size as well as their number in the cysts; often found in generations of either two, four or eight cells, therefore, closely resembling what is observed in Nile tilapia (Schulz *et al.*, 2005, 2010) and zebrafish (Leal *et al.*, 2009). On the other hand, in Atlantic cod, the undifferentiated and differentiated spermatogonia have been referred to as early and late spermatogonia respectively (Almeida *et al.*, 2008). Other reports suggest that primary spermatogonia give rise to secondary spermatogonia upon undergoing the first mitotic division (Hyder, 1969; García-López *et al.*, 2005). This may be true especially where the study did not take into account the two types of primary spermatogonia.

Ultimately, type A differentiated spermatogonia divide mitotically giving rise to secondary (type B) spermatogonia (Schulz *et al.*, 2005, 2010). In Lake Magadi tilapia, type B spermatogonia ensued subsequent to the fourth mitotic division from the single undifferentiated spermatogonium, implying that the earliest type B spermatogonial cyst comprised of

approximately sixteen cells. Further, successive mitotic divisions of type B spermatogonia resulted into several generations of the same, creating a pool of early and late type B spermatogonia separated by an intermediate form. The same phenomenon has been reported in zebrafish (Leal *et al.*, 2009). Similar to what has been observed in mammals (de Rooij and Russell, 2000), type B spermatogonia in fish divide more rapidly compared to type A (Schulz *et al.*, 2005). Considering the nuclear size, type B spermatogonia in this study were generally smaller compared to the preceding undifferentiated and differentiated primary spermatogonia, and there was a clear trend of decreasing cell size between early and late type B spermatogonia. It is generally accepted that germ cells usually decrease in size as they mature and, like Lake Magadi tilapia, this phenomenon has also been recorded in Nile tilapia (Schulz *et al.*, 2005) and zebrafish (Leal *et al.*, 2009). Concomitant with decrease in size, histologically, there was a sequential and successive increase in the amount and staining intensity of chromatin material from early to late type B spermatogonia. In addition, there was re-distribution and formation of foci of electron-dense chromatin material in the respective cell nuclei. In these spermatogonia, there were inter-mitochondrial dense substances observed close to the mitochondria. Presence of inter-mitochondrial dense substance in secondary spermatogonia has been reported in other fish species, for example, *Sorubim lima* (Quagio-Grassiotto and Carvalho, 1999), *Thalassoma bifasciatum* (Koulish *et al.*, 2002), cardinal fish (Fishelson *et al.*, 2006), *Boleophthalmus pectinirostris* (Chung, 2008) and *Pampus argenteus* (Chung *et al.*, 2010). The amount of inter-mitochondrial dense substance in secondary spermatogonia of *A. grahami* was generally less compared to that observed in primary spermatogonia, contrasting sharply with what was reported in cardinal fish (Fishelson *et al.*, 2006)

The most advanced type B spermatogonia in Lake Magadi tilapia differentiated to primary spermatocytes after undergoing the final mitotic division in a similar fashion as previously reported by Nóbrega *et al.* (2009). The abundant spermatocytes in the testis of *A. grahami* were those under prophase I of the first meiotic stage of spermatogenesis. This characteristic has also been reported in the cardinal fish (Fishelson *et al.*, 2006). The first meiotic prophase is normally prolonged as a result of the intense gene expression required for meiotic specific proteins and, the mRNAs required later during spermiogenesis (Eddy, 1998). Based on the nuclear diameters, the sizes of primary spermatocytes of *A. grahami* were generally comparable to those of secondary spermatogonia. Among the primary spermatocytes, pachytene spermatocytes were the largest; a finding which is in general agreement with what is found in the Nile tilapia (Schulz *et al.*, 2005, 2010) and zebrafish (Leal *et al.*, 2009). In guppies, however, zygotene spermatocytes have the largest volume (Schulz *et al.*, 2010) while diplotene spermatocytes are the largest in mammals (França and Russell, 1998). Ultrastructurally, inter-mitochondrial dense substances, initially seen in preceding germ cells, were not observed in primary spermatocytes of *A. grahami*. This is in conformity with earlier reports which suggested that 'nuage' aggregates begin to disperse during the first meiotic prophase, when primary spermatocytes begin forming (Koulish *et al.*, 2002). In *A. grahami*, the most notable feature in primary spermatocytes was the gradual condensation and re-organization of the chromosomal material within the nucleus appearing mainly as short, double stranded structures (synaptonemal complexes) frequently observed in pachytene spermatocytes. The occurrence of synaptonemal complexes in this fish was consistent with its localization in the black porgy, *Acanthopragus schegeli* (Gwo and Gwo, 1993), *Sorubim lima* (Quagio-Grassiotto and Carvalho, 1999), *Thalassoma bifasciatum* (Koulish *et al.*, 2002) and

Pampus argenteus (Chung *et al.*, 2010). In other teleosts, however, the synaptonemal complexes are more evident in other spermatocytes rather than pachytene. In cardinal fish, for example, synaptonemal complexes begin forming at leptotene and become fully noticeable at zygotene stage (Fishelson *et al.*, 2006), a phenomenon also observed in *Boleophthalmus pectinirostris* (Chung, 2008).

Synaptonemal complexes form when paternal and maternal chromosomes are aligned in the correct position by specific synaptonemal complex proteins, to facilitate the occurrence of meiotic cross-over (Costa and Cooke, 2007; Schulz *et al.*, 2010). In *A. grahami*, few synaptonemal complexes were seen in the diplotene phase presumably because of their disintegration followed by dispersion prior to diakinesis. It has been suggested previously that synaptonemal complexes disappear after formation to facilitate genic change (Quagio-Grassiotto and Carvalho, 1999).

Interestingly, the cytoplasm of primary spermatocytes presented mitochondria which appeared smaller with slightly dense matrix and developed cristae, perhaps, reflective of their general advancement compared to those in the primary and secondary spermatogonia, which appeared larger with ill-defined cristae. In addition, these cells contained numerous, round, membrane bound vesicles distributed within the cytoplasm, especially in the diplotene stage. Membrane bound vesicles have been reported in primary spermatocytes of *Sorubim lima* and, together with elongated mitochondria, appeared destined to the midpiece of spermatozoon (Quagio-Grassiotto and Carvalho,1999). Furthermore, cytoplasmic bridges appeared to be a common characteristic of primary spermatocytes of *A. grahami*. This is not unusual since it has been reported in the past

that germ cells in many teleosts exhibit cytoplasmic bridges due to incomplete cytokinesis during mitosis and meiosis (Loir *et al.*, 1995; Nóbrega *et al.*, 2009; Schulz, *et al.*, 2005, 2010), and may express specific features consistent with specific germ cell type (Quagio-Grassiotto and Carvalho, 1999). Cytoplasmic bridges have also been described in other groups of animals (Dym and Fawcett, 1971; Gondos, 1973; Billard, 1984; Weber and Russell, 1987).

Primary spermatocytes in teleosts undergo the first meiotic division to form secondary spermatocytes (Fishelson *et al.*, 2006; Chung, 2008) whose life-span is generally very short (Koulish *et al.*, 2002). Therefore, the occurrence of relatively few cysts observed in the testis of Lake Magadi tilapia containing secondary spermatocytes may probably be as a result of the short life-span associated with these cells. Considerable evidence has been accumulated suggesting that a number of teleosts also have secondary spermatocytes with short life-spans including, *Sarotherodon niloticus* (Silva and Godinho, 1983), *Fundulus heteroclitus* (Selman and Wallace, 1986) and *Thalassoma bifasciatum* (Koulish *et al.*, 2002), as well as mammals (Russell *et al.*, 1990). Compared to spermatogonia and primary spermatocytes, secondary spermatocytes in *A. grahami* were generally smaller in size corroborating information from other studied teleosts (Schulz *et al.*, 2005, 2010; Fishelson *et al.*, 2006; Leal *et al.*, 2009; Chung *et al.*, 2010). In teleosts, secondary spermatocytes undergo a quick second meiotic division to form spermatids (Quagio-Grassiotto and Carvalho, 1999; Koulish *et al.*, 2002; Fishelson *et al.*, 2006). The transition of spermatids to spermatozoa during spermiogenesis in teleosts entails complex morphological and, to some extent, chemical changes occurring in spermatids. These changes include nuclear elongation, condensation of chromatin material, cell elongation and flagellae

formation (Lou and Takahashi, 1989; Koulish *et al.*, 2002; Quagio-Grassioto *et al.*, 2003). Like other teleosts, spermiogenesis in *A. grahami* involved synchronized and stepwise morphological changes in spermatids within individual cysts resulting into specific identifiable developmental cell stages. Based on morphological characteristics, three main developmental stages of spermatids were recognized in the testis of Lake Magadi tilapia namely; early spermatids (type1), intermediate spermatids (type 2) and late spermatids (type 3). As spermiogenesis progressed, spermatid sizes decreased, perhaps, due to condensation of chromatin material within the nucleus and loss of part of the cytoplasm as residual body in the developing spermatids. As a consequence, spermatozoa resulting from the completion of spermiogenesis were the smallest of all the germ cells in the testis of *A. grahami*. Progressive decrease of germ cell sizes during spermiogenesis is a common feature known to occur in both teleosts (Schulz *et al.*, 2005, 2010; Leal *et al.*, 2009) and mammals (França and Russell, 1998).

Ultrastructurally, the entire process of spermiogenesis in *A. grahami* depicted a typical case of type I spermiogenesis where diplosome (centriolar complex) migration and growth of flagellum, both disposed tangential to the nucleus, as well as establishment of a cytoplasmic canal were a common occurrence. This was followed by complete nuclear rotation, resulting into a flagellum that penetrated medially in the cytoplasmic canal and perpendicular to the nucleus in the spermatozoon. This type of spermiogenesis has been extensively described in the past (Jamieson, 1991; Mattei, 1991), and frequently occur in teleosts (Spadella *et al.*, 2007; Chung, 2008; Chung *et al.*, 2010) resulting into type I spermatozoa. The characteristic morphology of the spermatozoon of Lake Magadi tilapia typified by a round head, short midpiece (about 1.5µm in

length) and a few mitochondria (approx. 10) are features reminiscent of a typical primitive (aquasperm) type of externally fertilizing sperm (Poirier and Nicholson, 1982; Mattei, 1988; Lo Nostro *et al.*, 2003). This finding compares favourably with previous reports on breeding and brooding behaviour where, among other things, it was indicated that Lake Magadi tilapia exhibits external fertilization (Coe, 1966; Seegers and Tichy, 1999). Other types of spermiogenesis including type II (shows no nuclear rotation with flagellum oriented parallel to the nucleus) and type III (shows no nuclear rotation with flagellum oriented perpendicular to the nucleus) have also been described in a number of fish species (Jamieson, 1991; Shahin, 2006, 2007; Quagio-Grassiotto and Oliveira, 2008). It is further believed that these types of spermatozoa formation results into diverse variety of spermatozoa which are specific and highly conserved among various taxonomic units in teleosts (Mattei, 1988; Schulz *et al.*, 2010). This phenomenon has, therefore, been exploited as a potent tool for phylogenetic analysis in fish (Jamieson, 1991; Quagio-Grassiotto and Oliveira, 2008; Schulz *et al.*, 2010). Members of fish in the order Perciformes, for example, have been shown to exhibit all types of spermatozoa, but type II appears to be the most frequent (Jamieson, 1991; Mattei, 1991). However, in the suborder Labroidei where *A. grahami* belongs, type I spermatozoa seems to take precedence (Gardiner, 1978; Lou and Takahashi, 1989; Mattei, 1991; Matos *et al.*, 1995; Lahnsteiner and Patzner, 1997; Quagio-Grassiotto *et al.*, 2003).

The shape of the head, hence, the nucleus of the teleost spermatozoa appears to vary with respect to taxonomic unit of the fish. The rounded head of the spermatozoa of *A. grahami*, with no evidence of an acrosome or acrosomal vesicle, conforms to what is known in related fish species

belonging to the same family, the Cichlidae (Lou and Takahashi, 1989; Matos *et al.*, 1995; Quagio-Grassiotto *et al.*, 2003). However, this phenomenon appears to differ significantly with those of other families within the suborder Labroidei. The Embiotocid *Cymatogaster aggregata*, for example, possess spermatozoa with elongated heads (Gardiner, 1978), while those in the family Pomacentridae (Mattei, 1991; Lahnsteiner and Patzner, 1997) have kidney shaped heads. The head of *A. grahami* spermatozoon contained chromatin material characterized by highly condensed globular masses. In teleosts, especially during the advanced stages of spermiogenesis, chromatin material undergo gradual condensation processes, presumably as histones are replaced by the more basic protamines (Alfert, 1956; Louie and Dixon, 1972). This condensation generally takes two forms depending on species or taxonomic unit. It may either be a homogeneous condensation or form clusters of condensed chromatin globules of different sizes within the nucleus (Jamieson, 1991; Mattei, 1991). In Perciformes, the homogeneous chromatin condensation appears to be the most common (Gardiner, 1978; Gwo and Gwo, 1993; Lahnsteiner and Patzner, 1997; Koulish *et al.*, 2002; Fishelson *et al.*, 2006; Chung, 2008; Chung *et al.*, 2010). Similar chromatin condensation is also exhibited in other orders such as Cypriniformes (Fribourgh *et al.*, 1970), Pleuronectiformes (Medina *et al.*, 2000) and Siluriformes (Quagio-Grassiotto *et al.*, 2008; Spadella *et al.*, 2006). Another form of chromatin condensation, though rare, occurs in the order Characiformes, where condensed chromatin appears in form of floccus within the nucleus (Gusmão-Pompiani *et al.*, 2009). In *A. grahami*, the gradual compaction and condensation of chromatin material into foci of small electron-dense globules characterized the terminal stages of spermiogenesis. Consequently, the spermatozoa arising from the spermiogenic process bore highly compacted electron-dense chromatin globules, reflecting the clustered form

of chromatin condensation previously described by Jamieson (1991) and Mattei (1991). This form of chromatin condensation is also found in related cichlids, for example, Nile tilapia (Lou and Takashi, 1989), *Crenicichla saxatilis* (Matos *et al.*, 1995) and *Cinchla intermedia* (Quagio-Grassiotto *et al.*, 2003). The condensed chromatin globules within the nucleus of spermatozoa in *A. grahami* were numerous and appeared small in size, similar to observations in *Cinchla intermedia*, (Quagio-Grassiotto *et al.*, 2003), but contrasting those of *Oreochromis niloticus* where they appear relatively larger in size (Lou and Takahashi, 1989). A non-cichlid teleost, the golden grey mullet, *Liza aurata*, of the suborder Mugiloidei, also shows masses of condensed chromatin material in its spermatozoon head (Bruslè, 1981).

Nuclei of spermatozoa of most teleosts are reportedly penetrated by a nuclear fossa whose prominence varies among different taxonomic units (Jamieson, 1991; Mattei, 1991). In this regard, nuclear fossae in different families within the suborder Labroidei seem to have varying depths and orientation with respect to the nucleus. The Embiotocid, *Cymatogaster aggregata,* for example, shows no apparent nuclear fossa (Gardiner, 1978), while the family Pomacentridae presents a simple depression on the nuclear outline instead of an actual nuclear fossa (Mattei, 1991; Lahnsteiner and Patzner, 1997). In this study, the spermatozoa of *A. grahami* presented an actual nuclear fossa, extending deep into almost half the depth of the nucleus. This fossa appeared to lie longitudinal to the nucleus following complete rotation of the head. Similar findings regarding the nuclear fossa have been made in other related cichlids such as *Oreochromis niloticus* (Lou and Takahashi, 1989) and *Cinchla intermedia* (Quagio-Grassiotto *et al.*, 2003). However, unlike *A. grahami*, the nuclear fossae in these two cichlids are eccentrically

oriented within the nucleus owing to partial nuclear rotation they undergo. As in *A. grahami*, fish species of the family Labridae, exhibiting type I spermatozoa, also possess deep seated nuclear fossae (Lahnsteiner and Partzner, 1997) while some families under the order Siluriformes, exhibit complete nuclear rotation as well (Spadella *et al.*, 2007).

In the course of spermiogenesis, the spermatids in *A. grahami* exhibited gradual displacement of the cytoplasm towards the distal aspect of the cell. The cytoplasm ultimately surrounded the proximal part of the developing flagellum but separated from the latter by the cytoplasmic canal/channel forming the midpiece of the mature spermatozoa. Presence of the cytoplasmic canal/channel within the midpiece of spermatozoa has similarly been demonstrated in a number of teleosts and appears to vary with taxonomic groupings. These canals are completely lacking in Pomacentridae (Mattei, 1991; Lahnsteiner and Patzner, 1997), but present in Embiotocidae (Gardiner, 1978). In the spermatozoa of *A. grahami*, a cichlid, the short midpiece contained a relatively small symmetrical cytoplasmic canal. The distal aspect of the midpiece was limited by the plasma membrane ending blindly in a lobe-like pattern. Other cichlids such as *Oreochromis niloticus* (Lou and Takahashi, 1989) and *Cinchla intermedia* (Quagio-Grassiotto *et al.*, 2003) have also been shown to contain the cytoplasmic canal in their midpieces. However, unlike *A. grahami*, their midpieces are elongated distally forming cytoplasmic sheaths around the respective flagellae, with those of *Cinchla intermedia* having dilated edges at their distal-most ends. Conversely, fish species under the family Labridae, exhibiting type I spermatozoa as in *A. grahami*, have midpieces with cytoplasmic canals but lack cytoplasmic sheaths (Mattei, 1991; Lahnsteiner and Patzner, 1997). The lack of cytoplasmic sheath on the spermatozoa of *A.*

grahami may be considered to contribute to increased flexibility of the flagella, thereby providing more propulsive force to move the spawned spermatozoa in the harsh aquatic environment towards the eggs for fertilization to occur. This feature, therefore, may be presumed as one of the possible adaptive strategies employed by this fish in maintaining its reproductive capacity against the harsh environment. Within the midpiece of *A. grahami*, about 10 unfused spherical mitochondria that were arranged in two layers around the cytoplasmic canal were conspicuously present. This has close resemblance to the midpiece of other Cichlids where the number of mitochondria ranges between 8 to 10 (Lou and Takahashi, 1989; Matos *et al.*, 1995; Quagio-Grassiotto *et al.*, 2003). However, in other families within the suborder Labroidei, the number differs significantly from that of the Cichlidae family. For example, Labridae has 3 to 5 spherical mitochondria while Pomacentridae possesses 6, all located close to the nucleus at the proximal aspect of the flagellum (Mattei, 1991; Lahnsteiner and Patzner, 1997). On the other hand, the family Embiotocidae, represented by *Cymatogaster aggregata*, possesses 6 mitochondria arranged in 3 layers within the midpiece (Gardiner, 1978).

In teleosts, the tail/flagellum is a core component of the spermatozoon (Jamieson, 1991; Mattei, 1991) and, like mammals (Russell *et al.*, 1990), the building blocks of the flagellum are the microtubules, arranged as 9 peripheral doublets around a central pair and joined by nexin and dynein arms forming an axoneme (Jamieson, 1991; Mattei, 1991; Chung, 2008; Chung *et al.*, 2010). Spermatozoa of most teleosts possess a single flagellum but a few members of the order Batrachoidiformes, Gobiesociformes, Siluriformes, Myctophiformes and Lepidosireniformes (lungfishes) possess biflagellated spermatozoa (Jamieson, 1991; Mattei, 1991). Recently, some

members of the order Perciformes, were reported to have both mono and biflagellated spermatozoa (Lahnsteiner, 2003b; Fishelson *et al.*, 2006). Like the general characteristics of spermatozoa in most studied cichlids (Fishelson, 2003), the spermatozoa of Lake Magadi tilapia possessed a single flagellum, exhibiting 9+2 axonemal microtubule configuration. These observations on the flagellar axoneme of *A. grahami* are similar to those of other cichlids (Lou and Takahashi, 1989; Matos *et al.*, 1995; Quagio-Grassiotto *et al.*, 2003). A rather rare scenario, however, was demonstrated in the spermatozoa of the cichlid *Satanoperca jurupari*, where the spermatozoa were biflagellate (Matos *et al.*, 2002). The flagellar axoneme in the Lake Magadi tilapia was covered by the flagellar membrane, which was a continuation of the plasma membrane to the flagellum at the level of the midpiece. Related cichlids such as *Oreochromis niloticus* (Lou and Takahashi, 1989) and *Cinchla intermedia* (Quagio-Grassotto *et al.*, 2003) possess similar flagellar membranes, but which, unlike *A. grahami*, exhibit conspicuous lateral extensions referred to as side fins/ridges, thought to correspond to the undulating membranes occurring in a number of animal species (Lou and Takahashi, 1989). Based on the structural similarity of the flagellum of *A. grahami* with other teleosts as previously highlighted, it may be presumed that this structure in this fish, as in teleosts (Cosson *et al.*, 2008) and mammals (Russell *et al.*, 1990), provides motility for spermatozoa upon spawning.

5.2.1.1.2 Sertoli cells

In the testes of many teleosts, Sertoli cells rest on the basement membrane and separate the lamina propria from the germ cells in the lobular system (Grier, 1981, 1992; Loir *et al.*, 1995; Fishelson *et al.*, 2006). Some Sertoli cells, however, occur in the interstices of spermatocysts

exhibiting triangular shaped nuclei (García-López *et al.*, 2005). In *A. grahami*, Sertoli cells showed similar disposition as that of other teleosts, and occasionally, intercystic Sertoli cells were observed ensheathing two groups of germ cells at different developmental stages simultaneously, suggesting the capacity of these cells to perform double duty. This Sertoli cell phenomenon has also been observed in *T. bifasciatum* where it performs the dual function of enveloping both maturing germ cells and spermatogonia (Koulish *et al.*, 2002). This dual role has been attributed to the involvement of a partially intercommunicating network system derived from the same Sertoli cell participating in the envelopment of the two different spermatocysts (Koulish *et al.*, 2002). Ultrastructurally some teleosts have been shown to contain both light and dark Sertoli cells (Bruslé, 1987; Grandi and Colombo, 1997; Koulish *et al.*, 2002), but not in Lake Magadi tilapia. Like most studied teleosts (Grier, 1981, 1992; Loir *et al.*, 1995; Fishelson *et al.*, 2006; Schulz *et al.*, 2010), Sertoli cells in *A. grahami* produced extensions enclosing all developing germ cells, thereby forming respective spermatocysts. They possessed both basal and adluminal surfaces; a feature similarly observed in many other teleosts (Grier, 1992; Koulish *et al.*, 2002; Cinquetti and Dramis, 2003).

Ultrastructural features of Sertoli cells apparently vary according to the developmental stage of germ cells in *B. pectinirostris* and *P. argenteus* (Chung, 2008; Chung *et al.*, 2010). In *A. grahami*, this study found that Sertoli cells encysting primary spermatogonia and early spermatids exhibited more-or-less similar cytoplasmic characteristics, including free ribosomes, rough endoplasmic reticulum, mitochondria, and membrane-bound vesicles of varying sizes containing granular materials. These features are indicative of intense protein synthesis similar to

what has been reported in *P. martensi* (Cinquetti and Dramis, 2003). The main roles of Sertoli cells in teleosts include, but not limited to, nutrition of germ cells, phagocytosis and steroidogenesis (Gresik *et al.*, 1973). Previous studies have also shown that, in a number of teleosts, the cytoplasm of Sertoli cells surrounding spermatids contain large numbers of glycogen particles suggesting a nutritive role to the spermatids (Jun *et al.*, 2006; Chung, 2008; Chung *et al.*, 2010). These glycogen particles were, however, not observed in the Sertoli cells surrounding spermatids in this study. In addition, the cytoplasm of Sertoli cells and Leydig cells in some teleosts have been reported to contain numerous membrane-bound dense vesicles associated with lysosome system (Grier *et al.*, 1989; Koulish *et al.*, 2002; Cinquetti and Dramis, 2003) and lipid droplets (Gresik *et al.*, 1973; Lo Nostro *et al.*, 2003; Jun *et al.*, 2006; Chung, 2008; Chung *et al.*, 2010) presumably for steroidogenesis. This study did not critically examine the nature of the membrane-bound dense vesicles in Sertoli cells of *A. grahami*, hence, cannot adequately address the nature of these vesicles. Lipid droplets were, however, lacking, while smooth endoplasmic reticulum were hardly seen in Sertoli cells of *A. grahami* suggesting that these cells are less likely to be involved in steroid production in this fish. In this regard, *A. grahami* is not alone since it has been established that Sertoli cells of *Fundulus heteroclitus* (Selman and Wallace, 1986), *Padogobius martensi* (Cinquetti, 1994) and *Thalassoma bifasciatum* (Koulish *et al.*, 2002) also show no evidence of steroidogenesis in their cytoplasm. However, tests for enzymes involved in steroid synthesis in Sertoli cells of *A. grahami*, in addition to the ultrastructural evidence previously mentioned, may be necessary to verify the steroidogenic capacity of these cells.

In *A. grahami*, it was observed that cysts of type B spermatogonia and primary spermatocytes possess relatively thin Sertoli cell cytoplasmic processes compared to the thick ones enclosing advanced spermatids. In addition, the amount of vacuoles and phagosomes in Sertoli cell cytoplasmic processes appeared to increase as spermatids transform into spermatozoa. It is speculated that the increase in these cytoplasmic structures is due to the increased need for lysosomic resources required for catabolism of phagocytised degenerated spermatids, spermatozoa, or residual bodies shed off during the period of spermiogenesis. Indeed, the importance of Sertoli cells in phagocytosis of residual bodies and undischarged germ cells after spermiation is well documented (Koulish *et al.*, 2002; Lo Nostro *et al.*, 2003; Chung *et al.*, 2010). Therefore, the nature of encystment of advanced spermatids in *A. grahami* by the Sertoli cell cytoplasmic processes is not a new phenomenon and may be serving the same purpose as previously suggested.

At the terminal ends of the seminiferous lobules, Sertoli cells in *A. grahami* exhibited unique morphological features involving projection of extensive cytoplasmic processes into the lumen. This apparently led to the creation of extensive network of cytoplasmic projections accessing damaged spermatozoa and residual bodies within the lumen. Increased lysosomal activity in these cells was apparent, as evidenced by the presence of numerous heterophagic vacuoles of varying sizes containing degenerating germ cells and other phagocytised bodies within the cytoplasm. These observations seem to support previously documented results where Sertoli cells showed long and slender cytoplasmic processes in the lobule lumen encircling and phagocytizing damaged germ cells (Van Vuren and Soley, 1990; Cinquetti and Dramis, 2003; Chung, 2008;

Chung *et al.*, 2010). Furthermore, these Sertoli cells in *A. grahami*, were sitting upon a robust basement membrane exhibiting extensive finger-like evaginations into the cytoplasm of the same Sertoli cells. Such finger-like projections of the basement membrane into Sertoli cells have also been demonstrated in the teleosts; *Alphanius dispar* (Abraham *et al.*, 1980) and *Padogobius martensi* (Cinquetti, 1994; Cinquetti and Dramis, 2003) and mammals; atrophied rat testis (Ross and Grant, 1968) and man (Chakraborty *et al.*, 1976). In particular, the evaginations in *A. grahami* closely resemble structurally those of *Alphanius dispar*, hence, may be playing the same role of transporting metabolites to the Sertoli cells and ultimately to the germ cells (Abraham *et al.*, 1980).

On the proximal aspect of the collecting ducts in *A. grahami*, the cuboidal Sertoli cells resting on a robust basement membrane sharply contrasted with their counterparts in the lobular system; the latter being generally elongated. These Sertoli cells in the collecting ducts were apparently joined by junctional complexes including tight junctions apically and desmosomes mid-laterally. Presence of similar inter-Sertoli cell junctions have been described in many teleosts (Koulish *et al.*, 2002; Marina *et al.*, 2002; Cinquetti and Dramis, 2003; Batlouni *et al.*, 2005, 2009; de Montgolfier *et al.*, 2007). These junctions, particularly the tight junctions, have been associated with the formation of the blood-testis barrier in teleosts (Abraham, 1991; Loir *et al.*, 1995). In this study, therefore, the presence of occluding junctions in the Sertoli cells lining the proximal ends of the collecting ducts is a likely indication of the existence and continuation of the blood-testis barrier from the lobular system to this region restricting the interaction of cells of the immune system with spermatozoa, as occurs in most teleosts (de Montgolfier *et al.*, 2007). These

cuboidal shaped Sertoli cells in the Lake Magadi tilapia exhibited particularly pronounced lysosomic activity evidenced by numerous large autophagic and digestive vacuoles containing whole spermatozoa and other ingested cellular debris. Therefore, it is likely that these cells primarily play the role of phagocytosis, unlike those of the lobular system which may be involved in nutrition of germ cells, in addition to phagocytosis. Indeed, it has been reported in the past that the amount of glycogen particles and lipid droplets in Sertoli cells decreases during the period of spermiation (Chung, 2008; Chung *et al.*, 2010). Consequently, these Sertoli cells of the collecting ducts in *A. grahami* may, therefore, be involved in ridding the collecting ducts of damaged or deformed spermatozoa and residual bodies following spermiation. Similar observations have been reported in a number of teleosts (Cinquetti and Dramis, 2003; Lo Nostro *et al.*, 2003; Chung, 2008; Chung *et al.*, 2010) and mammals (Morales *et al.*, 1985).

5.2.1.2 Boundary tissue

5.2.1.2.1 Myoid cells

Myoid cells constitute an important component of the peritubular boundary tissue in both mammalian (Maekawa *et al.*, 1996), avian (Aire and Ozegbe, 2007) and teleostean testis (Grier, 1981; Koulish *et al.*, 2002; Cinquetti and Dramis, 2003) and are largely considered to offer structural integrity to the seminiferous tubules or lobules in these animal species. This not only holds true for *A. grahami*, since they form a major component of the lobular boundary tissue but, in addition, also form important structural components of the tunica albuginea. Myoid cells have similarly been reported to occur in the testicular capsule of *Thalassoma bifasciatum* (Koulish *et al.*, 2002). The incorporation of myoid cells into the tunica albuginea of *A. grahami* as well as

other teleosts has brought a new dimension to these cells in teleosts, as opposed to mammals (Maekawa *et al.*, 1996) where they are only limited to the intertubular boundary tissue. The general orientation of the myoid cells along the longitudinal axis of the boundary wall was typical in *A. grahami* and in conformity with most studies in teleosts (Grier, 1981; Loir *et al.*, 1995; Lo Nostro *et al.*, 2004; Fishelson *et al.*, 2006). Ultrastructurally, the boundary tissue of Lake Magadi tilapia exhibited myoid cells disposed in one to three layers, similar to *Padogobius martensi* (Cinquetti, 1994). Information regarding the organization of myoid cell layers in most teleosts appears to be scanty. Nevertheless, in mammals, the organization of myoid cell layers varies among species (Bustos-Obregon, 1976; Christl, 1990). In this regard, myoid cells in the laboratory rodents such as mice, rats and hamsters (Maekawa *et al.*, 1996) and the rufous sengi (Kisipan, 2009) have single layers. Conversely, large vertebrates such as bulls, rams, rabbits and man have more than one layer (Maekawa *et al.*, 1996); a feature which appears to resemble what is observed in *A. grahami*. It has been postulated that mammalian myoid cells secrete a number of substances, among them extracellular components (Fibronectin, type I and IV collagens and proteoglycans) some of which may be involved in laying down the basal lamina (Maekawa *et al.*, 1996). In this study, the location of myoid cells (close to the basal lamina separated only by thin single layered collagen fibers) lends credence to the possibility that these cells may participate in laying down the collagen fiber layer and formation of the basal lamina. The presence of single layered collagen fibers between contiguous and succeeding layers of myoid cells, and between laterally placed myoid cells and the basal lamina was conspicuous in *A. grahami* and seems to support previous observations in a number of teleosts (Grier, 1981; Koulish *et al.*, 2002;

Cinquetti and Dramis, 2003; Lo Nostro *et al.*, 2004). The collagen fibers, in this study, appeared to bind adjacent myoid cell layers to the basal lamina through hemidesmosomes.

Myoid cells in both mammals (Virtanen *et al.*, 1986) and teleosts (Lo Nostro *et al.*, 2004) have been reported to possess characteristics consistent with those of smooth muscle cells. In this respect, therefore, myoid cells contain abundant actin and myosin filaments, besides desmin, vimentin and α-actinin (Virtanen *et al.*, 1986; Maekawa *et al.*, 1996). The orientation of these microfilaments appears to vary with animal species. In the rat and ground squirrel, for example, the filaments run both longitudinally and transversely with respect to the long axis of the cell thereby exhibiting a lattice-like pattern (Maekawa *et al.*, 1996). In this study, however, the microfilaments, abundant in proportion, were oriented in the longitudinal axis of the cell. This is in agreement with the general pattern of microfilaments in myoid cells observed in many teleosts (Grier, 1981; Koulish *et al.*, 2002; Cinquetti and Dramis, 2003; Lo Nostro *et al.*, 2004). The spatial organization and association of the myoid cell with its surrounding tissue as well as the ultrastructural endowments and disposition of its organelles, forms the basis of its primary function in teleostean testis. Therefore, it is postulated that these cells aid in the initiation of a continuous contractile network within the interlobular boundary tissue and testicular capsule, thereby eliciting a wave of force that facilitates expulsion of spermatozoa from the lobules into spermatic ducts during spawning (Grier *et al.*, 1989; Loir *et al.*, 1995; Koulish *et al.*, 2002; Cinquetti and Dramis, 2003). Presence of synaptic nerve endings on plasma membranes of myoid cells (Loir *et al.*, 1995) further reinforces the fact that these cells rely on nervous input to initiate contractions. Similarly, testicular myoid cells in Lake Magadi tilapia may be presumed to have

contractile activity comparable to those of other teleosts owing to their ultrastructural similarity. The continuous contractile activity of these cells along the lamina propria is aided by the presence of hemidesmosome junctions between contiguous cells (Koulish *et al.*, 2002). This activity presumably aids in expulsion of spermatozoa within the lobule lumen towards the excurrent duct system. Clusters of pinocytotic vesicles that were observed along the plasma membranes of myoid cells in *A. grahami* are indicative of active endocytosis. This feature has similarly been observed in a number of teleosts, including *Alphanius dispar* (Abraham *et al.*, 1980), *Padogobius martensi* (Cinquetti, 1994; Cinquetti and Dramis, 2003) and *T. bifasciatum* (Koulish *et al.*, 2002). The nuclei of myoid cells in Lake Magadi tilapia were more-or-less pleomorphic, sometimes ovoid or elongated with obvious indentation and scarce heterochromatin; features that show close resemblance to those of the nuclei in the teleost *Padogobius martensi* (Cinquetti, 1994).

The incidental finding of buds in some myoid cells of Lake Magadi tilapia appearing to sprout from the medial surfaces into the interlobular tissue presents a unique feature in teleostean testis. This feature has, so far, not been previously described either in mammalian or teleostean testes. Therefore, the significance of this phenomenon in myoid cells of Lake Magadi tilapia remains to be elucidated.

5.2.1.3 Interstitial tissue

5.2.1.3.1 Leydig cells

Leydig cells in the testis of *A. grahami* occurred singly or in small clusters within the interstitial compartments of the subcapsular and interlobular tissues, reflecting a pattern frequently observed in most teleosts (Loir *et al.*, 1989; Pudney, 1999; Koulish *et al.*, 2002; Cinquetti and Dramis, 2003; Chung, 2008; Chung *et al.*, 2010). Like some teleosts, (Grier, 1981; Lo Nostro *et al.*, 2004), these clusters of Leydig cells also occurred in the interstitium around the efferent ducts and near blood vessels. In some teleosts, however, the spatial distribution of these cells seems to vary with species and is not entirely limited to the interstitial tissue as in *A. grahami*. In two species of gobies, namely *Glossogobius olivaceus* and *Gobius jozo*, for example, they occur as a central glandular mass surrounded by lobules and, as accumulations lying along the entire length of the mesorchial side of the testis respectively (Pudney, 1999). In *Poecilia latipinna* (Grier, 1981) and *Lebistes reticulatus* (Lo Nostro *et al.*, 2004), however, Leydig cells occur at the periphery of the testis rather than within the intertubular spaces contrasting sharply with the results of this study. The ultrastructural features observed in the present study including an elaborate smooth endoplasmic reticulum, numerous mitochondria with well developed cristae and lipid droplets are features frequently encountered in many teleosts (Grier, 1981; Cinquetti and Dramis, 2003; Lo Nostro *et al.*, 2004; Nóbrega and Quagio-Grassiotto, 2007; Chung, 2008; Chung *et al.*, 2010). These features have been associated with the capacity of these cells to synthesize and secrete steroids in both mammalian (Fawcett, 1986) and teleostean testes (Loir *et al.*, 1995; Cinquetti and Dramis, 2003). Lipid droplets are presumed to be the precursors of cholesterol; an integral component in steroidogenesis (Haider, 2004). It is likely, therefore, that

these droplets observed in the Leydig cells of *A. grahami* are playing the same role in steroidogenesis. Surprisingly, lipid droplets are not a common feature of Leydig cells in reproductively mature testis of *Cichlasoma nigrofasciatum* (Nicholls and Graham, 1972), *Pterophyllum scalare* and *Haplochromis burtoni* (Grier, 1981), *Esox lucius* and *Esox niger* (Grier *et al.*, 1989), *Thalassoma duperrey* (Hourigan *et al.*, 1989), *Synbranchus marmoratus* (Lo Nostro *et al.*, 2004), *Kareius bicoloratus* (Jun *et al.*, 2006) and *Serrasalmus spiropleura* (Nóbrega and Quagio-Grassiotto, 2007). In Lake Magadi tilapia, Leydig cells may, therefore, be considered as the true androgen producing cells in the testis owing to their ultrastructural features that are consistent with this activity.

5.2.1.3.2 Connective tissue and blood vessels

In the testis of Lake Magadi tilapia, fibroblasts and collagen fibers are important components of the connective tissue. Fibroblasts were localized in the testicular capsule as well as interlobular tissue, and their presence in the testicular tissue of this fish, as in many other teleosts (Lo Nostro *et al.*, 2004), may be presumed to be related to their involvement in the secretion of collagen fibers and the basement membrane separating the lobular and interlobular compartments. Collagen fibers, on the other hand, were the most prominent connective tissue fibers forming the stroma in the testis of *A. grahami*. Similarly, in other teleosts, the testicular interstitial tissue is known to contain a predominance of collagen fibers (Cinquetti and Dramis, 2003; Lo Nostro *et al.*, 2003, 2004).

Small and large blood vessels observed in the interstitial tissue, at the cortical and medullary regions of the testis of Lake Magadi tilapia, is a feature commonly encountered in a number of

teleosts (Grier, 1981; Lo Nostro *et al.*, 2003, 2004). Therefore, it may be reasoned that this vascular system in the testicular tissue of Lake Magadi tilapia facilitates continuous supply of nutrients to various components of the testis thereby enabling their normal functioning.

The existence of nodular structures within the interstitial tissue between the efferent ducts at the medullary region in this study was more-or-less an incidental finding. A number of studies conducted in teleosts have reported the existence of nodular structures mainly within the reticulo-endothelial supporting matrix of hemopoietic tissues (Agius and Roberts, 2003). In addition, similar nodules have also been reported in other visceral organs including the gonads, and have largely been observed to contain macrophages (Agius and Roberts, 2003; Lo Nostro *et al.*, 2004) and granulocytes (Grier and Taylor, 1998). In teleost testes, they have been identified in *Centropomus undecimalis* (Grier and Taylor, 1998), *Synbranchus marmoratus* (Lo Nostro *et al.*, 2003, 2004) and *Solea senegalensis* (García-López *et al.*, 2005). The incorporation of pigments such as melanin, lipofuscin, hemosiderin and ceroids into these nodules has consequently led to their naming as melano-macrophage centers, MMC's (Wolke, 1992; Agius and Roberts, 2003). In Lake Magadi testis, these nodules contained several spheroidal cellular aggregates, enclosed in a fibrous capsule but, unlike other teleosts, the pigmentation was not obvious compromising their possible naming as melano-macrophage centers (MMC). It has been postulated that these nodules arise following stress or environmental changes (Peters and Schwarzen, 1986; Macchi *et al.*, 1992). In this connection, therefore, it may be argued that the frequent changes and rather extreme environmental conditions witnessed in Lake Magadi may, most likely, contribute to the formation of these nodules in the testis of *A. grahami*. Consequently, the existence of these

nodules may be a pointer to the extremely stressful conditions under which this fish survive on a daily basis.

5.2.2 The excurrent duct system

The excurrent duct system in Lake Magadi tilapia was generally organized into two parts; the intratesticular and extratesticular ducts with no identifiable accessory glands. The intratesticular ducts were confined in each lobe, comprising several collecting and efferent ducts, and a single main testicular duct. The extratesticular ducts, on the other hand, comprised the common spermatic duct and the genital papilla. This organization of the efferent duct system is in conformity with the general pattern in most studied teleosts (Billard, 1986; Lahnsteiner *et al.*, 1993a, b, 1994; Lahnsteiner, 2003a; Lopes *et al.*, 2004; García-López *et al.*, 2005; Lacerda *et al.*, 2006). The collecting ducts, in this study, were identified on the basis of diminishing germinal epithelium and transformation of the Sertoli cells from elongated, in the lobular system, to cuboidal. In Lake Magadi tilapia, each testicular lobe had, on its dorsal aspect, a longitudinally oriented main testicular duct that received spermatozoa from several efferent ducts distributed along the entire length of the testicular lobe. Each main testicular duct ran along the length of the respective testicular lobe to connect with the unpaired common spermatic duct caudally, with the latter running ventral to the urethra, which it opens together with at the urogenital papilla placed further caudally. This close association of the main testicular duct and the common spermatic duct in *A. grahami* is in agreement with what has been reported in the Nile tilapia (Lacerda *et al.*, 2006) but sharply contrasts with what is known to occur in some marine fishes. In such marine fishes, for example, *Trachinus draco* and *Uranucopus scaber,* the main testicular duct originates

from the cranial third of the caudally placed testis with respect to the genital papilla, to be joined cranially by the ureter forming a single common duct which opens exteriorly via the genital papilla (Lahnsteiner, 2003a). It also contrasts with observation in some marine fishes such as *Diplodus sargus, Thalassoma pavo* and *Sparisoma cretense* which have paired main testicular ducts emanating from the cranially placed testis in relation to the genital papilla, to be joined caudally by the ureter forming a single duct, which eventually opens exteriorly through the genital papilla (Lahnsteiner, 2003a).

The histological presentation of the main testicular duct of *A. grahami* with unfolded lumen and monolayered epithelium surrounded by connective tissue, compares favourably with those of fresh water teleosts such as Salmonidae (Lahnsteiner *et al.*, 1993a), Esocidae (Lahnsteiner *et al.*, 1993b), Cyprinidae, (Lahnsteiner *et al.*, 1994) and marine water teleosts (Lahnsteiner, 2003a). However, the apocrine secretion reportedly exhibited in marine as well as fresh water fish was not present in the main testicular duct of Lake Magadi tilapia. Four different species-specific histological features of the spermatic duct (common spermatic duct) epithelium in some marine teleosts have been demonstrated namely; monolayered folded, monolayered unfolded, multilayered folded and multilayered unfolded (Lahnsteiner, 2003a). In *A. grahami*, the epithelium of the common spermatic duct was folded with simple cuboidal epithelium, therefore, showing close resemblance to those of the marine teleosts *Uranucopus scaber, Trachinus draco* and *Sparisoma cretense* (Lahnsteiner, 2003a). In Lake Magadi tilapia, the increased folding of the common spermatic duct in the spawning fish compared to the non-spawning one appears to be a strategy to increase more luminal space and surface area, presumably for storage of

spermatozoa. This is in agreement with the findings in marine fish (Lahnsteiner, 2003a). In addition, the epithelium in the common spermatic duct of a spawning *A. grahami* appears to assume a secretory role (evidenced by the secretory material within the crypts and the duct lumen) whereupon the mode of secretion is invariably apocrine as previously reported in fresh water fishes (Lahnsteiner *et al.*, 1993a, b, 1994) and marine fishes (Lahnsteiner, 2003a). In this study, therefore, spawning seems to trigger increased epithelial folding and secretory activity, suggesting that the common spermatic duct plays a role similar to that of epididymis in mammals; that of seminal fluid secretion, sperm maturation and storage prior to ejaculation through the genital papilla (Russell *et al.*, 1990).

In this study, mature breeding males possessed very prominent genital papilla compared to the immature males and female fish. A similar morphological arrangement has been reported in *Oreochromis karongae*, where the male reproductive system exhibited a prominent accessory organ called tassel at its end (Msiska, 2002). In this study, therefore, this morphological feature became important in sexing the fish.

5.3 Conclusion and recommendations

The breeding and brooding behavior of Lake Magadi tilapia observed in this study confirms earlier studies that indeed this fish, like other tilapias, are maternal mouth brooders. The behavior relating to construction of breeding nests by males, followed by swimming displays by males, ostensibly to attract females, are typical characteristics of tilapias.

The gross anatomical disposition of the testes in this fish where they lie longitudinally within the coelomic cavity and connect with the genital papilla by a duct system, also points to the common similarity with most studied teleosts. Presence of extremely dark pigmented parietal peritoneum which completely enclosed all the abdominal viscera in both sexes may be presumed to play a crucial role in protecting the highly susceptible gonads from the UV radiation experienced in the lake. However, further investigations are required to test this hypothesis.

The testicular structure of Lake Magadi tilapia was of the unrestricted lobular type, with complete cystic spermatogenesis resembling most Perciformes. The histological and ultrastructural investigations of the testis revealed active spermatogenesis despite the harsh environmental conditions within the lake. Ultrastructural investigations of spermiogenesis and sperm structure have revealed some characteristics consistent with other members of the family; Cichlidae. These characteristics include condensation of spermatid chromatin material into multiple electron-dense globules and presence of isolated mitochondria (about ten) around the proximal aspect of the sperm flagellum. These characteristics are now becoming more noticeable as more studies on cichlid reproduction continue to be done and, therefore, may be phylogenetically significant for this taxonomic clade. However, additional data from other members of this family are recommended to better test this hypothesis.

Presence of nodular structures in the medullary region of the testis in *A. grahami* may be indicative of the extremely stressful conditions this fish is facing. Whether or not these nodules

play any role in maintaining testicular milieu for normal testicular functions in this fish, in the vicinity of the harsh environmental conditions deserves further investigation.

Incidentally, some of these morphological features observed in the reproductive organs of Lake Magadi tilapia appears fairly unique and are not frequently encountered in other cichlids which are known to inhabit fresh water environments. Consequently, these features may be considered to be some of the possible adaptive strategies employed by this fish in maintaining its reproductive potential in the face of the harsh environment they subsist in. These features include:

1. A fairly high gonado-somatic index (GSI) reflecting the high rate of spermatogenesis and consequently, high spermatozoa output per unit body weight ensuring availability and therefore, increased chance for fertilization to occur, which in this fish, takes place externally.
2. The black pigmented parietal peritoneum which is considered to act as a screen against the high UV radiation experienced in the lake region, thereby protecting the testis from the adverse effects of this radiation on germ cells and consequently spermatogenesis.
3. Presence of nodular structures within the interstitium of efferent ducts may also be considered to be playing some adaptive role, which in this species remains to be investigated.
4. The ultrastructure of the spermatozoon of Lake Magadi tilapia lacks a cytoplasmic sheath (the distal extension of the midpiece over the flagellum to about ¾ of its length observed in most cichlids). The absence of this structure in the spermatozoon of *A. grahami* may be

considered as an adaptive strategy to allow for more flexibility of the flagellum, hence, generating increased motility of the entire spermatozoon towards the egg while in water, thereby increasing the chances of fertilization to occur.

This study has therefore provided insights into some aspects of the male reproductive system and the process of spermatogenesis in Lake Magadi tilapia, which subsists in one of the harshest aquatic conditions on earth. It is hoped that where gaps of knowledge remain, further studies will be undertaken to elucidate them.

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