EVALUATION OF THE DEVELOPMENTAL MORPHOLOGY OF THE EYEBALL OF THE RED SOKOTO GOAT (*Capra hircus*)

A DISSERTATION SUBMITTED TO THE SCHOOL OF POSTGRADUATE STUDIES OF THE UNIVERSITY OF NIGERIA IN PARTIAL FULFILLMENT FOR THE AWARD OF THE DEGREE OF MASTER OF SCIENCE IN VETERINARY ANATOMY

BY

NLEBEDUM, U.C.

(PG/M.Sc./07/48006)

DEPARTMENT OF VETERINARY ANATOMY

FACULTY OF VETERINARY MEDICINE

UNIVERSITY OF NIGERIA,

NSUKKA.

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CERTIFICATION

This is to certify that this project was carried out by NLEBEDUM UCHENNA. C. in the Department of Veterinary Anatomy, University of Nigeria, Nsukka. The work is original and has not been submitted either wholly or in part for the award of any degree or diploma of this University or any other University.

It is submitted in partial fulfillment for the award of Master of Science degree of the University of Nigeria, Nsukka.

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Prof. D.N. Ezeasor	**Prof. I.C. Nwaogu**
(Supervisor)	**(Head of Department)**
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Prof. B.I. Onyeanusi	**Prof. C.O Nwosu**
(External Examiner)	**(Dean of Faculty)**

DEDICATION

To my loving wife, best friend and "sister"- Ugoma;

Daddy, Mummy, Obi and Nomso my other "sister"

ACKNOWLEDGEMENT

Glory and honour to almighty God for His love, protection and sustenance.

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ABSTRACT

This study traced the developmental histology of the eyeball of the Red Sokoto goat during the prenatal and postnatal stages.

Thirty Red Sokoto goat fetuses, 10 neonatal and 10 adult goat heads obtained from the abattoir were used for this study. The fetuses were aged using the standard formula while those of the adult goats were estimated by dentition. The eyes were dissected from the head and assigned to five groups (A-E). Eye samples were routinely processed for histological studies. Sections were examined with a light microscope and photographed with a Moticam digital camera attached to a microscope. Corneal thickness was measured in all groups and data obtained were analysed using analysis of variance. Variant means were separated using the least significant method (LSD) and significant difference was accepted at probability level ($p < 0.05$).

The corneal primordium was present in the eyes of fetuses in group A. The anterior and posterior epithelia were of the simple cuboidal type. The corneal epithelium became bilayered while the primordium of the limbus and the sclera appeared. In group B, the Descemetôs membrane was visible. All the layers of the sclera except the lamina fusca were fully differentiated. The differentiating lamina cribrosa appeared at this stage. In group C, the Bowmanôs layer was observed. The limbus, lamina cribrosa and sclera were well differentiated with the entire scleral layers present. In group D, the corneal epithelium had more cellular layers and pigments were observed in limbus. In group E, the corneal epithelium was of the non keratinized stratified squamous type. The Descemetôs membrane was thick. Apart from the cornea, all other regions of the fibrous coat had more melanocytes and pigments in group E. The central and peripheral corneal thicknesses of the adults (242.29 ± 23.95; 179.34 ± 8.37) were significantly higher ($p < 0.05$) than those of the fetuses of 2nd (68.50 ± 7.14; 71.20 ± 6.93), 3rd (90.47 ± 8.85; 68.35 ± 4.73) and 4th (81.71 ± 14.11; 88.87 ± 15.26) months of gestation and also that of the neonate (91.61 ± 15.81; 90.99 ± 9.08). In the eyes of group A, the iris was present as well as the iridopupilary membrane. The annular sinusoid was also present, choroidal differentiation was also initiated. Also pigment cells appeared in the stroma of the iris along with the dilator pupilary muscle myocytes. The ciliary body appeared as ciliary folds covered by a bilayered epithelium. The formation of the trabecular meshwork had been initiated while the iridocorneal angle was still closed. The angular aqueous plexus was seen at this stage. The choroid also started differentiating. In

group B, the constrictor pupillary muscle had appeared while the iridopupillary membrane had started regressing. The granula iridica also had started forming and the pectinate ligament appeared. The formation of the tapetum fibrosum was initiated at this stage. In group C, the ciliary folds formed the ciliary processes. The iridocorneal angle opened at this stage with the dorsal angle opening before the ventral one. In group D, the iridopupilary membrane had broken down and the anterior iridal surface was thrown into folds. The ciliary cleft was also formed at this stage. In group E, the crypts of Fuschs were seen on the anterior iridal surface. Few radially and circularly oriented muscle fibers were also seen in the ciliary body and the suprachoroidal space also appeared. The retinal primordium was present in the eyes of group A as well as the commencement of differentiation into retinal layers and cell types. The retinal cell types and layers appeared in the eyes of group B. The physiologic cup appeared in the eyes of group C at the optic nerve head. In group E, the rows of cells in the outer nuclear layer and inner nuclear layer of the retina were fewer than those in group D. Glial cells were seen at the optic nerve head at this stage. In group A, the lens capsule was present. The lens epithelium was of the pseudostratified and simple columnar types centrally and equatorially respectively. This changed to simple cuboidal and simple columnar centrally and equatorially in groups C and D. In group E, the lens epithelium was made up of simple cuboidal type centrally and simple columnar type at the equator. Hyaloid vessels were present in group A, became smaller and sparse in group C, few were seen on the posterior surface of the lens and equator in group D and they were absent in group E.

The development of the nervous and vascular layers progressed posterior-anteriorly while that of the fibrous layer progressed anterior-posteriorly. Also the pigmentation of the different parts of the eye increased with fetal growth and was highest in the adult. This study has shown that by late 3rd trimester, the RSG eyeball has acquired a full complement of the nervous, vascular and fibrous layers along with other structures necessary for vision. Therefore it essentially had the same morphology as in the adult and appeared capable of carrying out the function ascribed to the eyeball in the adult.

CHAPTER ONE

1.1 INTRODUCTION

Veterinary ophthalmology is a recognized discipline of veterinary medicine. Ocular examination is important in most clinical examinations and the understanding and diagnosis of ocular disorders rely on knowledge of normal ocular structure and physiology.

The eye of vertebrates is a highly specialized extension of the brain with a camera-like arrangement that provides sensitivity to light, good visual acuity and a wide field of vision. It is a complex and highly developed photosensitive organ that permits an accurate analysis of the form, light intensity and colour reflected from objects (Junqueira and Caneiro, 2005). The eye is a composite collection of tissues with similarities to the morphology and functions of tissues found in most other systems; therefore it provides a good opportunity for the examination of different tissue types without invasive techniques since there are ocular manifestations of many systemic diseases.

The eye has largely retained the same basic components and manner of development along the evolutionary path even with diverse animal habits and habitats. However, each component of the eye has undergone some changes primarily due to ecologic reasons (Samuelson, 1999).

The position of the eye in the head is related to the animalôs environment, habit and method of feeding. Generally, predatory species have eyes set well forward while those that are hunted carry their eyes more laterally. The eye position of predatory animals provides binocular vision that allows for concentration on near objects and also for perception of depth (Dyce *et al*., 2002) while the laterally positioned eyes of the hunted animals (preys) allows for a very wide field of vision and also for scanning the horizon for predators even when the animal has its head down during grazing (Samuelson, 1999).

The shape of the pupil is oval in the horizontal plane in herbivores. This feature further widens the animalôs field of vision and enhances early detection of predators.

Most domestic animals except the pig posses a reflective layer called the tapetum lucidum. This lies in the dorsal part of the fundus within the inner capillary layer. In the dog and cat, this layer is called tapetum cellulosum while in the ruminants this layer is called the tapetum fibrosum. The tapetum is believed to be a nocturnal adaptation since it improves the animalôs vision in dim light (Khaled, 2003; Miller, 2008b; Dyce *et al*., 2002). It improves their vision in poor light conditions such as at dusk or dawn (mesopic vision) which is their most active period and which also coincides with the most active period for most of their predators.

1.2 STATEMENT OF PROBLEM

From available literature, no study was found on the developmental morphology of the eyeball of Red Sokoto goat or any other breed of goat in Nigeria at prenatal, neonatal and adult stages of developments. However, the Red Sokoto goat breed is the most predominant goat breed in Nigeria accounting for 60% of Nigerian goat population estimated at 34 Million (Federal Department of Livestock and Pest Control Services,1992). The relevance of goats is greatest in developing countries where they meet socio-economic, cultural and recreational needs (Solaiman, 2010). Ocular diseases in food animals had been stated to play a significant role in economic losses (Whittaker *et al.*, 1999; Potter *et al.,* 2008), as the eyes are essential for grazing and reproduction. In addition, ocular examination provides a non-invasive diagnostic tool since there are ocular manifestations of many systemic diseases and, the understanding and diagnoses of ocular disorders rely on the knowledge of normal ocular anatomy and physiology. The eyes of goats have also been frequently employed in in-vitro research, and for training novice surgeons (Dada and Sindu, 2000; Sudan *et al.,* 2002; Pawar and Majumdar, 2007).

Therefore, the study of the developmental morphology of the eye is important as it will contribute to the existing anatomical knowledge, serve as reference for further ophthalmic, pathological and physiological studies and also aid in diagnosis and treatment of ophthalmic and systemic diseases.

1.3 MAIN RESEARCH OBJECTIVE

To trace the developmental histology of the caprine eyeball during the prenatal and postnatal stages.

1.4 SPECIFIC OBJECTIVES

1. To study the histology of the development of the eyeball of the Red Sokoto goat during the prenatal stage of development.

2. To study its further developmental morphology during the postnatal stage.

CHAPTER TWO

LITERATURE REVIEW

2.1 EMBRYOLOGY OF THE EYEBALL

The vertebrate eye is derived from three sources, the neural ectoderm, surface ectoderm and the neural crest derived mesoderm. The development and differentiation of the eye rely on sequential interaction between these basic tissues (Chow and Lang, 2001; Thompson *et al*., 2010).

The first morphological sign of eye development in vertebrates is a bilateral pair of shallow grooves on either side of the midline at the expanded cranial end of the still open neural folds. These grooves eventually form the optic vesicle with a cavity that is continuous with the forebrain. The optic vesicle grows laterally protruding into the surrounding peri-ocular mesenchyme. They extend towards and make contact with the overlying surface ectoderm displacing the mesenchyme between them and the surface ectoderm.

Mechanical influences of cytoskeleton and extracellular matrix as well as localized proliferation and cell growth contribute to the expansion of the optic vesicle (Cook, 1999). Inductive signals between the optic vesicle and the surface ectoderm at this point probably induce the formation of the lens placode. These signals could be mediated by crystalline proteins as high level expression of these protein families coincides with the lens placode formation (Cvekl and Piatigorsky, 1996; Wride, 1996). Coordinated invagination of the lens placode and the optic vesicle results in the formation of the lens vesicle and a double layered optic cup (Chow and Lang, 2001). The inner layer of the optic cup facing the lens forms the neural retina while the outer layer forms the retinal pigment epithelium (RPE). The rim of the optic cup later becomes the edge of the pupil (Ofri, 2008c).

The process of invagination forms a groove at the ventral extremity of the optic vesicle. This groove runs continuously from the ventral-most region of the neural retina and along the ventral aspect of the optic stalk to the junction of the optic stalk with the neural tube. This groove is known as the optic (choroid) fissure (Bron *et al.*, 1997).

Blood vessels develop in the nearby mesenchyme, and form the hyaloid vessels. These vessels enter and run through the optic fissure to provide an intraocular vascular system for the developing eye. This vascular system later atrophies and new intraocular supply develops. The optic fissure also provides an exit route for axons from the developing retina. The optic fissure closes during embryonic life (Bron *et al*., 1997; McGeady *et al.*, 2007). The accessory layers of the eyeball organize from the surrounding mesenchyme. The outer layer is more compact and specializes into the fibrous layer ï sclera and cornea. The inner, looser layer organizes into the vascular choroid. It also contributes to the formation of the ciliary body and iris (McGeady *et al.,* 2007).

2.1.1 DEVELOPMENT OF THE CORNEA

Mesenchymal cells in the region between the lens and the surface ectoderm become arranged into two layers and the resultant cavity between them forms the anterior chamber of the eye. The remaining ectoderm of the skin after separation of the lens vesicle differentiates into the corneal epithelium (Rüsse and Sinowatz*,* 1998; Slatter, 2001). The surface ectoderm also forms the primary stroma and mesothelium of the cornea. The inner wall of the anterior chamber forms the irido-pupillary membrane which, in its central part, is in contact with the anterior surface of the lens (McGeady *et al*., 2007). Loosely arranged mesenchyme fills the future anterior chamber and gives rise to corneal endothelium and stroma. The iridopupillary membrane in front of the lens later disappears completely, providing communication between the anterior and posterior eye chambers.

2.1.2 DEVELOPMENT OF THE SCLERA

The superficial mesenchymal layer surrounding the optic cup differentiates into an outer fibrous layer ï the sclera of the eye, a tough and thin opaque layer with high tensile strength. The sclera is continuous with the dura mater which ensheaths the optic nerve at the point where the nerve enters the optic foramen of the skull (McGeady *et al*., 2007).

2.1.3 DEVELOPMENT OF THE CHOROID

The choroid is a highly vascularised and pigmented layer surrounding the eye. It is located between the sclera and the retina. It develops from two embryonic tissues, the mesoderm and cranial neural crest cells (Zhao and Overbeek, 2001). The endothelial cells of choroidal blood vessels are of mesodermal origin while all other cells including stromal cells, melanocytes and pericytes are derived from neural crest cells (Noden, 1982; Etchevers *et al*., 2001). During early eye development, tubes and spaces form in the periocular mesenchyme next to the optic vesicle. These tubes are lined by endothelium of mesodermal origin. As the eye develops, they expand from the central axis to the caudal end of the optic vesicle and form a plexus (Zhao and Overbeek, 2001). Pigmentation of choroidal melanocytes is seen in the 90mm CRL bovine fetus, occurs in late gestation and is completed at birth in the human embryo and; begins soon after birth and is completed by two weeks of age in the mouse (Bistner *et al.,* 1973, Torczynski, 1982, Zhao and Overbeek, 2001).

2.1.4 DEVELOPMENT OF THE IRIS AND CILIARY BODY

The ciliary body and iris are derived from the distal tip of the optic cup at the point where the inner and outer cup layers meet (Beebe, 1986). Mesenchymal tissue on the anterior surface of the non-visual portion of the retina contributes to the formation of the connective tissue elements of the iris and the dilator and sphincter muscles of the pupil. The region of the non-visual retina between the iris and the visual retina forms the cilliary processes (McGeady *et*

al., 2007). Mesenchymal cells between the cilliary processes and the lens form the suspensory ligaments of the lens. A thin layer of mesoderm ï the irido-pupillary membrane is formed by the inner wall of the anterior chamber. This membrane, in its central part, is in contact with the anterior surface of the lens. This membrane breaks down during the late fetal period.

2.1.5 DEVELOPMENT OF THE LENS

The lens is formed from the lens vesicle which is derived from the lens placode. The lens vesicle is lined by a single layer of cuboidal cells. The cells of the anterior wall remain cuboidal while the cells of the posterior wall become elongated and grow towards the anterior wall of the vesicle. As the cells elongate, they obliterate the cavity of the vesicle. The elongated cells transform into transparent cells with large quantities of proteins called crystallins. The elongated cells also lose their nuclei and other organelles leaving intact outer membranes, an inner cytoskeleton of proteins and transparent cytoplasm composed of crystallins (McGeady *et al*., 2007; Ofri, 2008a).

2.1.6 DEVELOPMENT OF THE RETINA

The retina is derived from the apposed walls of the optic cup. The outer thinner wall which is separated from the inner wall by the intra-retinal space differentiates into the pigmented layer of the retina and also contributes to the formation of the ciliary body and iris.

Two distinct areas of differentiation develop in the inner layer of the retina. A narrow zone bordering the rim of the cup, the non-visual retina, remains thin walled and subsequently contributes to the formation of the ciliary body and iris. The remaining zone, referred to as the visual retina, differentiates mostly into photoreceptive and impulse transmitting neurons. This differentiation begins near the optic stalk. The line of demarcation between these two regions makes a wavy circle called ora serrata.

The neuroepithelial cells of the inner wall of the optic cup proliferate and differentiate giving rise to the specialized layers of the visual retina which includes the photoreceptors (rods and cones), the bipolar and ganglion cells and the supportive glial cells. There is a sequential development of the various cell types. Ganglion cells and horizontal cells are generated first followed by cone photoreceptors cells. Rod photoreceptor cells, bipolar cells and Müller glia develop last (Levine *et al*., 1997, McGeady *et al*., 2007).

2.1.7 DEVELOPMENT OF THE OPTIC NERVE

The axons of the ganglion cells converge to a point where the optic stalk leaves the optic cup. The axons infiltrate the optic stalk and form the optic nerve which surrounds the hyaloid vessels within the optic stalk. Branches of these hyaloid vessels vascularise the retina. The cells of the optic stalk differentiate into neurologlial supporting tissue. Glial cells migrate into the optic nerve and form the primitive optic disc. Myelinization of the optic nerve begins at the optic chiasm and reaches the optic disc after birth (Cook, 1999).

2.2 GROSS ANATOMY OF THE EYEBALL

The eye is composed of the globe (bulbus oculi), conjuctivae, lacrimal apparatus, nictating gland, the eye lids (palpebrae), optic nerve, ocular and extra-ocular muscles and the bones that form the orbit (Diesem, 1975; Dyce *et al*., 2002). The eye ball encloses several compartments containing refractive media.

The position of the eye in the head is related to the animalôs environment, habit and method of feeding. Generally, predatory species have eyes set well forward while those that are hunted carry their eyes more laterally. The eye position of predatory animals provides binocular vision that allows for concentration on near objects and also for perception of depth (Dyce *et al*., 2002).The laterally positioned eyes of the hunted animals allows for a very wide

field of vision and also for scanning the horizon for predators even when the animals has its head down during grazing (Samuelson, 1999).

The eye ball is a slightly asymmetrical sphere. The central points of the corneal and scleral curvatures are called the anterior and posterior poles, respectively, and the line joining them is called the geometric or optic axis. The visual axis is the line from the centre of the most sensitive area of the retina to the object viewed (Miller, 2008a).The mean mediolateral and anterior-posterior circumference of the left and right eyeballs are 8.19cm, 8.36cm, 7.20cm, 7.18cm for the West African Dwarf Goat and 8.42cm, 8.58cm, 7.41cm,7.46cm for the Red Sokoto goat (Olopade *et al*., 2005).

The eye ball consists of three layers;

a. The outer fibrous tunic; made up of the posterior opaque sclera and the anterior transparent cornea.
b. The middle vascular tunic; made up of the choroid, ciliary body and iris
c. The inner nervous tunic (retina); made up of the sensory (visual) retina and the non-sensory (non-visual) retina.

The anterior compartment of the eye is filled with the aqueous humor and is divided into the anterior and posterior chambers. The anterior chamber is between the cornea and iris while the posterior chamber is between the iris and the lens. The posterior compartment of the eye is filled with the vitreous body and is between the lens and the retina (Dellman and brown, 1976; Miller and Christensen, 1964).

2.2.1 OUTER FIBROUS TUNIC

The fibrous tunic of the eye is made up of the posterior, opaque sclera and the anterior, transparent cornea. The anterior-most part of the sclera is covered by the translucent bulbar

conjunctiva. The point at which the cornea, sclera and bulbar conjunctiva meet is called the limbus. The fibrous tunic together with the intraocular fluid pressure, serve to maintain the shape and tugor of the eyeball.

2.2.1.1 CORNEA

The cornea is the anterior part of the eye globe. In domestic species, the horizontal diameter of the cornea is greater than the vertical diameter. This is especially marked in the large herbivores (Maggs, 2008). The cornea is very sensitive as it is the most densely innervated, surface tissue in the body with free nerve endings found near the anterior epithelium. In addition to their sensory functions, the corneal nerves also help maintain the functional integrity of the ocular surface releasing trophic substances that promote corneal epithelial homeostasis and by activating brainstem circuits that stimulate reflex tear production and blinking (Corneal reflex) (Dyce *et al*., 2002; Marfurt *et al*., 2010).

The cornea is transparent, permitting light rays to enter the globe (Bloom and Fawcett, 1970). The cornea is the most powerful optical refracting surface in the eye because of its curvature and transparency (Maggs, 2008; Khaled, 2003). The normal cornea is avascular. It receives its nutrients by diffusion from the aqueous humour, the precorneal tear film and the atmosphere as well as adjacent capillary beds at the limbus and bulbar and palpebral conjuctiva (Khaled, 2003; Maggs, 2008). The endothelium and posterior stroma of the cornea receive most of their nutrients from the aqueous humour while the tear film nourishes the anterior cornea which also gets oxygen via diffusion from the atmospheric oxygen.

The anatomic features that keep the cornea transparent are;

1 Its avascular nature
2 Relatively low cell density
3 Lack of pigments

4 Nonkeratinized surface epithelium maintained by preocular moisture film.

5 Maintenance of a relatively dehydrated state

6 A smooth optical surface (provided by the precorneal tear film).

7 A highly regular arrangement of stromal collagen fibrils (Dyce *et al*., 2002; Samuelson, 1999; Maggs, 2008).

2.2.1.2 SCLERA

The sclera forms the posterior part of the fibrous tunic and is the largest portion of the fibrous coat of the eye. In mammals, the sclera can be considered as viscoelastic fibrous connective tissue consisting of isolated and flattened fibroblast embedded in an extracellular matrix made up of collagen and elastic fibrils interspersed with proteoglycan molecules (Domenico *et al*., 2002).The sclera is made up of three layers, from outside to inside, they are the episclera, the scleral stroma (Sclera proper) and the lamina fusca. Nerve fibres, blood and lymph vessels pierce through the sclera at various locations.The colour of the sclera varies among species and individuals and depends on the thickness of the scleral stroma and the fat content along its outer boundary. It appears blue at the thin regions and yellow with increased fat content with the lamina fusca appearing brown due to the pigments from the adherent suprachoroidal layer (Samuelson, 1999).

Scleral thickness varies among species and different regions of the globe. It is thinnest near the equator (Khaled, 2003). In animals with well developed intrascleral plexus, it is thickest at the region of the intrascleral venous plexus while in ungulates, the region of the optic nerve entrance or the posterior pole is the thickest (Samuelson, 1999).

The optic nerve leaves the eye through a sieve-like perforation of the sclera at the posterior pole called the lamina cribrosa. Changes in tension on the lamina cribrosa due to glaucoma reduces the size of the spaces through which nerve axons pass thereby disrupting the

axoplasmic flow in individual nerve fibres, thus contributing to optic nerve degeneration in this disease (Maggs, 2008).

Around the point of emergence of the optic nerve, the sclera is pierced by the short posterior ciliary arteries and nerves which enter the choroid. The long posterior ciliary arteries and nerves pierce the sclera near the optic nerve and pass anteriorly around the eye to the ciliary body. Anterior ciliary arteries and vortex veins enter and leave the sclera in the region overlying the ciliary body (Dyce *et al.*, 2002; Maggs, 2008). The intrascleral venous plexus lies anteriorly in the outer portion of the scleral stroma. In some amphibians, birds, fish and lizard the sclera is largely made up of cartilage and/or bones.

2.2.2 MIDDLE VASCULAR TUNIC

Between the retina and the outer fibrous tunic is a vascular and pigmented layer called the uvea or uveal coat. The vascular layer is made up of three portions, the choroid, the ciliary body and the iris. The choroid and the ciliary body are both attached to the internal surface of the sclera. The iris and the ciliary body form the anterior uvea while the choriod is known as the posterior uvea. The uvea provides nutrition to the ocular tissues and also provides mechanisms for visual accommodation and reduction or exclusion of light (Bloom and Fawcett, 1970).

2.2.2.1 IRIS

The iris is a thin continuation of the ciliary body. It extends centrally to cover the anterior surface of the lens with its free edge outlining the pupil. The iris divides the anterior ocular compartment into the anterior and posterior chambers which communicate through the pupil. The shape of the pupil is oval in the horizontal plane in herbivores. The pupil can be reduced or expanded by the contraction or relaxation of the constrictor and dilator muscles of the iris. In this manner, the iris controls the amount of light entering the eye (Dyce *et al*., 2002,

Samuelson, 1999). Several round black masses exist along the upper edge of the pupil in herbivores. These masses vary in size and are called granula iridica or copora nigra. Smaller masses also exist on the lower edge of the pupils. These pigmented masses are extension of the posterior pigmented epithelium of the iris. They enhance the effectiveness of pupillary contraction.

Anteriorly, the iris has two zones, the central pupillary zone and the peripheral ciliary zone. A thickening of the iris between these two zones is called the collarete. The pupillary zone of the iris is typically darker than the ciliary zone (Miller, 2008b).The iris controls the amount of light entering the posterior segment of the eye through the pupil. The iris is suspended between the cornea and the lens and is attached at its outer margin to the ciliary body.

The colour of the iris varies among various breeds or species and among individuals. This colouration is due to pigmentation of the iridal stroma. The variation of colour is mostly due to the amount and type of pigmentation present as well as the degree of vascularization. The coloration of the irides of domestic animals tends to be dark, but can vary from dark brown to gold brown, gold, blue and blue-green (Samuelson, 1999).

2.2.2.2 CILIARY BODY

The ciliary body lies immediately posterior to the iris. It is the largest component of the anterior uvea. It is triangular in sagittal section with its apex continuing with the choroid, its inner side facing the vitreous body, the outer side facing the sclera and the base giving rise to the iris and iridocorneal angle (Dyce *et al.*, 2002; Samuelson, 1999; Miller, 2008b). The ciliary body is covered with two layers of epithelium. The inner layer of epithelium is non-pigmented while the outer layer is pigmented. The outer pigmented layer is continuous with similar epithelium on the posterior surface of the iris and the pigment epithelium of the retina.

A large portion of the ciliary body is occupied by the smooth muscle fibres of the ciliary muscle, blood vessels, connective tissues and nerves. The muscle fibres originate near the apex of the triangle and insert into the region of the ciliary cleft and trabecular spaces of the iridocorneal angle. The ciliary smooth muscle alters the shape of the lens for near and far vision (Tortora and Anagnostakos, 1981; Dyce *et al*., 2002). The ciliary body begins caudally at a sharply outlined dentate border, the ora serrata, which marks the boundary between the optic part (pars optica retinae) and the blind or ciliary part (pars ciliaris retinae) of the retina.

The ciliary body is topographically divided into an anterior pars plicata (corona ciliaris) and a posterior pars plana (orbiculus ciliaris). The pars plicata consists of a ring of ciliary processes which protrude into the posterior compartment. The zonular fibres (suspensory ligaments) which support the lens originate from the pars plana and between the ciliary processes (Miller, 2008b). The ciliary muscle is poorly developed in non primate species. Therefore these species have poor accommodative ability. The ciliary processes produce the aqueous humor and also are involved in lenticular accommodation. The anterior component of the ciliary body is formed by anterior chamber angle or filtration angle. This is formed by the junction of the corneoscleral tunic, base of the iris and an anterior recession of the ciliary body known as the ciliioscleral sinus or cleft (Samuelson, 1999). Pectinate ligaments span the opening of the ciliioscleral sinus from the pigmented corneoscleral junction to the root of the iris. A matrix of loose tissue strands, the trabecular meshwork is found behind the pectinate ligaments and within the ciliioscleral sinus.

Aqueous collecting channels are found adjacent to the meshwork. These channels empty into the intrascleral venous plexus and then the vortex veins. Aqueous humor, which is produced by ciliary body epithelial cells and vasculature, flows from the posterior chamber through the pupil into the anterior chamber and to the filtration angle. A balance between production of

aqueous humor and its drainage creates the normal intraocular pressure (IOP). This pressure is responsible for maintaining the shape and turgidity of the eye and also for maintaining a close adherence of the retina to the choroid (Samuelson, 1999).

The fibrous and vascular tunics are separated by the perichoroidal space except at the corneoscleral junction anteriorly and at the exit of the optic nerve posteriorly where they are firmly attached to each other (Leeson and Leeson, 1970). Blood supply to the ciliary body is derived from the two long posterior ciliary arteries and the anterior ciliary arteries. The long posterior arteries pass into the suprachoroidal space equatorially, they undergo several divisions which anastomose with branches of the anterior ciliary arteries to form the major arterial circle (Samuelson, 1999). The ciliary processes are mainly supplied by this major arterial circle. There are anatomic variations of this vasculature among mammals. In the rabbit and primates, two types of arterioles supply the major and minor processes while in other species, a single type of arteriole originates from the major arterial circle and supplies the processes (Matsuo, 1973; Morrison *et al.*, 1987a; Funk and Rohen, 1990). Interspecies variations also occur in the angioarchitecture of the ciliary processes. Ciliary processes in carnivores are supplied by one arteriole that is directed posteriorly throughout its length with capillaries extending to each process margin from where they empty into venous sinuses at the base of each process. Ungulates possess ciliary processes with many arterioles occupying the core of the ciliary process along with veins that drain into the choroidal circulation (Morrison *et al.*, 1987b). The musculature of the ciliary body is made up of smooth muscle fibres running mainly along a meridional plane in most mammals while in birds and other non-mammalian species, the ciliary body musculature is made of skeletal muscles that also run meridionally.

2.2.2.3 CHOROID

The choroid is a thin densely pigmented vascular tissue forming the posterior uvea. It joins the ciliary body anteriorly and lies between the retina and sclera posteriorly. The choroidal anterior margin joins the ciliary body along serrated junction known as the ora serrata in primates. In most domestic animals, this junction is not serrated and is called the ora ciliaris retinae. The choroid is the main source of nutrition for the outer layers of the retina, which are adjacent to it. It also absorbs light rays so that they are not reflected back out of the eyeball.The choroid receives its main arterial supply from the short posterior ciliary arteries, long posterior ciliary arteries and the anterior ciliary arteries and is drained by the vortex veins (Khaled, 2003; Miller, 2008b).The blood of these capillaries is responsible for the redness of the fundus when examined with an ophthalmoscope (Dyce *et al*., 2002).

In most domestic animals except the pig, a reflective layer ï the tapetum lucidum lies in the dorsal part of the fundus within the inner capillary layer. This reflective layer is an avascular layer between the capillaries and the network of larger vessels. In herbivores, the tapetum is of the fibrous type ï tapetum fibrosum, while in carnivores, it is cellular in nature ï tapetum cellulosum (Samuelson, 1999).The reflective properties of the tapetum is responsible for the distinctive colour of the fundi of different animals and is also responsible for an animalôs eyes ñshineò in the dark when it looks towards a light. This colour varies with breed, age, species and thickness of the tapetum. The tapetum is believed to be a nocturnal adaptation since it reflects light through the retina a second time thereby increasing the stimulation of the light sensitive receptor cells and thus improves the animal vision in dim light (Khaled, 2003; Miller, 2008b; Dyce *et al*., 2002).

2.2.3 INNER NERVOUS LAYER

2.2.3.1 Retina

The inner nervous layer lies only in the posterior portion. It covers the choroid. It is composed of a sensory portion (pars optica retinae) and a non sensory portion. The non sensory portion begins at the ora serrata and covers the ciliary body and the iris as the pars ciliaris retinae and the pars iridis retinae, respectively. The retina is responsible for the transduction of light into neuronal signals that are eventually perceived as a visual image (Samuelson, 1999; Ofri, 2008b). As indicated by its high oxygen consumption the retina has the highest rate of metabolism in the body (Anderson, 1968; Samuelson, 1999; Ofri, 2008b). Retinas of animals are classified according to the pattern of their vasculature into;

1 Holoangiotic,
2 Merangiotic,
3 Paurangiotic and
4 Anangiotic (Ofri, 2008b; Samuelson, 1999).

In holoangiotic retinas, blood vessels traverse most of the inner retinal surface. It is seen in most domestic animals including primates and ruminants. In merangiotic retinas, blood vessels are localized to the temporal and nasal parts of the inner retina. It is seen in rabbits. In paurangiotic retinas, the retinal vessels are minute and extend only a short distance from the optic disc leaving most of the retina avascular. It is seen in the horse and elephant. The anangiotic type of retina is seen in most non-mammalians including birds and reptile and some mammals including marsupials and bats (Samuelson, 1999).

2.2.4 OPTIC NERVE

The optic nerve in most domesticated animals lies inferior and lateral to the posterior pole of the globe. It extends from the globe to the optic chiasm. It is formed by ganglion cell axons, glial cells and septae which arise from the pia mater. It consists of intracranial, orbital and ocular portions. The intraocular optic nerve consists of retinal, choroidal and scleral portions. Many ciliary nerves and short posterior ciliary arteries surround the optic nerve. Axons of the retinal ganglion cells leave the nerve fibre layer, converge and form the optic nerve head (optic papilla or optic disc). From the optic nerve head, they pass through the choroid and sclera into the orbit. There is a central depression that is normally present within the optic papilla called the physiologic cup (Samuelson, 1999).

2.2.5 LENS

The lens is a transparent, avascular, biconvex structure that focuses sharp images on the retina. It has an anterior surface that is less curved than the posterior surface. The centers of the surfaces are called the anterior and posterior poles. The posterior surface of the lens is in contact with a depression in the vitreous called the hyaloid or patellar fossa. Its anterior surface is in contact with the posterior surface of the iris and fills the pupil. It is suspended by zonular ligaments arising from the ciliary processes and attaches to the lens capsule at the lens equator. It is also held in place by the patella fossa and the support of the iris.

To view near objects, animals accommodate through contraction of the ciliary body muscles. This contraction leads to changes in the shape and/or position of the lens (Ofri, 2008a). The lens is quite soft in young animals with only a small central dense nucleus which becomes more dense and larger with age thereby reducing the ability of accommodation as the lens ages. Some herbivores, marsupials, monotremes and many rodents have no accommodative

mechanisms (Prince, 1956). Ungulates accommodate weakly while primates and birds have good accommodative abilities (Samuelson, 1999; Ofri, 2008a).

2.3 HISTOLOGY OF THE EYE

2.3.1 OUTER FIBROUS TUNIC

2.3.1.1 Cornea

Animal corneas consist of five layers; from outside and moving inward, the epithelium, Bowmanôs layer (anterior limiting membrane); stroma (substantia propria), the descemetôs membrane (posterior limiting membrane) and the endothelium (mesothelium) (Banks, 1993).

Corneal thickness varies between species, breeds and individuals. In most domestic species, it is 0.56-1mm.The central corneal thickness in the bovine, canine, feline and porcine are thicker than the peripheral thickness; the reverse is true in horses (Gellat, 1991; Banks, 1993). Many factors influence the thickness of the cornea; hydration, intraocular pressure, sex, age, closed or opened eye, dead or living cornea, method of measurements, and disease condition among other factors (Samuelson, 1999; Almubrad *et al.,* 2010)

Water content of the corneal stroma ranges from 65% to 75%; collagen (75% of the dry weight) provides the main structural component while non collagenous proteins (5%) and glycosaminoglycans (1%) represent a small fraction (Fatt and Weissman, 1992). The cornea is profusely innervated with sensory nerves especially pain receptors. The cornea is supplied by the long ciliary nerve from the ophthalmic division of the trigeminal nerve (Mawas, 1951; Raghavan and Kacharoo, 1964). It also receives sympathetic innervation from cells located in ipsilateral cervical ganglion (Marfurt *et al.*, 1989). The superficial layers are mainly supplied with pain receptors, which are unsheated in the epithelium, while the stroma contains more pressure receptors (Patt and Patt, 1969; Samuelson, 1999).

The corneal epithelium covers the anterior corneal surface. It is of the nonkeratinized, stratified squamous type that is continuous with the bulbar conjuctival epithelium. It is about 25-40µm thick in the domestic carnivore, about 90µm think in the bovine cornea (Prince *et al*., 1960, Samuelson, 1999). The anterior epithelium consists of a single layer of columnar basal cells that lie on a thin basement membrane; two or more layers of polyhedral cells (wing cells) and two or more layers of non keratinized squamous cells. In larger animals, the layers of polyhedral and squamous cells are more numerous. There are microvillous projections on the surface of the superficial squamous cells that anchor the precorneal tear film (Shively and Epling; 1970a, Maggs, 2008). The basal cells are composed of tall columnar cells with a domed apex and flattened base. Their nuclei are apically located. The basal cells can still undergo mitoses. Lymphocytes may also be seen in the basal layer of the epithelium. The cornea is normally devoid of pigments except at the limbus (corneo-scleral junction). The basement membrane is PAS positive. The anterior limiting membrane is not distinct in most domestic species but it is prominent in primates and birds (Samuelson, 1999).

The stroma is the bulk of the cornea. It constitutes 90% of the corneal thickness. It consists of collagenous fibres arranged in regular layers. Flattened keratocytes (modified fibroblasts) are found between the layers. The keratocytes are flattened elongated and branched cells with little cytoplasm. The stroma is avascular and contains many unmyelinated nerves (Raghavan and Kacharoo, 1964; Banks, 1993). The ground substance, composed of chondroitin sulphate, keratan sulphate, hyaluronic acid, plays an important role in maintaining the transparency of the cornea by maintaining proper corneal hydration. The cornea loses its transparency when it imbibes excessive fluid (Almubrad *et al*., 2010).

The posterior limiting membrane separates the stroma from the endothelium. The posterior limiting membrane is PAS positive and also stains positively for elastic fibres (Banks, 1993). It also appears as a thick amorphous, highly refractile layer in H&E preparations. The

posterior limiting membrane is the basement membrane of the corneal endothelium. It is produced throughout life by the endothelium thus its thickness increases with age (Banks, 1993).

The corneal endothelium is the innermost layer of the cornea. It lies posterior to the limiting membrane. It is of mesenchymal origin and is composed of a single layer of low cuboidal or squamous cells. Corneal endothelium is continuous with that of the venous plexus through which aqueous humor is drained (Banks, 1993). Corneal endothelium do not regenerate, however, proliferation occurs mainly in the immature animal; up to 12% of the cells at birth and eventually drops to zero by the time of eyelid opening in altricial animals (James, 2004; Mac Callum *et al.*, 1983; Laing *et al.*, 1976; Von *et al.*, 1961). The endothelium functions to pump ions from the stroma into the aqueous humour. This largely contributes to the transparency of the cornea as it keeps the stroma relatively dehydrated. With age, the endothelial cell density reduces. The remaining cells spread out to compensate for the loss of cells. If the cell density falls below 500-800 cells/mm^2, the cells will be unable to remove water from the corneal stroma. This is known as corneal decompensation (Samuelson, 1999; Maggs, 2008). This leads to corneal edema and opacity.

2.3.1.2 Sclera

The sclera makes up a larger portion of the fibrous coat of the eye. The sclera joins with the cornea anteriorly to form the limbus. Its anterior part is covered by conjunctiva while the posterior part is covered by the tenonôs capsule. The sclera is composed of dense, fibrous, connective tissue rich in elastic fibres. The sclera is divided into three layers; the episclera is the outermost layer. This layer is transparent and is composed of loose highly vascular fibroblastic tissue that is continuous with the outer tenonôs capsule. Fibres from the episclera blends with the middle layer, the sclera proper. The sclera proper is made up of fibroelastic

tissue with sparse vasculature. The fibres run in different directions and are also irregularly spaced hence, its non-transparency (Maggs, 2008). Rostrally, the sclera fibres are oriented circularly around the optic axis, providing firm insertion points for the extra ocular muscles (Banks, 1993).

The innermost layer, the lamina fusca (dark layer), is made up of fibroblastic tissue rich in elastic fibres. This layer is brown because of pigment cells found in this layer. There are specie variations in the scleral thickness in different areas of the globe, particularly at the insertion of the extraocular muscles (Samuelson, 1999)

In the fish, birds and lizards, the sclera is mostly made up of cartilage. When present, the cartilage forms a cup that extends up to the peripheral margin of the cornea. In lizards and birds, it extends up to a ring of bony plates (scleral ossicles) located anterior to the ciliary body (Samuelson, 1999).

Near the posterior pole of the globe is a sieve-like scleral modification called the lamina cribrosa through which the fibres of the optic nerve exit the globe. It is the thickest region of the sclera in ungulates, (Samuelson, 1999). Increased intraocular pressure due to glaucoma may disrupt the axoplasmic flow in the optic nerve fibres thus contributing to the optic nerve degeneration in this disease (Maggs, 2008; Samuelson, 1999; Banks, 1993).

2.3.2 VASCULAR LAYER

The middle vascular layer is usually pigmented and highly vascularized. It is made up of the choroid, ciliary body and the iris. The iris is attached to the anterior portion of the ciliary body. The ciliary body and the choroid are attached to the inner surface of the sclera. The ciliary body and the iris are both refered to as the anterior uvea while the choroid is refered to as the posterior uvea.

2.3.2.1 Iris

The iris is divided into three layers; the anterior border layer; the stroma and smooth muscles and the posterior pigmented epithelial layer.

The anterior border layer also called the endothelial layer consists of fibroblasts, melanocytes and connective tissue fibres (Samuelson, 1999; Banks, 1993). The anterior border layer faces the anterior chamber and its discontinuous layer of delicate cells is continuous with that of the corneal endothelium (Khaled, 2003; Banks, 1993). The iris stroma is composed of fibroblasts, chromatophores, smooth muscles, blood vessels and loose collagenous tissue.

Dense sheats are formed around blood vessels and nerves (Shively and Epling, 1969). The iridal colour varies among individuals and species and depends upon the prescence, absence, amount of iridal pigmentation or type of iridal pigmentation and the degree of vascularization.

The smooth muscle fibres are arranged as sphincter (Sphincter pupillae muscle) and dilator (dilator pupillae muscle). The sphincter pupillae muscle is circumferentially arranged along the pupillary border while the dilator pupillae muscle extends from the iris sphincter to the iris periphery. These two muscles are striated muscles in birds and other non-mammals. The posterior surface of the iris is covered by two layers of epithelium that is continuous with the ciliary body epithelium. The posterior layer in heavily pigmented and is continuous with the non pigmented epithelial layer of the ciliary body. The anterior layer, which forms the myoepithelial cells of the dilator muscles, is continuous with the pigmented epithelial layer of the ciliary body. In the anterior layer, only the region of the cells containing the nucleus is pigmented.

In horses and ruminants, iridial granules (granula iridica) forms along the pupillary margin. Iridial granules are well vascularized cyst-like extensions of the pupillary edge of the stroma

and the epithelial layers covering the posterior iris surface. They are more prominent along the dorsal border of the pupil in ruminants and horses (Samuelson, 2007).

2.3.2.2 Ciliary body

The ciliary body is the anterior continuation of the choroid, and it lies just posterior to the iris. It begins at the ora serrata (the point at which the sensory portion of the retina stops) and continues to the iris. It is also part of the formation of the trabecular meshwork of the iris angle (Hees and Sinowatz, 2000; Dellmann and Brown, 1976). The ciliary body is triangular in cross section; one side facing the vitreous body and the lens, one side joining the sclera and the base of the triangle giving rise to the iris and iridocorneal angle (Miller, 2008b).

The pars plicata is made up of a ring of ciliary processes, these processes are 70-100 in number depending on the species (Prince *et al.*, 1960). Each process is made up of a central core of stroma and blood vessels covered by a double layer of epithelium. The processes are mostly absent in lower vertebrates (Duke- Elder, 1958; Prince, 1956).The ciliary body mostly consists of loose collagenous tissue rich in elastic fibres, a capillary network and ciliary muscles (Banks, 1993).The ciliary muscles consist of smooth muscle fibres. There are three predominant muscle fibre directions; meridional, radial and equatorial with the meridional fibres (muscle of Brücke) being the outermost, and the radial fibres inside them (Khaled, 2003). The circular muscles (Müllerôs muscle), though less numerous than the meridional fibres, are predominant in the nasal portion of the ciliary body, where they occur alone in the pig (Maximow and Bloom, 1955; Bloom and Fawcett, 1970; Patt and Patt, 1969; Dellman and Brown, 1976; Samuelson, 1999). In birds and other nonmammalian species, the muscles of the ciliary body are made up of skeletal muscles. The epithelial covering of the ciliary body is made up of bilayered cuboidal cells with the inner layer being nonpigmented and the outer layer pigmented. In ungulates, these epithelial cells are more columnar than cuboidal

(Samuelson, 1999). The ciliary nonpigmented epithelium is continous posteriorly with the sensory retina at the ora ciliaris retinae and with the posterior pigment epithelium of the iris anteriorly. The ciliary pigmented epithelium is continous with the retinal pigment epithelium posteriorly and the anterior pigment epithelial layer of the iris anteriorly where it forms the dilator papillae muscle (Samuelson, 1999).The tip of the ciliary processes lack the covering pigmented epithelium (Leeson and Leeson, 1970). The ciliary body musculature is innervated by both sympathetic and parasympathetic nerves (Gum, 1991; Miller *et al.*, 1964).

2.3.2.3 Choroid

The choroid is the posterior part of the uvea. It lies between the retina and sclera posteriorly and joins the sclera, at the ora ciliaris retinae anteriorly. It is a thick and much vascularized layer with an abundance of pigment cells. It is divided into four layers; the suprachoroidea, the large vessel layer, the medium-sized vessel layer, which also contains the tapetum lucidum in most domestic animals, and the choriocapillaris.

The suprachoroidea consists of avascular, elastic, pigmented connective tissue. Fibrocytes,melanocytes and macrophages can be found in this layer (Samuelson,1999). It is separated from the sclera by a space, the suprachoroidal space which is traversed by branching collagen lamellae attaching the choroid to the lamina fusca of the sclera.

The large vessel layer lies just internal to the suprachoroidea. It contains numerous melanocytes, fibrocytes, occasional macrophages and large vessels, most of which are veins, all embedded in loose connective tissues. The large veins are associated with a different bed of vessels that has been refered to as lacunae, sinusoids and even lymphatic vessels (Destefano and Mugnaini, 1997; Meriney and Pilar, 1987; Walls, 1942). The choroid functions as a black box, the major source of oxygen and nutrient to the retina and also as a

ñcooling systemò that dissipates the heat produced from the absorption of light (Auker *et al.*, 1982; Parver *et al.*, 1982; Samuelson, 1999).

The medium-sized vessel layer lies internally to the large-vessel layer. It consists of loosely arranged bundles of collagen interspaced with elastic fibres. The collagen bundles run parallel to the choroidal surface (Samuelon, 1999). Fibrocytes and melanocytes are the cell types found in this layer with the melanocytes being predominant in heavily pigmented individuals.

2.3.2.4 Tapetum lucidum

In domestic species, except the pig and chicken, the medium-sized vessel layer of the choroid contains a layer of reflective tissue called tapetum lucidum. The tapetum lucidum is a biologic reflector system that is considered to improve mesopic vision under low light condition by reflecting unabsorbed photons back to the photoreceptors thereby giving the photoreceptors a second chance at absorbing the photons (Schwab *et al*., 2002; Ollivier *et al.,* 2004; Shinozaki *et al.*, 2010). It is situated mainly in the dorsal half of the fundus of the eye where it assumes an L-Shape (functional area) to triangular shape (histological area) depending on the intensity of light (Shinozaki *et al*., 2010). In most fish the tapetum lucidum lies in the retina (Arnott *et al*., 1970, Nichol and Arnot, 1973). The choroidal tapetum in mammals is made up of regularly arranged collagenous fibres and occasional fibroblasts (tapetum fibrosum) in the ungulates, and of polyhedral cells (iridocytes) containing reflective crystals (tapetum cellulosum) in carnivores (Samuelson, 1999). The thickness of the tapetum is not uniform as it is multilayered in the center and thins out to a single cell layer at the periphery until the tapetum lucidum is replaced by typical stromal elements (Banks, 1993; Samuelson 1999).

The collagen fibrils of the tapetum fibrosum and the electron-dense rods in the cytoplasm of the cells of the tapetum cellulosum all have their long axis parallel to the retinal surface and also have the same diameter of 150nm. The two types of tapetum also have the same spatial arrangement that is similar to the arrangement seen in the corneal stroma (Bartolami *et al.,* 1979; Samuelson, 1999).

Small blood vessels interconnecting the medium-sized vessel layer and the choriocapillaris penetrate the tapetum lucidum at right angles. These vessels when viewed end-on with an ophthalmoscope appear as small focal dark spots called stars of Winslow (Miller, 2008).The tapetum lucidum develops late in altricial animals and is usually fully developed at birth in precocial animals.

2.3.2.5 Choriocapillaris

The choriocapillaris is the innermost layer of the choroid. It consists of a thin layer of fenestrated capillaries separated from the retinal pigment epithelium (RPE) by a basement membrane complex (Bruchôs membrane) (Samuelson, 1999; Samuelson, 2007). The fenestrations in the capillaries are on the retinal side of the choriocapillaris while the endothelium, nuclei and pericytes are located on the choroidal side of the capillaries (Banks, 1993; Khaled, 2003). These characteristics favour transport from the capillaries to the retinal pigment epithelium (RPE) (Dellman and Brown, 1976). In primates, pigs and animals without tapetum lucidum, the Bruchôs membrane is a five layered membrane comprising the basal laminae of the RPE and choriocapillaris endothelia, two adjacent layers of collagen and a layer of elastic fibres between them. In animals with tapetum lucidum, it is reduced to a three layered membrane made up of the two basal laminae and a layer of collagen between them (Samuelson, 1999).

2.3.3 NERVOUS TUNIC

2.3.3.1 Retina

The Retina, the inner layer of the globe, is composed of a photosensitive and a non-photosensitive portion. The photosensitive portion is the posterior portion and is referred to as pars optica retinae while the non- photosensitive portion is the anterior portion. It begins at the ora serrata and lines the ciliary body as the pars ciliaris retinae and iris as the pars iridis retinae.

The retina is made up of the following layers from outside inward:

1 The retinal pigment epithelium
2 The layer of rods and cones (visual cell layer)
3 External limiting membrane
4 Outer nuclear layer,
5 Outer plexiform layer
6 Inner nuclear layer
7 Inner plexiform layer
8 Ganglion cell layer
9 Optic nerve fibre layer
10 Internal limiting membrane (Ofri, 2008b; Dyce *et al.,* 1987; Samuelson, 1999)

2.3.3.1.1 Retinal Pigment Epithelium (RPE)

The RPE is a layer of squamous to cuboidal cells continuous with the outer pigmented epithelial layer of the ciliary body. Its basal part adheres to the choriod coat (Khaled, 2003; Samuelson, 1999). The base of the cell is characterized by invaginations with abundant mitochondria near these invaginations. The apex of these cells has numerous extensions that

envelop the tips of the photoreceptors. The cytoplasm of these cells contains numerous melanin granules but is devoid of these granules over the dorsal choroid that contains the tapetum lucidum (Junquiera and Carneiro, 2005; Samuelson, 1999). The RPE functions to absorb photons that escaped the rods and cones and also to phagocytize the shedded tips of the rods and cones (Banks, 1993; Samuelson, 1999; Junqueira and Carneiro, 2005).

2.3.3.1.2 Layer of rods and cones

The main visual cells of the eye are the rods and cones. The rods are more sensitive to light and mediate scotopic vision while cones respond to bright light and are responsible for vision during day light (Photopic vision) and colour vision (Wässle and Boycott, 1991; Samuelson, 1999). The visual cell layer contains the outer segments of the rods and cones. The rods and cones are both made up of an outer segment (the photosensitive part) and an inner segment which contains the nucleus and cytoplasmic organelles (Junqueira, and Carneira, 2005; Hees and Sinowatz, 2000). The outer segment is separated from the inner segment by a constriction. There is a basal body just below this constriction from which a cilium arises and connects the two segments. The outer segment of the rod cell is cylindrical and slender while that of the cone cell is cone shaped being wider than the rod at its base and tapers down to a rounded tip (Khaled, 2003). The major morphological differences between the rod cells and cone cells are the variations in their synaptic terminals and the membranous discs in their outer segments. Cone synaptic terminals (cone pedicles) are wider than the synaptic terminals (spherules) of the rod cells (Ahnelt and Kolb, 2000; Samuelson, 1999). The outer segments of the rods contain membranous discs that are separate from the adjacent discs and the cell membrane while the discs in the cone cells are continous with the cell membrane (Rodieck, 1973; Junqueira and Carneiro, 2005).

The discs of the rod cells contain the photopigment rhodopsin (visual purple). The discs of the cone cells contain a variety of the cone photopigment, iodopsin, which is sensitive to red, green, or blue regions of the visible spectrum (Junqueira and Carneiro, 2005). Birds have been reported to possess up to four or more cone photopigments, and even a pigment sensitive to the ultraviolet portion of the light spectrum (Chen *et al*., 1984; Waldvogel, 1990).

2.3.3.1.3 External Limiting Membrane

The external limiting membrane separates the inner segments of the rod and cone cells from their nuclei (outer nuclear layer). It is not a true membrane. It is made up of cell junctions (zonular adherens) that attach the inner segments of the visual cells to Müller cells. The Müller cells support and surround the cells between the external limiting membrane and the inner limiting membrane of the retina (Samuelson, 1999; Hees and Sinowatz, 2000).

2.3.3.1.4 Outer Nuclear Layer

This layer contains the cell bodies of the photoreceptors. The cone cell nuclei are usually found next to the external limiting membrane. There is great variation in the number of rows of nuclei according to the location in the retina and the species. The rod cell nuclei are smaller, round to oval and more heterochromatic while the nuclei of cone cells are generally larger, oval and more euchromatic. Müller cell processes, rod cell and cone cell axons and outer rod and cone connecting fibres can also be found in this layer (Samuelson, 1999).

2.3.3.1.5 Outer Plexiform Layer

This layer is mainly formed by the synapses between the rod and cone cells, bipolar, and horizontal cells (Morten and Berndt, 2006). The cone pedicles usually extend further (towards the vitreous body) into the outer plexiform layer (Shively *et al*., 1970b; Rodieck, 1973).

2.3.3.1.6 Inner Nuclear Layer

The inner nuclear layer contains the cell bodies of horizontal cells, bipolar cells, amacrine cells and Müller cells (Ofri, 2008b). The nuclei of the horizontal cells are located along the outermost margin of the inner nuclear layer while the nuclei of the amacrine cells are located along the innermost margin (Samuelson 1999). The nuclei of the Müller cells and the nuclei of the bipolar cells occupy the intermediate zone of the inner nuclear layer (Shively *et al*., 1970b; Sigelman and Ozanics, 1988). The horizontal cell nuclei are large, oval or round with a prominent nucleolus with more perinuclear cytoplasm than bipolar cells. The horizontal cells also possess wide horizontally oriented cell processes (Samuelson, 1999; Khaled, 2003). There are two distinct morphological types of horizontal cells; an axonless horizontal cell (Type A) synapsing with all types of cones (Gallego, 1986; Boycott *et al*., 1987) and another type,(Type B) which has an axon and synapses with rod spherules and also cone pedicles (kolb and Famiglietti, 1974).

There are two basic types of bipolar cells found in the mammalian retina, the cone bipolar cells and the rod bipolar cells (Cajal, 1893). The cell bodies of a single type of rod bipolar cells and a variety of cone bipolar cell types are housed in the inner nuclear layer (Samuelson, 1999). Both the rod bipolar cell and the cone bipolar cell types posses large round nuclei and characteristic dendritic tree in the outer plexiform layer, where they receive signals from the rods and cones respectively, and an axon in the inner plexiform layer that sends signals to ganglion and amacrine cells (Dowling and Boycott, 1966; Boycott and Dowling, 1969; Khaled,2003). Ultrastructurally, the axonal cytoplasm of bipolar cells can be identified by their microtubules; also their nuclei are more osmiophilic and smaller than those of the horizontal cells (Samuelson, 1999). Cone bipolar cells consist of many types; up to nine in the monkey and rabbit and even eleven in the cat (Cohen and Sterling, 1990; Boycott

and Wässle, 1991; Mills and Massey, 1992; Strettoi and Masland, 1995; Kolb and Nelson 1996).

In the retina of the rat which is 99% rods, it has been shown that at least 50% of the bipolar cell population is made up of cone bipolar cells (Euler and Wässle,1995) indicating that the cone bipolar cells may also be involved in impulse transmission from rod cells.

The amacrine cell has been described as a neuron without an axon (Cajal, 1893). The cells are located vitreally in the inner nuclear layer and are characterized by occasional, indented euchromatic nuclei. They have a more abundant cytoplasm than the bipolar cells being filled with mitochondria, polysomes and rough endoplasmic reticulum, neurofilaments and tubules (Samuelson 1999). The amacrine cells provide integration in the inner plexiform layer making synapses with the bipolar, ganglion and other amacrine cells (Dowling and Boycott, 1966). Amacrine cells found in the ganglion cell layer of the retina are called displaced amacrine cells (Wong and Hughes, 1987; Vaney *et al*., 1991; Chang and Straznicky, 1992). These displaced amacrine cells have been established to be present in all retinas and comprise up to 20% of all amacrine cells depending on the retinal location and the species (Wong and Hughes, 1987; Wässle *et al*., 1987; Masland, 1988; Vaney, 1990).

There is another type of neuron found in the inner nuclear layer, the interplexiform cell that is not seen in all retinas. This cell has connections with the bipolar cells and horizontal cells and provides feedback mechanism from the inner retina to the outer synaptic layer (Samuelson, 1999).

The Müller cells (retinal glial cells) are elongated, branching cells that extend from the internal limiting membrane to the external limiting membrane of the retina. Their nuclei have denser chromatin than other nuclei in the inner nuclear layer. They tend to have more cytoplasm and are located in the outer portion of the inner nuclear layer. Müller cells are

functionally similar to neuroglia as they support, nourish and insulate the neural cells and fibres of the retina (Samuelson, 1999; Junqueira and Carneiro, 2005). The vitreal ends of the Müller cells possess end feet, which have phagocytic abilities (Nishizono *et al*., 1993).

2.3.3.1.7 Inner Plexiform Layer

This layer is made up of axons of bipolar, amacrine, horizontal cells and dendrites of ganglion cells (Ofri, 2008b). This later is thicker and well developed in cone dominated retinas (i.e. diurnal species) (Samuelson 1999; Samuelson, 2007). The dendrites of the ganglion cells are the only processes in the inner plexiform layer without synaptic vesicles. They are pale and contain microtubules, mitochondria and endoplasmic reticulum. The amacrine processes are also pale and contain synaptic vesicles and mitochondria. They are the most numerous processes in the inner plexiform layer due to the profuse aborization of their axons (Samuelson, 1999; Junqueira and Carneiro, 2005). The axons of the bipolar cells also contain mitochondria and numerous synaptic vesicles.

2.3.3.1.8 Ganglion Cell Layer

This layer contains the cell bodies of the retinal ganglion cells, displaced amacrine cells, neuroglial and retinal blood vessels. It is usually one cell thick except in the region of the area centralis and visual streak where two or three cell layers can be seen. In primates it can be up to six to nine cell layers (Samuelson, 1999).Ganglion cells in cresyl-violet stained retinae can be identified by the presence of abundant Nissl substance in a copious cytoplasm, a clear nucleolus in the nucleus and a polygonally shaped cell body (Gonzales-Soriano *et al*., 1997). The ganglion cells can be divided into two main types, the monosynaptic (small) type with dendrites synapsing with cones and the diffuse type with dendrites synapsing with many bipolar cells (Leeson and Leeson, 1970).

2.3.3.1.9 Nerve Fibre Layer

This layer is made up of non-myelinated axons of the ganglion cells that run parallel to the retina coursing towards the optic nerve head at the posterior pole of the globe. Neuroglial cells, large retinal vessels and inner tips of the Müller cells are also seen in this layer. The sizes of the axons vary with the large axons originating from the large ganglion cells (Samuelson, 1999).

2.3.3.1.10 Inner Limiting Membrane

This is composed of a basal lamina and the fused terminations of Müller cell fibres. The outer vitreous body has close association with this layer as vitreal fibres insert into the basement membrane, (Banks 1993; Samuelson, 1999; Samuelson, 2007).

2.3.4 Optic Nerve

The axons of the ganglion cells exit near the posterior pole of the globe and form the optic nerve head (optic disc, optic papilla). The axons then pass through the choroid and the sclera into the orbit. The optic nerve is formed by the axons of the ganglion cells, glial cells and septae arising from the Pia mater. In most species, the axons at the disc are myelinated. The axons leaving the globe are myelinated axons that form a bundle enclosed within a dural sheat that is continuous with that of the brain. Pial sheat immediately invests the nerve. This pial sheat is separated from the dural sheat by the arachnoid sheat. The septae arising from the pial sheat divides the nerve into bundles (Samuelson, 1999; Samuelson, 2007). The glial cells of the nerve are evenly distributed throughout the fibre bundles (Prince *et al*., 1960).

2.3.5 LENS

The lens is an important dioptric medium of the eye. It is devoid of blood vessels and pigments as these would decrease transparency. It is composed of the lens capsule, lens

epithelium and lens fibres. The lens capsule is the basement membrane for the lens epithelium inside the anterior lens capsule (Samuelson, 2007). The thickness of the capsule varies between regions, with the anterior pole and being thicker than the posterior pole. The lens capsule possesses elastic properties but lacks elastic fibres in its make up consisting mainly of type IV collagen (Gellat and Samuelson, 1982; Barnard *et al.,* 1992).

The lens epithelium, in adults, is confined to the anterior surface of the lens beneath the capsule. They are squamous to cuboidal at the pole and columnar near and at the equator. In early life, the epithelium is cuboidal both at and near the pole (Samuelson *et al*., 1987). They lose their nuclei as they mature and move centrally. The epithelium of the posterior surface of the lens is absent in adults as it forms the embryonic primacy lens fibres (Banks, 1993; Samuelson, 1999). In older animals, the cells at the anterior pole become more and more squamous. There is a proliferative zone within the epithelium just anterior to the lens. The cells in this zone transform into lens fibres. The lens fibres are hexagonal in shape with ball and socket junctions formed at the six angular regions. There are also many gap junctions in addition to the ball and socket junctions (Philipson *et al.,* 1975). The lens fibres form a U-Shaped cell enlongating towards the posterior and anterior poles of the lens. These fibres meet other fibres from the opposite side to form the posterior and anterior lens sutures (lens stars). These sutures are usually Y-shaped anteriorly and an inverted Y-shape posteriorly (Ofri, 2008a; Banks 1993). As each lens fibre completes its development, it loses its nucleus and other cellular organelles and appears senescent (Samuelson, 2007).

The central position of the nucleus of each of the developing lens fibres is retained therefore the difference in the lengths between the younger and older developing lens fibres creates the lens bow configuration of nuclei at the equator of the lens.. The portion of lens formed during embryonic development is known as the embryonic nucleus. It is in the center of the lens. The fetal nucleus, adult nucleus and cortex respectively occur as concentric zones of lens

fibres from the embryonic nucleus outwards (Banks, 1993; Samuelson, 1999). The lens fibres are formed throughout life.

CHAPTER THREE

MATERIALS AND METHODS

3.1 ACQUISITION OF SPECIMEN

Thirty Red Sokoto goat fetuses, ten RSG neonates and ten adult RSG heads were used for this study. The entire specimens were of abattoir origin. The adult animals were inspected for ocular diseases before slaughter. The eyes obtained were divided into five groups based on their ages. The first three groups, comprising of ten fetal eyes each, represented the 2nd (31-60 days), 3rd (61-90 days) and 4th (91-120 days) months of gestation. The 4th group was made up of ten neonate eyes and the last group (Group 5) comprised of ten adult goat eyes.

3.2 AGEING

The crown-rump lengths of the fetuses were measured using a piece of thread and a meter rule and the age determined using the formula GA=2.032CRL+36.35 (Nwaogu and Anya, 2009). Where GA is gestation age in days and CRL is crown-rump length in centimeters. The ages of the mature goats were estimated by dentition (Dyce *et al.,* 2002).

3.3 TISSUE PREPARATION

Following decapitation, the fetal heads were processed whole or the eyes were dissected out depending on the age of the fetal specimen. The eyes were dissected out of the adult heads according to the methods of Keller (1975) and the ocular adnexia trimmed off. Thereafter, the fetal eyes were fixed in 10% buffered formalin for 16-24 hours. The adult eyes were fixed in Davidsonôs fixative for 8-24 hours (Presnell *et al.,* 1997). After fixing, the eye tissues were dehydrated in graded series of ethanol, cleared in xylene and embedded in paraffin wax. The blocks were sectioned in transverse plane at 5-6εm using LEICA microtome. The sections

were mounted on glass slides and Stained with H&E, PAS, AB and AB/PAS procedures at pH 2.5 (Bancroft and Stevens, 1977) and examined under the light microscope.

3.4 PHOTOMICROGRAPHY

Selected tissue slides were photographed with Moticam® digital camera attached to a microscope.

3.5 HISTOMORPHOMETERY

The thicknesses of the central and peripheral cornea in sections from all the stages were measured using an ocular micrometer at a magnification of X 400.

3.6 STATISTICS

Data obtained from the measurement of the corneal thickness were analysed using analysis of variance. Variant means were separated using the least significant method (LSD) and significant difference was accepted at probability level (p ’ 0.05).

CHAPTER FOUR

RESULTS

4.1 FETUSES OF ABOUT 45 DAYS

4.1.1 FIBROUS TUNIC

4.1.1.1 Cornea

In fetuses of about 45 days of age, the cornea had an anterior epithelium that was made up of a layer of cuboidal cells centrally which transformed into bistratified squamous epithelium towards the limbus (Fig.1). The epithelium was continuous with the epithelium of the eye lid bud.

The stroma was made up of loosely arranged mesenchymal connective tissue and fibroblasts (Fig.1). It also presented a slightly AB positive extracellular matrix with PAS positive cells (Fig.2). It was continuous with the mesenchyme surrounding the optic cup (Fig.3). Mesenchymal cells contained pigment granules within their cytoplasm in the region of the future limbus. The endothelium was composed of low cuboidal to flat cells (Fig.4). The Bowmanôs membrane and Descemetôs membrane were not seen.

The primordium of the sclera was represented by the slightly PAS positive outer region of the mesenchymal condensations continuous with the corneal stroma at the equator of the optic cup. (Fig.5)

4.1.2 VASCULAR TUNIC

4.1.2.1 Iris

The iris was represented by a clump of mesenchymal cells at the rim of the optic cup. This clump of mesenchyme was confluent with the mesenchyme of the stroma of the cornea

(Fig.6). The iridopupillary membrane was seen bridging these two iridal primordia anterior to the lens. The vessels of the iridopupillary membrane were continuous with vessels of the hyaloid system thus forming the tunica vasculosa lentis (TVL) (Fig.7). Melanocytes were seen within the mesenchymal cells in the iridal primordia.

4.1.2.2 Choroid

The choroidal layers were not fully differentiated. However, there were condensations of mesenchyme at the equator of the optic cup that represented its primordium and also that of the sclera. This condensation was continuous with the tuft of the developing iris and the corneal stroma anteriorly (Fig.8). The choriocapillaris was represented at this age by the annular sinusoid (a narrow vessel surrounding the optic cup). This vessel separated the developing choroid and the pigment cells of the developing retina (Fig.9). It was lined by mesenchymal cells and was continuous with the vessels of the iridopupillary membrane. A short distance from the rim of the optic cup, this vessel presented enlargements within the choroidal condensation representing the ciliary body (Fig.9). Blood vessels were present in the mesenchyme in the choroidal region.

4.1.3 NERVOUS TUNIC

4.1.3.1 Retina

The retina was made up of two layers ï the outer retinal pigment epithelium and the inner neuroblastic layer (Fig.10). The outer retinal pigment epithelium was a layer of cuboidal cells with pigment granules in their cytoplasm. From the equator to the rim of the optic cup, these cells became taller assuming a columnar shape with their pigment granules mostly within their apical cytoplasm. The RPE in the posterior region, dorsal to the exit of the optic nerve (tapetal region), had fewer pigment granules in their cytoplasm (Fig.13).

The inner neuroblastic layer was divided into two zones; an outer nuclear zone that contained columnar cells in a pseudostratified arrangement and an inner anuclear zone (marginal zone) (Fig.11). Mitotic figures were seen in the outer region of the outer nuclear zone close to the RPE (Fig.10). However, the outer nuclear zone showed signs of separation into two nuclear layers by the primordium of the transient layer of Chievitz centrally (Fig.12).

The developing optic nerve at this stage lacked the connective tissue sheaths and septae. The lamina cribrosa was also absent at the optic nerve head at this stage (Fig.13).

4.1.4 LENS

A vaguely defined lens capsule was observed at this age (Fig.14). The anterior lens epithelium was a mixed type of epithelium. Centrally, it was composed of cuboidal cells and columnar cells. Some of the cuboidal cells were sitting on other cuboidal cells while the columnar cells traversed the thickness of the epithelium. Towards the equator, it presented a pseudostratified arrangement and was thicker and more packed with cells. The epithelium extended slightly beyond the equator where it had fewer cells that were columnar in shape and were pseudostratified. The cells at this region had euchromatic nuclei. At this point also, the cells were taller and formed the lens fibres. The lens bow was obvious at this age (Fig.15). The lens was surrounded by vessels. Anteriorly, vessels of the iridopupillary membrane were closely associated with the anterior epithelium. Posteriorly and laterally, vessels from the hyaloid system were also closely associated with it. These vessels anastomosed and formed the tunica vasculosa lentis (TVL) (Fig.6).

4.2 FETUSES OF ABOUT 59 DAYS

4.2.1 FIBROUS TUNIC

4.2.1.1 Cornea

The corneal epithelium was made up of a bilayered stratified squamous epithelium (Fig.16). The epithelial covering in the limbal region was thicker and was made up of stratified cuboidal epithelium (Fig.17). The Bowmanôs and Descemetôs layers were not defined. The stroma was more compact being more regularly arranged especially in the anterior and posterior regions where mesenchymal cells had differentiated into fibroblasts (Fig.18). The anterior region was slightly PAS positive. The endothelium was composed of low cuboidal cells (Fig.18). At the limbal region, few pigment cells were present (Fig.17).

4.2.1.2 Sclera

Although the scleral primordium was present, the layers of the sclera were absent at this stage (Fig.19). At the point of exit of the optic nerve, glial cells were present and formed channels that guided nerve fibres into the optic nerve. The scleral meshwork (cribriform plate) was absent. Fibroblasts were oriented meridionally in this region. Posteriorly, the developing sclera appeared as a region of condensation of mesenchyme around the developing choroid (Fig.20). It was composed of fibroblasts and few melanocytes oriented parallel to the surface. It contained few blood vessels and nerves most of which were in the outer region of the developing sclera (Fig.21). Developing extraocular muscles were seen within the developing sclera. The developing sclera contained collagen fibres (Fig.21).

4.2.2 VASCULAR TUNIC

4.2.2.1 Iris

The iris at this age was short with a well vascularised stroma composed of loose connective tissue containing fibroblasts and spherical pigment cells (Fig.22). Developing smooth muscle cells containing pigment granules were also seen in the stroma of the developing iris in the pupillary region. They were radially oriented and were in close association with the posterior pigmented epithelium of the iris (Fig.23). The anterior surface of the iris had acquired a double layered epithelium composed of an anterior layer of fibroblasts and a posterior layer of spindle shaped cells few of which had pigment granules within their cytoplasm (iridal endothelial layer)(Fig.23). This endothelial layer was continous with the endothelium of the cornea at the iridocorneal angle (Fig.23). The iridopupillary membrane was present with its blood vessels emanating from the pupillary margins of the irides being continous with the annular sinusoid surrounding the optic cup (Fig.23).

4.2.2.2 Ciliary body

The ciliary body at this age consisted of the ciliary folds and the developing iridocorneal angle. The ciliary body musculature was absent (Fig.24). Few melanocytes were also present. It was mostly made up of fibroblasts and differentiating mesenchymal cells (Fig.25). There were enlargements and branching of the annular sinusoid in the ciliary body with the branches going into the core of the ciliay folds. The epithelial covering was bilayered and was continuous with the retina posteriorly and the bilayered posterior epithelium of the iris anteriorly. The layers were made up of columnar cells with outer layer being pigmented and the inner layer being non-pigmented. The pigment granules masked the nuclei of the cells of the outer layer while the cells of the non-pigmented inner layer had oval euchromatic nuclei (Fig.26).

The stroma of the ciliary folds contained sinusoids (branches from the annular sinusoid), loose connective tissue, fibroblasts and mesenchymal cells. They were covered by a double layered epithelium; an outer layer of pigmented columnar cells and an inner layer of cuboidal to tall cuboidal non-pigmented cells (Fig.26).

The iridocorneal angle was closed anteriorly at the iridocorneal junction (Fig.23) however, the differentiation of its trabecular meshwork had commenced by the condensations of the cornea, sclera and the iris (Fig.27).

The iridal region, which was on the inner and anterior aspect of the iridocorneal angle, presented larger spaces within the developing connective tissue beams as well as extensive vascularization while the corneoscleral region, which was on the outer and posterior aspect, presented few small spaces. The vessels of the angular aqueous plexus were seen between these two regions (Fig.27).

4.2.2.3 Choroid

The choroid at this age was represented by a region of mesenchyme around the optic cup anteriorly while posteriorly, fibroblasts were seen within the mesenchymal cells (Fig.28). The annular sinusoid (choriocapillaris) was enlarged at this age (Fig.19). More pigment cells also appeared along with a proliferation of blood vessels within the stroma made up of fibroblasts and few differentiating mesenchymal cells (Fig.29). In the tapetal region, the fibroblasts were larger and their nuclei were more euchromatic than those in the non tapetal region. There was a transitional region between the tapetal and non-tapetal regions. Most of the cells of the retinal pigment epithelium overlying this region contained sparse pigment granules while some cells were completely devoid of the pigments giving the epithelium in this region a sieve like appearance (Fig.30). The choroid was represented anteriorly by the iridocorneal mesenchymal condensations.

4.2.3 NERVOUS TUNIC

4.2.3.1 Retina

The neuroblastic layer of the retina was still partially divided into two layers; the inner neuroblastic layer and the outer neuroblastic layer by the primordium of the transient layer of Chievitz (Fig.31). Anteriorly, the division into the two neuroblastic layers was not seen (Fig.28). The developing nerve fibre layer was also present (Fig.31).

Spherical cells were present in the inner margin of the inner neuroblastic layer. These cells also formed a layer on the vitreal side of the inner neuroblastic layer (Fig.32). Most of these migrating and differentiating spherical cells were euchromatic. The inner region of the outer neuroblastic layer also presented spherical cells within the inner zone of this layer (Fig.32). Most of the retinal cells were still columnar in shape (Fig.32). Large blood vessels were observed within the developing nerve fibre layer.

4.2.4 OPTIC NERVE

At this stage of development, the optic nerve had connective tissue sheaths and connective tissue septae separating the nerve bundles (Fig.33). Glial cells were present in the optic nerve (Figs.33, 35). Few fibrocytes were seen oriented perpendicularly to the nerve fibres at the cribiform plate region (Figs.34, 35). The hyaloid vessel was also observed surrounded by cells displaced towards the vitreous from the optic stalk (Fig.36). No pigmentation was present in the lamina cribrosa (Figs.34, 35).

4.2.5 LENS

A well defined PAS positive lens capsule was present around the lens (Figs.37, 38).The anterior lens epithelium was of the simple columnar type centrally but changed to a pseudostratified columnar type at and near the lens equator (Figs.39, 40). The lens bow was

present and was formed by the nuclei of the lens fibres (Fig.40). The tunica vasculosa lentis, formed by the hyaloid vessels, was present around the lens and anatomosed with the iridopupillary membrane anteriorly (Figs.38, 40, 41).

4.3 FETUSES OF ABOUT 75 DAYS

4.3.1 FIBROUS TUNIC

4.3.1.1 Cornea

The corneal epithelium was composed of a basal layer of low cuboidal cells and a superficial layer of flattened cells that were sparsely distributed. The Bowmanôs membrane was not visible (Fig.42).

The stroma contained fibrocytes, fibroblasts and few differentiating mesenchymal cells sandwiched inbetween layers of collagen (Fig.43). The fibrocytes and fibroblasts had slightly euchoromatic nuclei. These cells were oriented parallel to the corneal surface. The endothelium was made up of low cuboidal to flattened cells. A thin Descemetôs membrane was present (Fig.44).

At the limbus, the anterior epithelium was slightly thicker. Blood vessels and few melanocytes were present in the stroma. Fibroblast and fibrocytes were also present within a slightly irregularly arranged collagen fibres (Fig.45, Fig.46).

The sclera was better defined and contained fibroblasts with more collagen fibres arranged parallel to the surface. Spindle shaped melanocytes were also seen between the collagen fibres (Fig.47). The episclera was present at this stage and was made up of loose connective tissue containing fibroblasts and few differentiating mesenchymal cells (Fig.48). Blood vessels and nerves were also present with the nerves seen especially around the insertion of extraocular muscles (Fig.47). The lamina fusca was not seen.

4.3.1.2 Lamina cribrosa

The development of the lamina cribrosa was obvious at this stage as fibroblasts were observed oriented meridionally across the optic nerve bundles from the choroid (Fig.49, Fig.50). Collagen fibres were present and were oriented in the same manner (Fig.50). Few melanocytes were observed (Fig.50).

4.3.2 VASCULAR TUNIC

4.3.2.1 Iris

The iris was more elongated than it was at day 59. It had an anterior epithelium composed of melanocytes and fibroblasts. Its stroma was made up of loose connective tissue containing fibroblasts, melanocytes and blood vessels (Fig.51). The constrictor pupillary muscle was present and was circularly oriented. From the pupillary margin, it extended to a point about half way the length of the developing iris, where its myocytes merged with the anterior layer of the double layered pigmented posterior epithelium. Its myocytes contained pigment granules (Fig.52, Fig.53). Many blood vessels were seen just posterior to the anterior epithelium of the iris (Fig.54, Fig.55). Nerve bundles were also present towards the base of the iris. The iridopupillary membrane was still present but the size of its vessels was smaller and the membrane was more fibrous (Fig.56). The tip of the iris was covered by the posterior pigmented epithelium which had cysts in it (granular iridica). The developing pectinate ligament was present at this stage (Fig.57, Fig.58).

4.3.2.2 Ciliary body

The ciliary body at this age was composed of the developing ciliary processes, iridocorneal angle and the ciliary body musculature (Fig.59). The anterior ciliary folds, just at the base of the iris, branched into numerous processes while the folds caudal to them did not (Fig.59).

The double layered epithelium covering the ciliary body and processes was composed of an external pigmented columnar cell and internal non pigmented columnar to cuboidal cells. The internal non pigmented layer of cells was cuboidal over the processes (Fig.60). The core of the processes contained loose connective tissue and blood vessels that arose from the vessels within the ciliary body (Fig.60).

The iridocorneal angles were fully differentiated and open (Fig.57). However, the dorsal iridocorneal angle seemed to open later than the ventral iridocorneal angle. Smooth muscle cells of the ciliary body musculature were present at this age in the posterior region of the ciliary body (Fig.61). The angular aqueous plexus (AAP) was well differentiated (Fig.57).

4.3.2.3 Choroid

The choroid at this stage had differentiated into a layer composed of loose connective tissue containing fibroblasts and spindle shaped melanocytes oriented parallel to the surface (Fig.62). Large blood vessels were present in the outer region of this layer. These vessels were connected to the choriocapillaris by smaller vessels traversing the layer. Fibroblasts and fibrocytes were seen stacked up over the tapetal region, just outside the choriocapillaris, especially at the area dorsal to the optic nerve representing the initiation of the formation of the tapetum fibrosum (Fig.63, Fig.64).

4.3.3 NERVOUS TUNIC

4.3.3.1 Retina

At this stage, the nerve fibre layer was present with blood vessels within it (Fig.65). The ganglion cell layer was also present throughout the retina. This layer contained ganglion cells at different stages of differentiation. The ganglion cell layer was separated from the outer neuroblastic layer by a plexiform layer containing cell fibres (Fig.65). Peripherally, the inner

region of the outer neuroblastic layer of the retina presented spherical cells that were located inwards away from the neuroblastic layer (developing inner nuclear layer) while in the central retina, the spherical cells had completely separated from the outer neuroblastic layer, with a plexiform layer (outer plexiform layer) between them, representing the inner nuclear layer of the retina. Columnar cells were present in this inner nuclear layer (Fig.66, Fig.67). A single layer of spherical cells were also seen on the outer margin of the outer neuroblastic layer (developing outer nuclear layer). Columnar cells were present in the outer neuroblastic layer along with the other cell types found in the layers (Fig.66, Fig.67).

4.3.4 THE LENS

The lens was surrounded by a PAS positive capsule (Fig.68). Centrally, the anterior epithelium was composed of a single layer of cuboidal cells which became columnar near and at the equator where the epithelium became pseudostratified columnar, and formed the lens bow (Fig.69). The nuclei of the epithelial cells and the lens fibres were euchromatic (Fig.69). It did not have epithelial cells on the posterior surface. The lens was surrounded by the vessels of the hyaloid system (tunica vasculosa lentis) (Fig.70, Fig.71).

4.4 FETUSES OF ABOUT 105 DAYS

4.4.1 FIBROUS TUNIC

4.4.1.1 Cornea

The anterior epithelium was made up of stratified squamous epithelia. By day 105, this epithelium had 3 cell layers. The basal layer was made up of cuboidal cells with centrally located euchromatic nuclei. The other two layers were comprised of spindle shaped cells with less euchromatic nuclei (Fig.72). The basement membrane of the anterior epithelium was vaguely defined with H & E but was demonstrated by PAS (Fig.72, Fig.73). At the limbus,

the PAS positive sclera merged with the alcian blue positive corneal stroma (Fig.74). The corneal stroma lost its regular arrangement as it merged with the scleral stroma. The epithelial layer increased up to 4 cell layers and thus was thicker. Few of the epithelial cells at the limbus contained pigments and blood vessels were seen in the region (Fig.75).

The corneal stroma was regularly arranged with only fibroblasts sandwiched in between collagen layers except at the limbal region where melanocytes and blood vessels were present and the collagen bundles were also irregularly arranged (Fig.75, Fig.76). The endothelium was composed of cuboidal cells sitting on a PAS positive Descemetôs membrane (Fig.77).

4.4.1.2 Sclera

The layers of the sclera were fully differentiated. The lamina fusca was next to the choroid and contained numerous melanocytes oriented parallel to the surface. The sclera proper was composed of packed regularly arranged collagen bundles that contained fibroblasts, fibrocytes and scattered melanocytes. The episclera was made up of loose connective tissue containing blood vessels and nerves (Fig.78, Fig.79). Extraocular muscle insertions were seen in the episclera usually associated with nerves (Fig.79).

The lamina cribrosa was well differentiated at this age with collagenous fibres extending across the optic nerve bundles from the sclera. Fibrocytes and few melanocytes were present in the lamina cribrosa (Fig.80, Fig.81).

4.4.2 VASCULAR TUNIC

4.4.2.1 Iris

The iris at this stage was longer. Its stroma contained numerous blood vessels especially in the anterior stroma. Melanocytes, fibroblasts and nerves were also contained within the stroma (Fig.82, Fig.83). The pupillary muscles were also fully differentiated with the

circularly oriented constrictor pupillary muscle appearing more developed. It ran from the iridal tip to about two-third the way toward the base of the iris (Fig.84). The dilator pupillary muscle was made up of smooth muscle cells that were in close association with the double layered pigmented posterior epithelium (Fig.84). These cells had extensions that linked the dilator muscle to the spinchter muscles. The double layered posterior pigmented epithelium had covered the tip of the iris with cysts within it forming the granular iridica (Fig.84). This posterior epithelium was continuous with the double layered epithelium of the ciliary body which had an inner non pigmented layer and an outer pigmented layer (Fig.85). The iridopupillary membrane was still present with its vessels deriving supply from the vessels within the anterior iridal stroma.

4.4.2.2 Ciliary body

The ciliary processes of the ciliary body were longer and more extensive. The caudal ciliary folds extended into ciliary processes. The double layered epithelium covering the ciliary processes and ciliary body was made up of two cell layers. The outer layer is pigmented and columnar while the inner layer is non pigmented (Fig.86). The inner non pigmented layer over the pars plana, was made up of pseudostratified columnar cells (Fig.87) which gradually changed to simple columnar cells towards the processes and eventually to simple cuboidal cells over the processes. In the crypts between the processes, the inner layer was composed of columnar cells. The vessels draining the capillary arcades of the ciliary processes were larger (Fig.85).

The iridocorneal angle was open at this age and the pectinate ligament was also seen at the base of the iris (Fig.88). The spaces of the meshwork of the iridocorneal angle were larger and were lined by trabecular cells (Fig.89). Few melanocytes were seen in the ciliary body along with fibroblasts, nerves and blood vessels (Fig.86, Fig.87).

The ciliary body musculature was fully differentiated consisting mostly of meridionally oriented smooth muscle cells running from the posterior region of the meshwork to the ora serrata (Fig.86).

4.4.2.3 Choroid

The choroid at this stage had all of its layers present. The choriocapillaris was adjacent to the retinal pigment epithelium. It was connected to the large choroidal vessels on the outer portion of the choroid by smaller vessels traversing the choroid at perpendicular angles (Fig.90). The choroid was composed of loose connective tissue containing fibroblasts, fibrocytes and numerous elongated spindle shaped melanocytes oriented parallel to the retinal surface (Fig.91, Fig.78).

The tapetum fibrosum was well differentiated at this stage as layers of regularly arranged collagen fibres in the dorsal portion of the posterior choroid. Fibroblasts and occasional melanocytes were seen within these layers (Fig.92).

4.4.3 NERVOUS TUNIC

4.4.3.1 Retina

The retina at this stage had all the 10 retinal layers present (Fig.90). The region of interdigitation between the retinal pigment epithelium and the tips of the rods and cones was AB positive. The outer nuclear layer had numerous spherical cells arranged in 8 ï 9 layers with the outer row of cells being larger and more euchoromatic (cone nuclei) and the rest of the rows containing smaller and more heterochoromatic cells (Rod nuclei).

The inner nuclear layer was separated from the outer nuclear layer by a thin outer plexiform layer (Fig.90). It contained the cell bodies of horizontal cells, bipolar cells, Müller cells and amacrine cells arranged in 5-6 layers.

The inner plexiform layer was thicker than the outer plexiform layer and separated the inner nuclear layer from the ganglion cell layer which consisted of a single layer of retinal ganglion cells. Few neural supportive cells were also seen in this layer. The nerve fibre layer extended from the ganglion cell layer to the internal limiting membrane (Fig.90).

Blood vessels were observed in all the retinal layers except the outer nuclear layer, layer of rods and cones and the retinal pigment epithelial layer. Large blood vessels were present mainly within the nerve fibre layer and ganglion cell layer while capillaries were seen mainly within the inner nuclear layer (Fig.93).

4.4.4 OPTIC NERVE

The axons of the ganglion cells converged at the optic nerve head and exited the eye ball as the optic nerve. The physiologic cup (a depression within the optic nerve head) was seen at this stage (Fig.94). These axons were surrounded by inner and outer connective tissue sheaths corresponding to dura mater and pia mater. They were also separated into nerve bundles by connective tissue septae from the inner connective tissue sheath. Blood vessels were seen between bundles running within the septae. Neuroglial cells were seen within the nerve bundles of the optic nerve (Fig.95, Fig.96).

4.4.5 LENS

The lens was surrounded by a PAS positive capsule that was thicker anteriorly than posteriorly. Centrally, the anterior epithelium was composed of cuboidal cells however these cells became columnar cells near the equator where they elongated into the lens fibres (Fig.97). The posterior surface of the lens was not lined by any epithelium (Fig.98).The tunica vasculosa lentis was still present but the vessels were smaller and sparsely distributed (Fig.97, Fig.98).

4.5 NEONATE

4.5.1 FIBROUS TUNIC

4.5.1.1 Cornea

The anterior corneal epithelium was the non keratinized stratified squamous type with 3-4 layers of cells. The basal cells were tall cuboidal cells with basally located euchromatic nuclei some of which had perinuclear halo around them. The superficial cell layer consisted of flat cells with elongated heterochromatic nuclei. The cells found between the basal and superficial layers (wing cells) had oval to elongated nuclei with their long axis parallel to the corneal surface. The cells adjacent to the superficial layer presented more heterochromatic nuclei than cells close to the basal layer (Fig.99). Peripherally, at the limbus, the basal cells of the anterior epithelium became columnar cells with slightly more heterochromatic nuclei. Pigment granules were observed within the cytoplasm of some cells in this region. The anterior epithelium in this region presented more cell layers and was also thicker (Fig.100). The basement membrane of the anterior epithelium was vaguely defined with H&E (Fig.100).

The stromal cells (keratocytes) were more euchromatic and larger than those of the adults especially in the anterior region of the stroma adjacent to the bowmanôs layer (Fig.99). The stroma reacted positively to both Periodic acid-Schiff and Alcian blue techniques (Fig.101). The internal limiting membrane (Descemetôs membrane) was thinner than that of the adult and also reacted positively with PAS (Fig.101). The corneal endothelium was made up of a single layer of eosinophilic cuboidal cells with round to oval slightly euchromatic nuclei (Fig.102).

4.5.1.2 Limbus

At the limbus, pigment cells were observed within the stroma of the cornea (Fig.100). Cells of the basal layer were more heterochromatic and also contained pigments. The stroma lost its regular arrangement in this region and became irregular. This region also contained blood vessels (Fig.100).

4.5.1.3 Sclera

The sclera was composed of collagenous bundles interspaced with fibrocytes and melanocytes. It consisted of the episclera, sclera proper and the lamina fusca.

The episclera was made up of irregular connective tissue and also contained blood vessels and nerves. Extraocular muscles inserted into this layer. The sclera proper constituted the bulk of the sclera. The lamina fusca was adjacent to the choroid and was heavily pigmented with the pigment cells oriented parallel to the surface (Fig.103). The sclera reacted positively to PAS procedure.

4.5.1.4 Lamina cribrosa

The lamina cribrosa was formed by astrocytes and collagenous fibres. The astrocytes were seen on the vitreal surface of the lamina cribrosa where they formed channels that guided and supported the axons of the ganglion cells as they turned into the scleral portion of the lamina cribrosa. The scleral and choroidal aspects of the lamina cribrosa were made up of meridionally oriented collagenous fibres that were sparsely pigmented (Fig.104).

4.5.2 VASCULAR TUNIC

4.5.2.1 Iris

The neonate iris consisted of three layers; the anterior epithelial layer (endothelial layer), the stroma and the posterior pigment epithelial layer (Fig.105). The stroma contained the dilator (Fig.105) and constrictor pupillae muscles (Fig.106). The posterior pigment epithelium extended to the pupillary border of the iris to form the granula iridica (Fig.106).Remnants of the iridopupillary membrane was seen around the granula iridica (Fig.106). The endothelial layer was made up of fibroblasts and melanocytes that were oriented parallel to the surface of the iris (Fig.105).The anterior iridal surface was irregular but lacked the crypts of Fuschs seen in the adult. The stroma contained loose collagenous fibres, melanocytes and fibroblasts. The melanocytes were mostly enlongated and had scanty pigments granules in their cytoplasm unlike in the adult (Fig.105). The iris was well vascularised especially along the anterior region (Fig.105).The sphincter pupillae muscle was observed at the pupillary region of the iris within the stroma while the dilator pupillae muscle blended with the former near the pupillary region, extended about two-third of the way towards the periphery then blended with the anterior layer of the posterior pigment epithelium. Before the point of blending with the posterior pigment epithelium, strands of smooth muscle cells were observed running from the dilator muscle to the anterior layer of the posterior pigment epithelium (Fig.105). Some of the smooth muscle cells had pigment granules within their cytoplasm. These granules got more numerous at the region of attachment to the posterior pigment epithelium. The posterior layer of the iris was composed of a bilayered pigmented epithelium that was continuous with the epithelial covering of the ciliary body (Fig.105).

4.5.2.2 Ciliary body

The ciliary body included the iridocorneal angle, ciliary processes and the ciliary body musculature. It was composed of loose connective tissue and smooth muscles. Numerous blood vessels were also present along with melanocytes and nerves (Fig.107). The ciliary body was lined by bilayered epithelium with the outer layer being pigmented and the inner layer being cuboidal and non-pigmented (Fig.107). The layers were continuous with the posterior epithelial layer of the iris anteriorly and also with the retina posteriorly.

The ciliary processes had a core of blood vessels and loose connective tissue stroma with a covering of bilayered epithelium. The cells of the inner layer of the epithelium were non pigmented cuboidal to columnar cells with round to oval euchromatic nuclei. Fibrocytes were also observed in the stroma.

The ciliary body musculature was mainly made up of meridionally oriented smooth muscle fibres running from the posterior extent of the ciliary cleft towards the ora serrata.

The iridocorneal angle extended into the ciliary body and formed the ciliary cleft. It consisted of a meshwork of connective tissue beams and open spaces lined by fusiform shaped cells (trabecular cells). It was divided into two regions; a small anterior region with large spaces just posterior to the pectinate ligament and a bigger posterior region with small elongated spaces within the connective beams. This meshwork had vessels associated with it. The pectinate ligament was also seen at the base of the iris (Fig.107).

4.5.2.3 Choroid

The choroid was made up of connective tissue, fibroblasts and numerous melanocytes. It was between the retina and the sclera.

The vessel layer was composed of medium and large-sized vessels, loose connective tissue stroma and numerous spindle-shaped melanocytes that had their long axis parallel to the retina (Fig.108). In the dorsal portion of the posterior choroid, the vessel layer contained a layer of regularly arranged collagenous fibres ï tapetum fibrosum (Fig.108). This layer was between the vessel layer and the choriocapillaris (Fig.108). Interconnecting vessels ran through the tapetum fibrosum connecting the vessel layer and the choriocapillaris. Fibrocytes and occasional melanocytes were seen within the tapetal layer.

The innermost layer of the choroid was the choriocapillary layer. It contained a layer of capillaries that were in close association with the retinal pigment epithelium (Fig.108).

4.5.3 NERVOUS TUNIC

4.5.3.1 Retina

The retina consisted of a sensory portion (pars optica retinae) and a non sensory portion. The non sensory portion which started at the ora serrata lined the inside of the iris (pars iridis retinae) and the ciliary body (pars ciliaris retinae). The sensory portion extended from the optic disc to the ora serrata.

The sensory portion was made up of 10 layers. From outside inward these layers were,

1. Retinal pigment epithelium
2. Layer of rods and cones (visual cell layer)
3. Outer limiting membrane
4. Outer nuclear layer
5. Outer plexiform layer
6. Inner nuclear layer

7. Inner plexiform layer
8. Ganglion cell layer
9. Nerve fibre layer
10. Inner limiting membrane (Fig.108)

The retinal pigment epithelium was made up of eosinophilic cuboidal cells. These cells had basal euchromatic nuclei that were centrally located. Their nucleoli were also prominent. The apical surface of the cells presented extensions that surrounded the tips of the rods and cones. The cells were densely packed with pigment granules (Fig.109). However in the tapetal region, the RPE was devoid of pigments (Fig.108). Pigment cells over the transition region, between the tapetal and non tapetal regions, presented less pigmentation and also had alternating groups of pigmented and non pigmented cells.

The layer of rods and cones (visual cell layer) contained the outer and inner segments of the photoreceptors. The apical processes of the retinal pigment epithelium interdigitated with the outer segments of the photoreceptors. This region of interdigitation was AB positive (Fig.110). The rod cells were long and slender while the cone cells were wide at the base and tapered to a blunt tip.

The visual cell layer was separated from the outer nuclear layer by a thin PAS positive line ï the outer limiting membrane (Fig.110). The outer nuclear layer contained the nuclei of the rods and cones arranged in 7-8 rows centrally but tapered down to 2-3 rows peripherally at the junction of the sensory and non sensory retina. The outer nuclear layer contained more nuclei than in the adult. The nuclei were also heterochromatic and similar unlike in the adult (Fig.111).

The outer plexiform layer was between the outer nuclear layer and the inner nuclear layer. It was composed of the dendrites of the horizontal and bipolar cells and the synaptic terminals of the rods and cones. Capillaries were seen in this layer (Fig.111).

The inner nuclear layer was composed of euchromatic nuclei with prominent nucleoli. These cells were arranged in 5-7 rows centrally but tapered down to 1-2 rows of nuclei peripherally where the sensory retina changes into non sensory retina. Capillaries were also seen in this layer (Fig.111).

The inner plexiform layer was thicker than the outer plexiform layer and contained the processes and synapses between the cells of the inner nuclear layer and the ganglion cell layer. This layer also contained few euchromatic cells and capillaries (Fig.111).

The ganglion cell layer was composed of a single layer of retinal ganglion cells and neuroglial cells. In the dorsal portion of the retina, over part of the tapetal region, there was an area that contained large retinal ganglion cells arranged in 3-4 layers. The ganglion cells in this region were larger with intense eosinophilic cytoplasm (Fig.111).

The nerve fibre layer contained the axons of the ganglion cells, large retinal blood vessels and neuroglial cells. The axons of the ganglion cells ran parallel to the retinal surface (Fig.111).

The inner limiting membrane was made up of the fused endfeet of the Müller cells. Their end feet flared out and fused with each other to form the inner limiting membrane (Fig.111). This membrane was closely related to the cortex of the vitreous body. Few hyalocytes, fibrocytes and small heterochromatic cells were seen sandwiched between the cortex of the vitreous body and the inner limiting membrane of the retina. The inner limiting membrane was Alcian blue positive (Fig.110).

4.5.4 OPTIC NERVE

The optic nerve head was formed by the convergence of the fibres of the ganglion cells near the posterior pole of the eye ball (Fig.104). Glial cells formed passages that guided the fibres into and through the lamina cribrosa. The optic nerve was formed by the axons of the ganglion cells, associated glial cells and connective tissue cover that sent septae into the nerve. Blood vessels were seen within the connective tissue septae. The PAS positive septae divided the optic nerve into bundles (Fig.112).

4.5.5 LENS

The lens has a thick PAS positive capsule, an anterior epithelium and hexagonally shaped lens fibres (Fig.113). The lens capsule was thicker anteriorly than posteriorly. The anterior epithelium was made up of a layer of cells that were cuboidal with euchromatic nuclei centrally but became columnar with slightly heterochromatic nuclei at the equator where they elongated and formed the lens fibres (Fig.114). These cells elongated anterio-posteriorly with their apical and basal portions extending anteriorly and posteriorly respectively. The nuclei of the individual cells retained their central positions during the elongation thereby creating a curved arrangement of their nuclei - Lens bow (Fig.114). The lens fibre nuclei were oval and slightly heterochromatic (Fig.114). Blood vessels from the hyaloid vasculature were seen on the posterior surface of the lens and at the equator (Fig.114). Zonular fibres were seen attached to the lens at the equator (Fig.114).

4.6 ADULT

4.6.1 FIBROUS TUNIC

4.6.1.1 Cornea

The anterior corneal epithelium was of the stratified squamous type with up to 5-8 layers of cells. The basal cells were eosinophilic columnar cells with apically located heterochromatic nuclei. The nuclei were surrounded by perinuclear halo. Mitotic cells were observed in the basal layer of cells along with occasional lymphocytes. Cells with centrally located nuclei were observed sitting on the basal layer of cells. These cells (wing cells) were hexagonally shaped with spherical and slightly euchromatic nuclei. This layer of cells was about 3 cells deep. The superficial layer of cells was about 3 cells deep and consisted of flattened (squamous) cells. These flattened cells were eosinophilic with darkly stained nuclei (Fig. 115). The cornea was observed to be thicker centrally than at the periphery. This increase in thickness was due to the increase in the number of layers of the polyhedral wing cells and the superficial squamous cells as the basal cell remained a single layer of cells in all regions. Peripherally at the limbus, the basal cells of the anterior epithelium were pigmented. The basement membrane (Bowmanôs membrane) was vaguely defined with H&E but reacted positively with Periodic Acid-Schiff reaction.

The stroma was composed of regularly arranged collagen fibres with flattened cells (Keratocytes) between them. These cells were elongated with little cytoplasm. The stroma also presented a high degree of pigmentation in the region of the limbus. The stroma reacted positively with PAS and Alcian blue with the anterior stroma being predominantly Alcian blue positive than PAS positive and the posterior stroma being predominantly PAS positive than Alcian Blue positive. The internal limiting membrane (Descemetôs membrane) was a thick homogenous membrane that appeared as a thick amorphous membrane and highly

refractile layer with H&E and also reacted positively with PAS. The corneal endothelium was composed of a single layer of low cuboidal to flattened cells that were slightly eosinophilic with mildly stained nuclei lying parallel to the internal limiting membrane (Fig.116)

4.6.1.2 Limbus

At the limbus, the corneal stroma contained pigment cells with a thick overlying epithelium. The epithelium in this region was thicker than the adjacent corneal epithelium and contained pigment granules. The basement membrane was thrown into folds. The stroma in this region lost the regular arrangement of the corneal stroma and appeared like irregular dense connective tissue (Fig.117)

4.6.1.3 Sclera

The sclera was made up of bundles of collagenous fibres that reacted faintly with PAS technique (Fig.118).It was composed of three layers; the episclera, the sclera proper and the lamina fusca.

The episclera was the outermost and was made up of irregular connective tissue. This layer contained blood vessels and nerves (Fig.119). It was continuous anteriorly with the connective tissue of the bulbar conjuctiva and posteriorly with the connective tissue of the tenonôs capsule. The sclera proper made up the bulk of the sclera. The collagen bundles in this layer were mainly oriented parallel to the surface while some of the bundles were interwoven. Fibrocytes and few melanocytes were seen in-between the collagen bundles. The innermost layer, lamina fusca had numerous pigment cells between the collagen fibres. These pigment cells were long and arranged parallel to the surface. This layer was next to the choroid (Fig.120).Very few blood vessels were seen in the sclera.

4.6.1.4 Lamina cribrosa

The lamina cribrosa was composed of collagenous fibres and astrocytes. The anterior lamina cribrosa (vitreal aspect) was mostly formed by astrocytes while the posterior lamina cribrosa (scleral aspect) was mostly made up of heavily pigmented collagenous fibres that were meridionally oriented. The astrocytes and fibrocytes in the anterior lamina cribrosa formed channels that supported and guided the nerve bundles as they turned into the scleral canal from the nerve fibre layer (Fig.121)

4.6.2 VASCULAR TUNIC

4.6.2.1 Iris

The adult caprine iris consisted of three layers; an anterior epithelial layer (endothelial layer), a middle layer of connective tissue stroma, which contained two smooth muscles - dilator pupillae muscle and sphincter papillae muscle and a posterior layer of pigmented epithelium (Fig.122). The posterior pigmented epithelial layer extended forward to the edge of the iris to form a mass called granula iridica. This mass was composed of cyst-like extensions of varying sizes that were lined by pigmented epithelium. The cysts contained connective tissue fibres, fibrocytes, capillaries and few smooth muscle cells that were seen at the basal region of the granula iridica (Fig.123).

The anterior epithelial layer was composed of fibroblasts and melanocytes. No basement membrane was observed under these cells. The melanocytes and fibroblasts were oriented parallel to the iris surface (Fig.124). The anterior surface of the iris was thrown into folds with the ventral iris bearing smaller but more numerous folds than the dorsal iris which bore larger and less numerous folds. In between the folds, there were invaginations of the anterior epithelial layer into the stroma of the iris. These invaginations (Crypts of Fuchs) extended to various depths into the stroma (Fig.124).

The middle layer, the iris stroma, was composed of loosely arranged fine collagenous fibres, chromatophores and fibroblasts (Fig.123). Nerves and numerous blood vessels were present. The blood vessels were surrounded by concentrically arranged collagenous fibres (Fig.124).The sphincter pupillae muscle was composed of circularly oriented smooth muscle fibres. It was seen at the edge of the iris within the iridal stroma capped by the granula iridica. It occupied mostly the central portion of the iris stroma with some of the fibres extending posteriorly and anteriorly. The dilator pupillae muscle was composed of a thin sheat of smooth muscle cells in the posterior region of the iridal stroma. It blended with the sphincter muscle near the pupil and extended to the periphery of the iris. It was intimately associated with the posterior layer of the pigmented epithelium (Fig.122).

The posterior layer of pigmented epithelium was composed of densely pigmented dome shaped cells that had different numbers of layers in different regions of this layer. Clumps of the epithelial cells were also observed at different regions. The posterior pigmented epithelium was continuous with the ciliary body epithelium (Fig.126).

4.6.2.2 Ciliary body

The ciliary body included the ciliary processes, the ciliary body musculature and the iridocorneal angle (Fig.126). It was mostly made up of loose connective tissue, smooth muscles and numerous blood vessels. Melanocytes and nerves were also present (Fig. 127). Plasma cells were observed within the trabecular meshwork and the ciliary body musculature. The epithelial covering was of the bilayered columnar type but gradually became cuboidal towards the ciliary processes (Fig.128).The inner and outer layers of the epithelium were non-pigmented and pigmented, respectively. The pigmented layer was continuous with the retinal pigmented epithelium posteriorly and with the anterior pigmented epithelial cell layer

of the iris anteriorly. The non-pigmented layer was continuous with the sensory retina posteriorly and with the posterior pigmented epithelium of the iris anteriorly.

The ciliary processes had a core of blood vessels and connective tissue stroma covered by a double layered epithelium; an inner cuboidal, non-pigmented cell layer and an outer layer of pigmented cuboidal cells. The cells of the inner cell layer were euchromatic. Fibroblasts were present in the stroma of the ciliary processes (Fig.129).

The ciliary body musculature consisted mainly of meridionally (running from the ciliary body towards the ora serrata) oriented smooth muscle fibres that were peripherally located (Fig.128), few circularly oriented myocytes and few radially oriented myocytes.

The iridocorneal angle extended posteriorly from the junction of the base of the iris and the cornea into the ciliary body (Ciliary cleft).It consisted of numerous open spaces within a reticular network of connective tissue beams. It was divided into two regions. The outer region which presented smaller spaces while the inner region presented larger spaces that got smaller posteriorly where the two regions merged (Fig.130). The spaces within this meshwork were lined by squamous cells (Fig.131). The iridocorneal angle was observed to be devoid of collagen bundles unlike the ciliary body musculature where numerous collagen bundles were observed oriented in different directions. A band of long collagenous strands (Pectinate ligament) was observed anteriorly at the junction of the base of the iris and the cornea. These collagen strands anchored the anterior base of the iris to the inner surface of the peripheral cornea (Fig.130). It was lined anteriorly by fibrocytes from the iridal part up to its attachment to the cornea where it was lined by few cuboidal cells. The posterior surface was lined by squamous cells.

4.6.2.3 Choroid

The choroid was located between the retina and the sclera. Its outer layer, the suprachoroidea was attached to the lamina fusca of the sclera by collagenous lamellae. These collagenous lamellae traversed a space, the suprachoroidal space, between the suprachoroidea and the lamina fusca. The suprachoroidea was composed of connective tissue, numerous melanocytes and fibroblasts (Fig.132).

The vessel layer was made up of large and medium-sized vessels and loose connective tissue stroma with abundant melanocytes most of which were enlongated to oval in shape (Fig.133).The vessel layer in the dorsal portion of the choroid contained a layer of regularly arranged collagenous fibres, the tapetum fibrosum. This layer was interposed between the vessel layer and the choriocapillary layer with interconnecting vessels running through it at right angles from the vessel layer to the choriocapillary layer (Fig.133).It was oriented parallel to the retinal surface. Its thickness varied from being multilayered to thinning down to a single layer in regions close to non-tapetal region. Fibrocytes were interposed between the layers of collagenous fbers (Fig.133).

The choriocapillary layer was the innermost layer of the choroid. It was made up of a layer of capillaries that were adjacent to the retinal pigment epithelium. It was only separated from the cells of the retinal pigment epithelium by a complex of the basement membranes of the endothelial cells of the capillaries and the retinal pigment epithelial cells (Fig.133).

4.6.3 NERVOUS TUNIC

4.6.3.1 Retina

The retina was composed of a sensory portion (pars optica retinae) and a non sensory portion which started at the ora serrata and covered the ciliary body and the iris as the pars ciliaris

retinae and the pars iridis retinae respectively. The sensory retina extended from the optic disc to the ora serrata where it was reduced to the bi-layered epithelial covering of the ciliary body.

The sensory portion was composed of 10 layers. The layers from outside inward were

1. Retinal pigment epithelium.

2. Layer of rods and cones (Visual cell layer)

3. Outer limiting membrane.

4. Outer nuclear layer.

5. Outer plexiform layer.

6. Inner nuclear layer.

7. Inner plexiform layer.

8. Ganglion cell layer.

9. Nerve fibre layer

10. Inner limiting membrane (Fig.134).

The retinal pigment epithelium was made up of eosinophilic cuboidal cells. These cells had a basally and centrally located round to oval euchromatic nuclei with prominent nucleoli. The apical surface of these cells presented cytoplasmic processes that projected inwards to interdigitate with the rods and cones (Fig.135). This region of interdigitation was alcian blue positive while the rods and cones were slightly PAS positive (Fig.136).The cells were densely pigmented. The pigment granules were located in the midportion to apical cytoplasm above the nuclei and within the processes. However, cells above the tapetum fibrosum

(tapetal region) were devoid of pigment granules (Fig.135).Cells found in the transition region between the tapetal region and the non tapetal region contained less pigment granules in individual cells and also presented alternating groups of cells containing pigments and groups devoid of pigments thus presenting a sieve-like arrangement (Fig.133).The tapetal cells underlying this transitional region gradually thinned down to one layer in the region close to the non tapetal region.

The layer of rods and cones contained the outer parts of the photoreceptors called the outer and inner segments (Fig.136). The outer segments of the rods and cones were surrounded by the apical processes of the retinal pigment epithelium. This region was AB positive. Each of the rods and cones were made up of an outer segment, inner segment, nuclear region and the synaptic region. The rods cells were long and slender while the cone cells were wide at the base and tapered down to a blunt tip. They were both eosinophilic (Fig.135).

A very thin eosinophilic line ï outer limiting membrane was seen separating the rods and cones from the outer nuclear layer (Fig.137).The outer nuclear layer contained the nuclei of the rods and cones arranged in 6-7 rows centrally but tapered down to 1-2 rows peripherally. The nuclei of the cones which were large and round were located close to the outer limiting membrane. The rest of the rows of nuclei in this layer were mostly made up of the smaller round to oval nuclei of the rod cells (Fig.137).

The outer plexiform layer was located on the vitreal side of the outer nuclear layer. It was a thin layer that separated the outer nuclear layer from the inner nuclear layer. It was composed of the synaptic terminals of the rods and cones and the dendrites of the horizontal and bipolar cells. Capillaries were seen in this layer (Fig.134).

The inner nuclear layer was composed of the amacrine, bipolar, horizontal and Müller (supporting glial) cells. These cells were arranged in three rows centrally but tapered down to a layer of cells peripherally.

The inner plexiform layer was notably thicker than the outer plexiform layer. This layer comprises the cell processes of the cells of the ganglion cell layer and the inner nuclear layer. Capillaries were seen within this layer. Occasional heterochromatic cells were also seen in this layer.

The ganglion cell layer was composed of a single layer of retinal ganglion cells, neuroglial cells and retinal blood vessels. The ganglion cell bodies were large and had large eccentrically positioned euchromatic nuclei, prominent nucleolus and copious cytoplasm (Fig.138).

The nerve fibre layer was composed of the unmyelinated axons of the ganglion cells. These axons were arranged parallel to the retinal surface. It was a thick layer that also contained large blood vessels (Fig.134)

The inner limiting membrane was made up of the fused end feet of the Müller cells. The end feet of these cells flared out on the vitreal surface of the retina and fused with the adjacent Müller cells to form the inner limiting membrane (Fig.138).This layer was closely associated with the outer vitreous body. This membrane was AB positive (Fig.136)

4.6.4 OPTIC NERVE HEAD

The axons of the ganglion cells converged near the posterior pole of the globe and formed the optic nerve head (optic papilla). A central depression (physiologic cup) was observed at the optic nerve head (Fig.121).At the optic nerve head, glial cells were seen forming channels that guided bundles of axonal fibres from the ganglion cells into the lamina cribrosa

(Fig.121). The optic nerve was formed by the axonal fibres of the ganglion cells, neuroglial cells and connective tissue septae arising from pia mater. Blood vessels were seen within the connective tissue septae. These septae were PAS positive and divided the optic nerve into optic nerve bundles (Fig.139)

4.6.5 LENS

The lens was made up of a thick lens capsule, an anterior lens epithelium and lens fibres (Fig.140). The lens capsule was PAS positive and thicker anteriorly than posteriorly. The anterior lens epithelial cells were composed of a layer of cells just inside the anterior lens capsule. The cells were cuboidal to squamous centrally but became low columnar at the equator where they elongated to form the lens fibres with their apical portion and basal portion extending anteriorly and posteriorly respectively. This arrangement created a curved line of nuclei (lens bow) at the lens equator since the nuclei of the individual fibres retained their central positions (Fig.141). The lens fibre nuclei were heterochromatic and oval in shape with their long axis in the anterior posterior orientation (Fig.141). Zonular fibres were attached to the lens at the equator (Fig.141).

FIGURES

FIG.1 Section of the anterior eye (limbus region) of a 44 days old fetus; Cuboidal cells (white arrow); squamous cells (black arrow); eyelid bud **EB;** corneal stroma **ST.** H&E

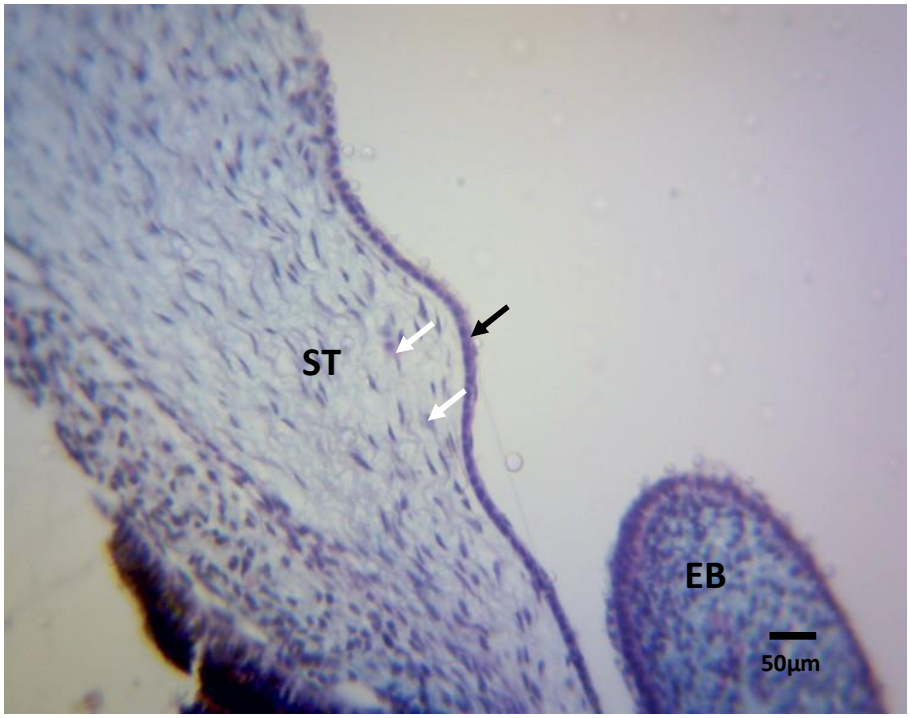

FIG.2 Section of the anterior eye of a 45 days old fetus; eyelid bud **EB,** corneal stroma **ST,** corneal epithelium (black arrow). Note the AB positive extracellular stromal matrix and the PAS positive cells (white arrows). AB-PAS. pH 2.5

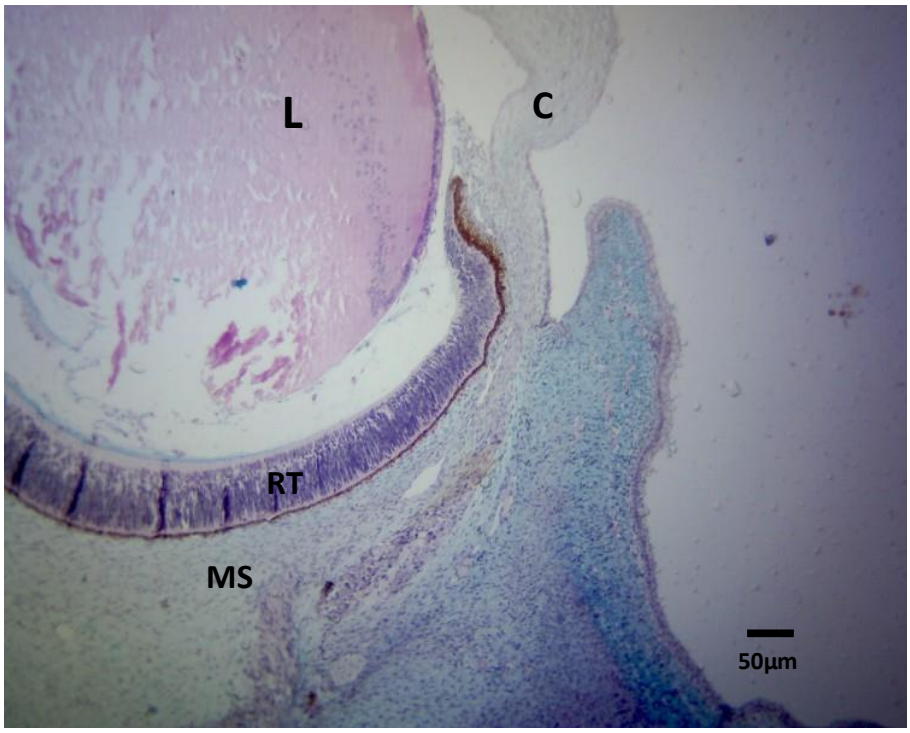

FIG.3 Section of the eye of a 45 days old fetus; lens **L;** cornea **C;** retina **RT;** mesenchyme surrounding optic cup **MS**. AB-PAS. pH 2.5

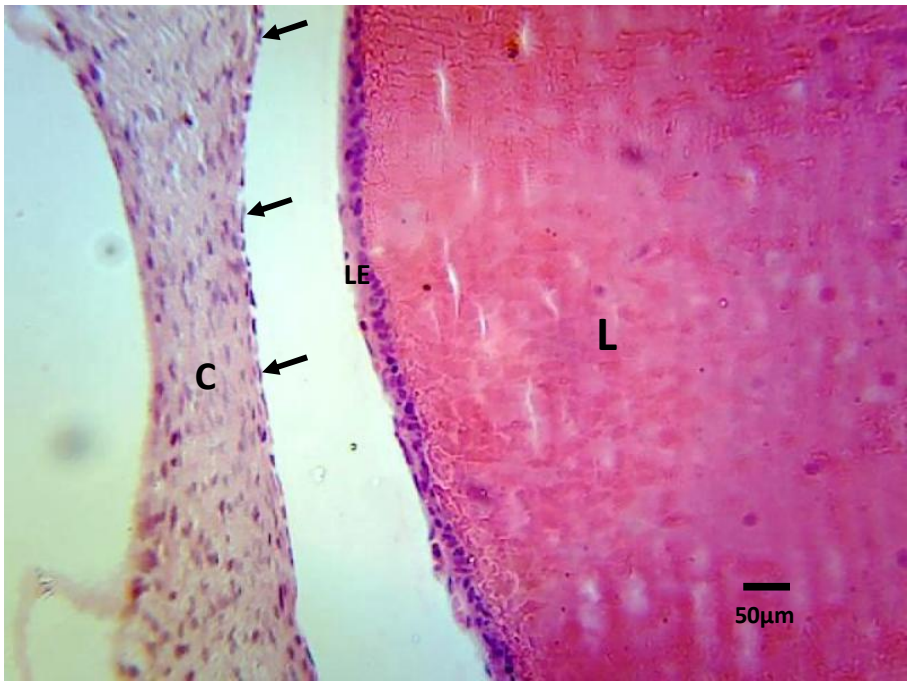

FIG.4 Section of the cornea and lens of a 45 days old fetus; lens **L;** anterior lens epithelium **LE;** cornea **C:** corneal endothelium (arrows). H&E.

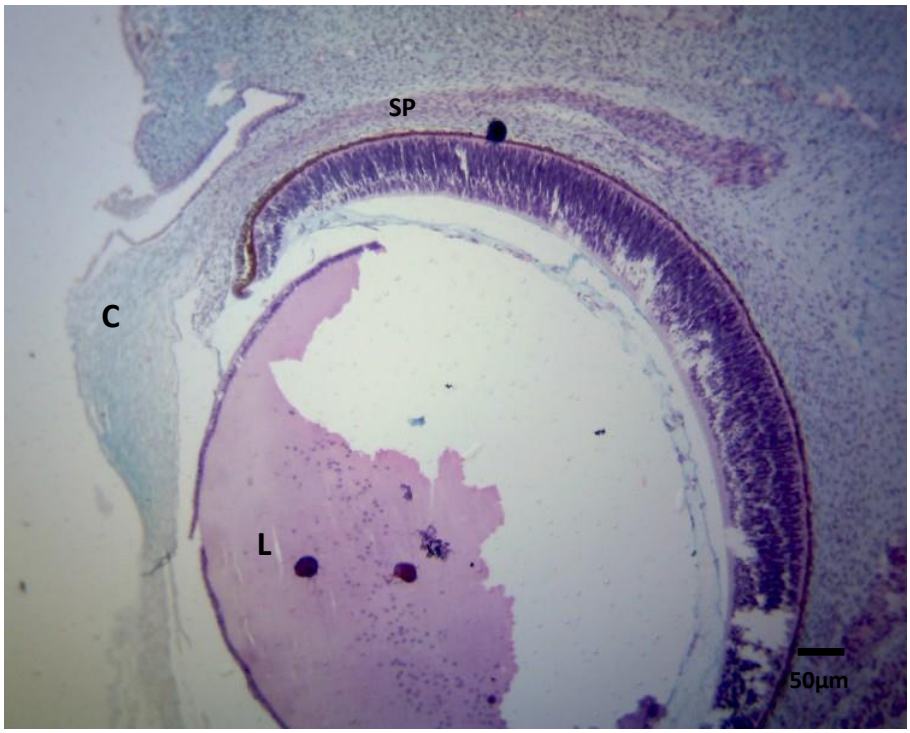

FIG.5 Section of the eye of a 45 day old fetus; lens **L;** cornea **C;** scleral primordium **SP.** AB-PAS. pH 2.5

FIG.6 Section of the eye of a 45 day old fetus; cornea **C,** lens **L,** iris (white arrow), iridopupillary membrane (black arrow); hyaloid vessels (arrowheads),neuroblastic layer of the retina **R,** periocular mesenchyme **PM. Note:** The hyaloid vessels and vessels of the iridopupilary membrane make up the tunica vasculosa lentis. H&E.

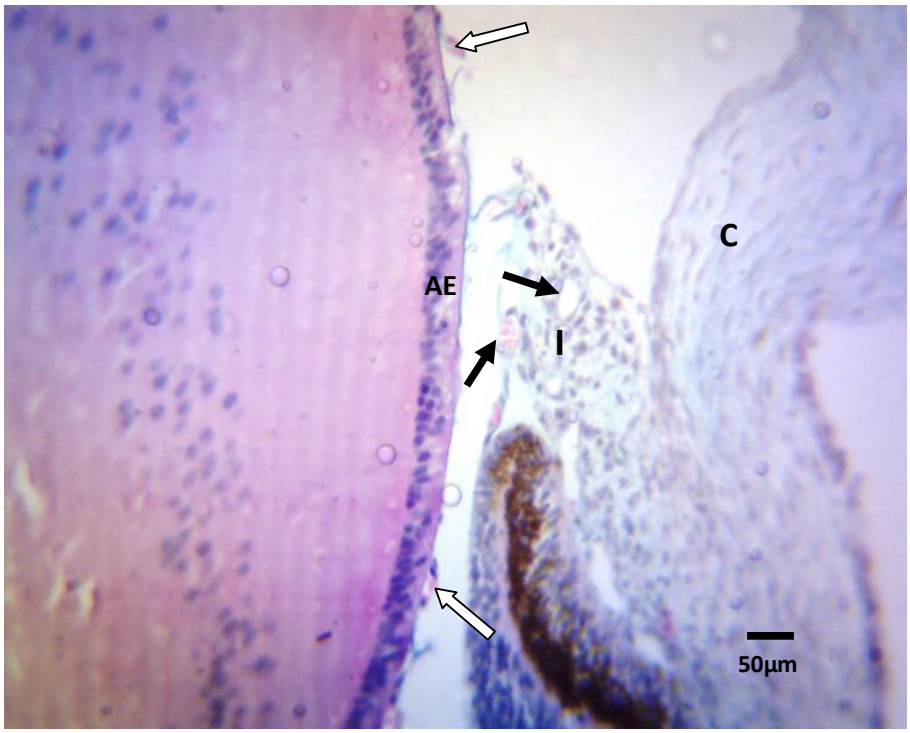

FIG.7 Section of the eye of a 45 day old fetus; Cornea **C,** Iris **I**, Iridopupilary membrane vessels (black arrows), anterior lens epithelium **AE,** hyaloid vessels (white arrows)**.** AB-PAS. pH 2.5

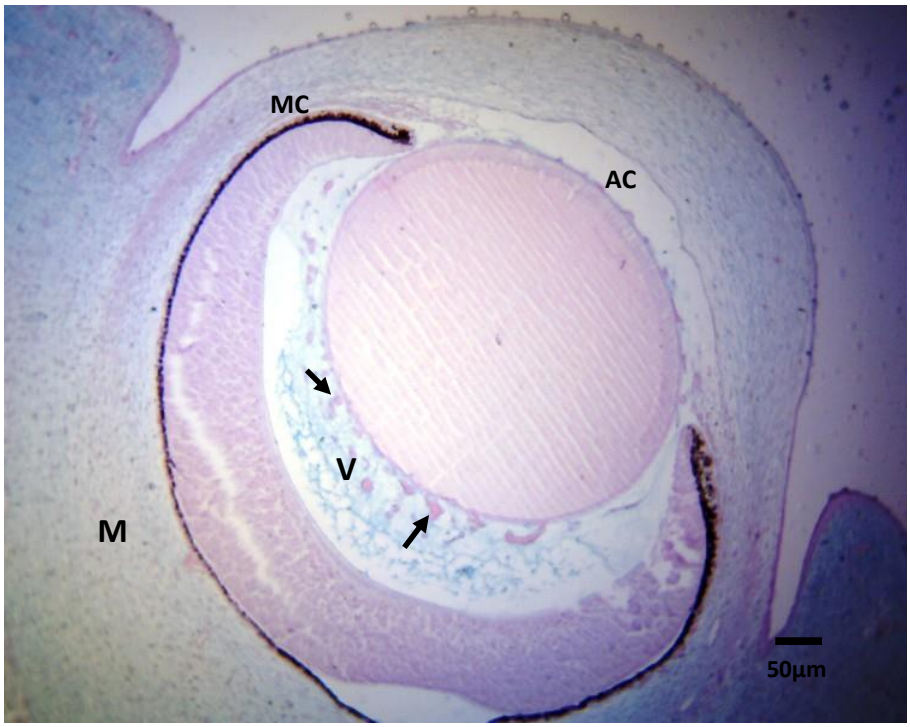

FIG.8 Section of the developing globe (Day 45 fetus)showing the mesenchymal condensations **MC** representing the choroid and the sclera; the anterior chamber **AC;** vitreous body **V** containing vessels (arrows) from the hyaloid system; undifferentiated mesenchyme **M.** AB-PAS. pH 2.5

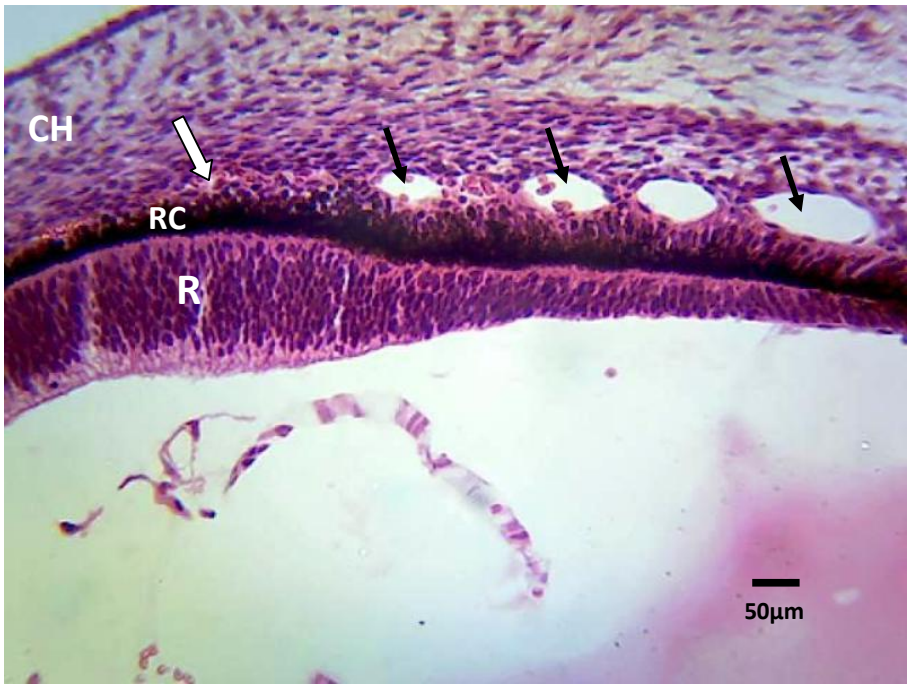

FIG.9 Section of the fetal anterior eye (Day 45 fetus) showing the developing choroid **CH,** retina **R,** retinal pigment cells **RC;** annular sinusoid, representing the choriocapilaris, (white arrow); enlargements of the annular sinusoids in the area where the ciliary body will later form (black arrows). H&E

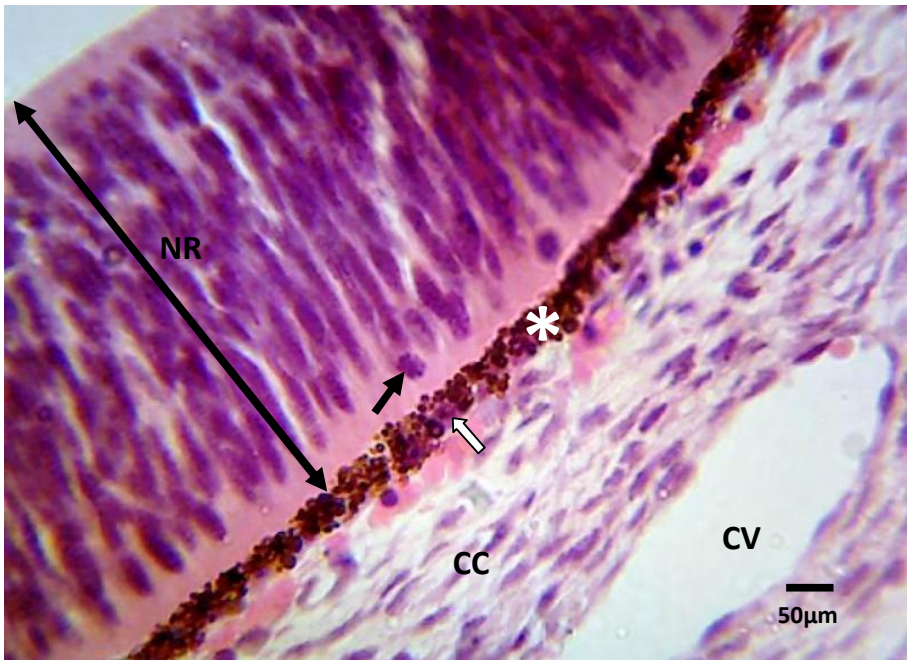

FIG.10 Section of the fetal eye (Day 45 fetus) showing the retinal neuroblastic layer **NR;** retinal pigment epithelium (asterix); mitotic cells in the neuroblastic layer (black arrow); differentiating mesenchyme in the choroidal area **CC;** choroidal blood vessel **CV;** nucleus of a cuboidal cell of the retinal pigment epithelium (white arrow).H&E.

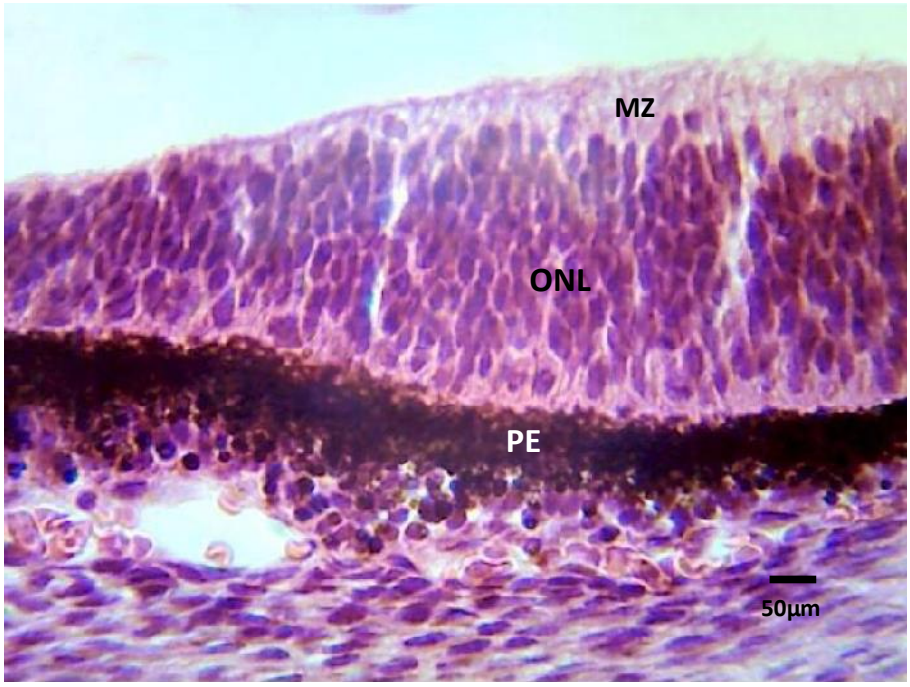

FIG.11 Section of the fetal peripheral retina (Day 45 fetus) showing the outer nuclear layer **ONL,** containing columnar cells**;** the inner anuclear layer (marginal zone) **MZ,** The retinal pigment epithelium **PE.**H&E.

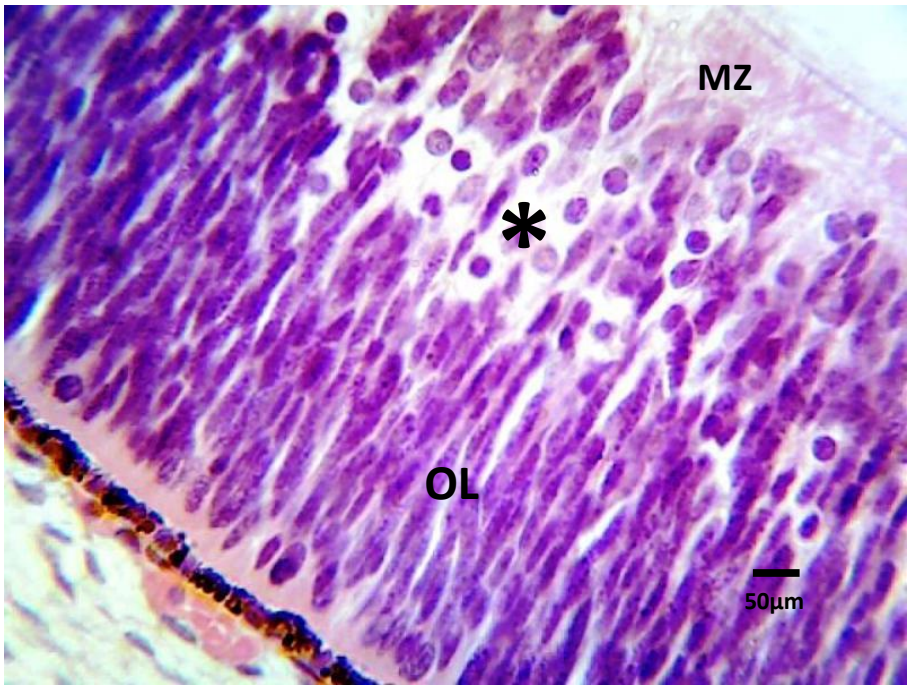

FIG.12 Section of the fetal central retina (Day 45 fetus) showing the outer nuclear layer **OL,**containing columnar cells**;** the inner anuclear layer (marginal zone) **MZ;** primordium of the transient layer of Chievitz (asterix)**.** H&E.

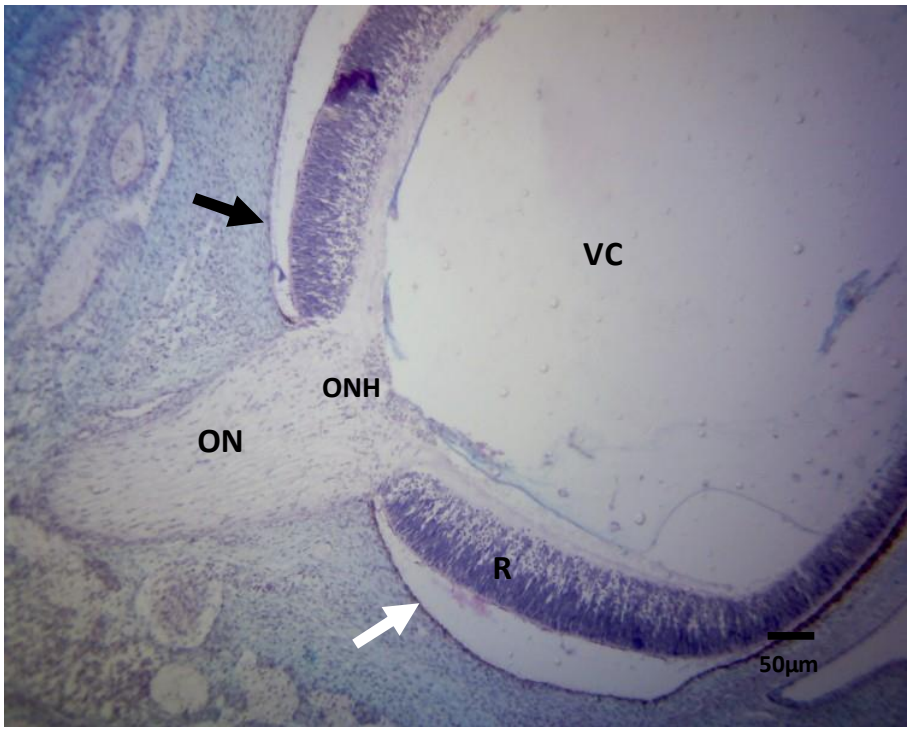

FIG.13 Section of the fetal globe (Day 45 fetus) showing the retina **R;** vitreous chamber **VC;** optic nerve **ON;** optic nerve head **ONH**, tapetal retinal pigment epithelium (black arrow); non tapetal retinal pigment epithelium (white arrow).Note the absence of the lamina cribrosa at the **ONH**. AB-PAS. pH 2.5

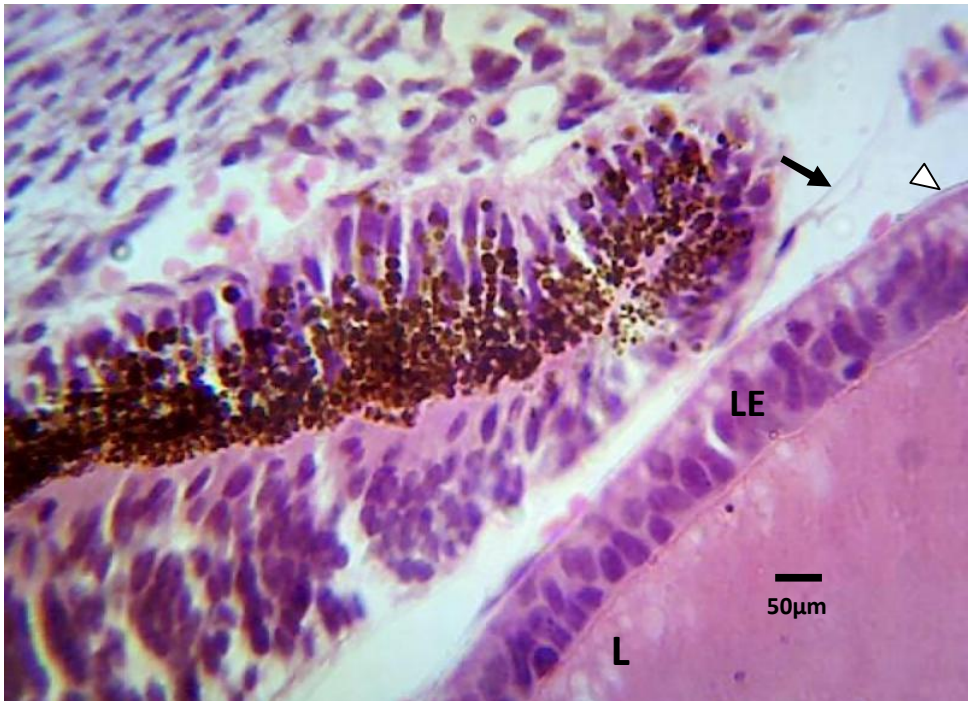

FIG.14 Section of the anterior eye (Day 45 fetus) showing the lens fibres **L,** the thin lens capsule (arrow head), iridopupillary membrane (black arrow), the lens epithelium **LE** containing cuboidal and columnar cells. H&E

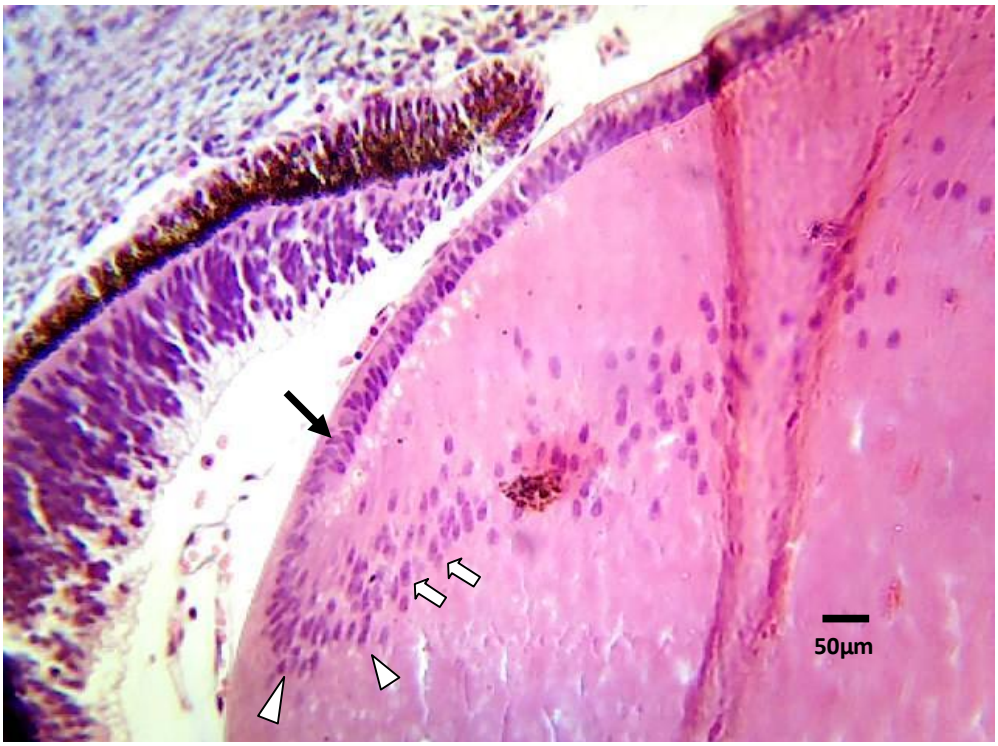

FIG.15 Section of the anterior eye of a 45days old fetus, showing the lens bow (arrow heads), lens epithelium at the lens equator containing pseudostratified columnar cells (black arrow), nuclei of newly formed lens fibres (white arrows). H&E

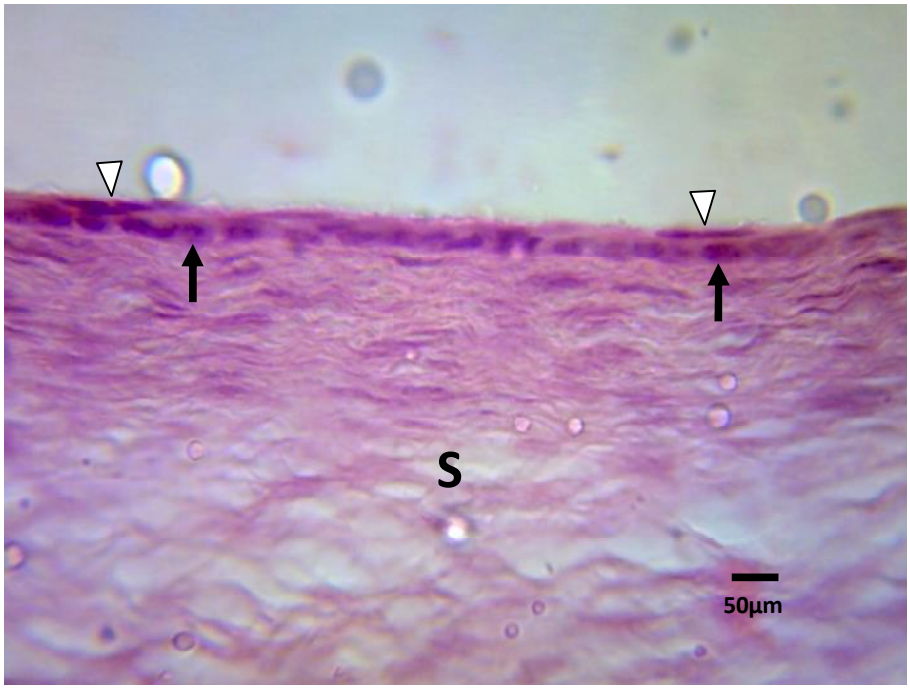

FIG 16 Section of a day 59 fetal cornea (central region) showing the anterior corneal epithelium composed of the basal layer of cuboidal cells (arrow heads) and the superficial layer of spindle shaped cells (arrows), corneal stroma **S.** H&E

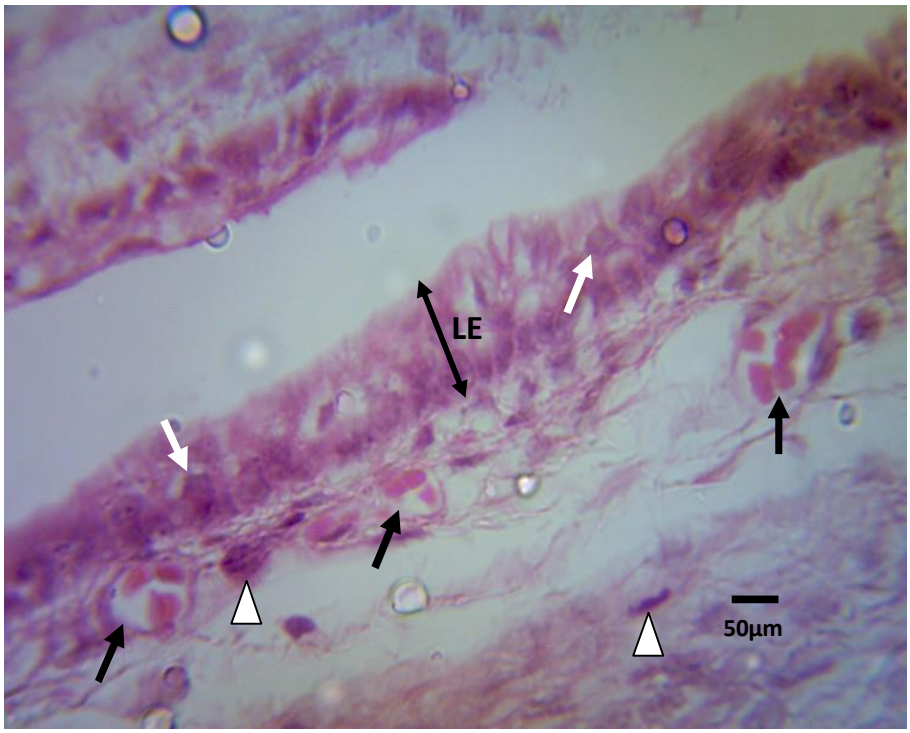

FIG. 17 Section of the limbus (Day 59 fetus) showing the cuboidal cells (white arrows) forming the layers of the epithelium **LE,** blood vessels (black arrows), pigment cells (arrow heads). H&E

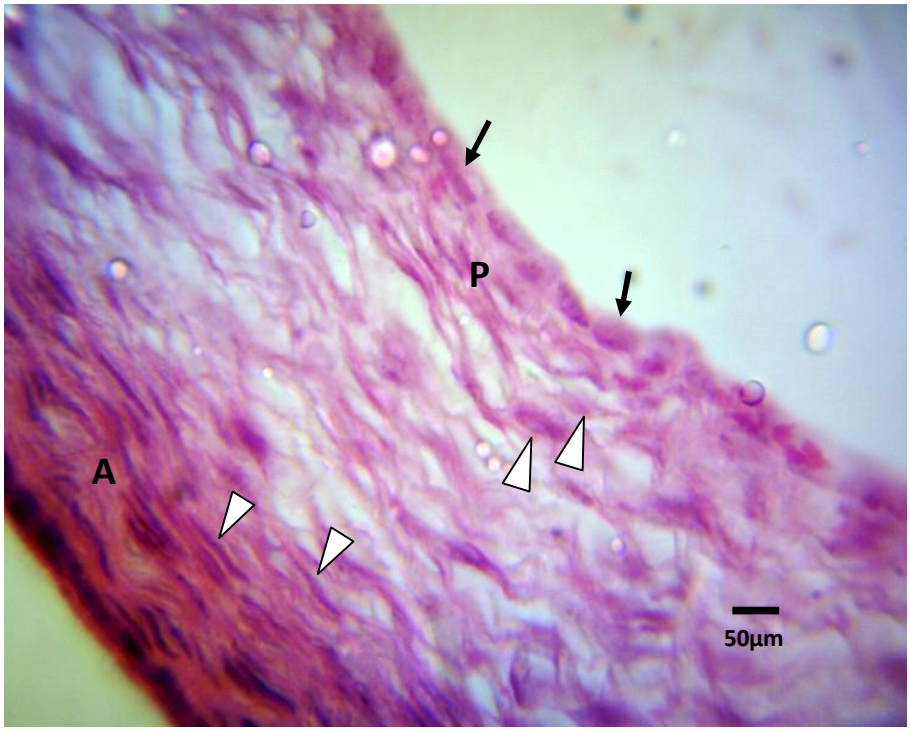

FIG.18 Section of a day 59 central cornea showing the compact anterior **A** and posterior **P** regions of the corneal stroma containing fibroblasts (arrow heads), corneal endothelial cells (black arrows).H&E

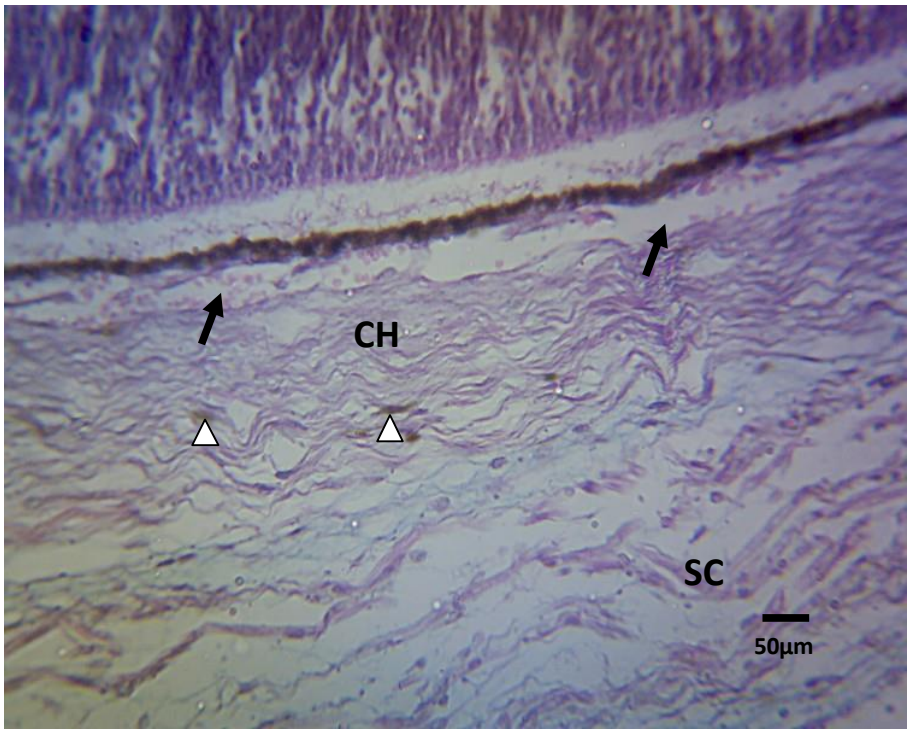

FIG.19 Section of the posterior globe (Day 59 fetus) showing the differentiating choroid **CH,** sclera **SC,** annular sinusoid (arrows), pigment cells (arrow heads) within the developing choroid. AB-PAS. pH 2.5

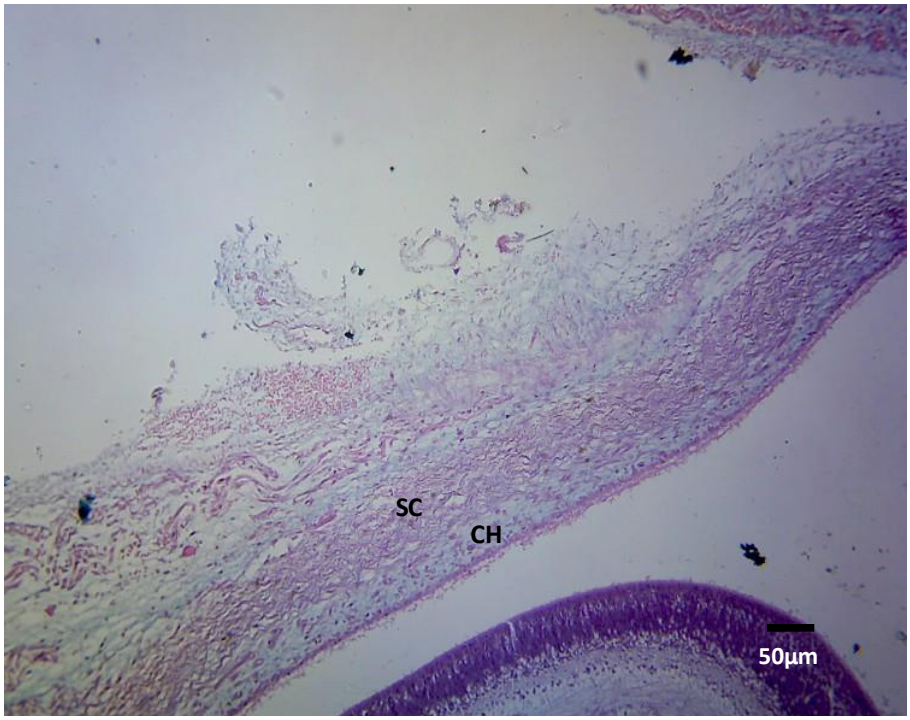

FIG.20 Section of the posterior globe (Day 59 fetus) showing the PAS positive scleral primordium, **SC** surrounding the AB positive choroid **CH.**AB-PAS. pH 2.5

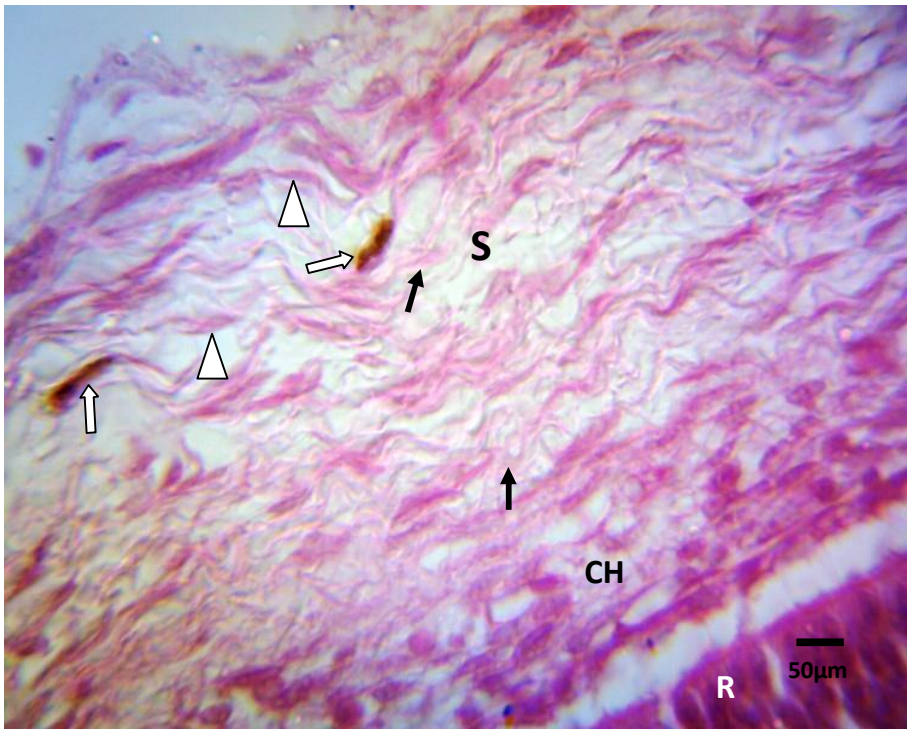

FIG.21 Section of the posterior globe of a day 59 fetus showing the developing choroid **CH,** developing retina **R** and developing sclera **S** containing melanocytes (white arrows), fibroblasts (arrow heads) laying down collagen fibres (black arrows).H&E.

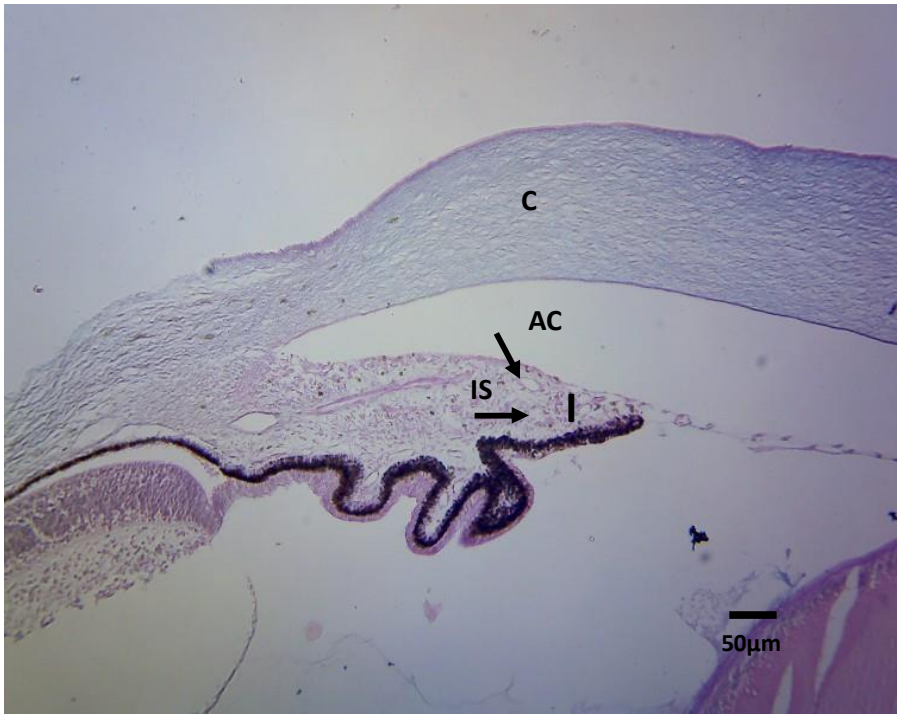

FIG.22 Section of the anterior globe(Day 59 fetus) showing the Iris **I,** blood vessels within its stroma (arrows), anterior chamber **AC,** cornea **C,** iridal stroma **,IS**,composed of loose connective tissue**.** AB-PAS. pH 2.5

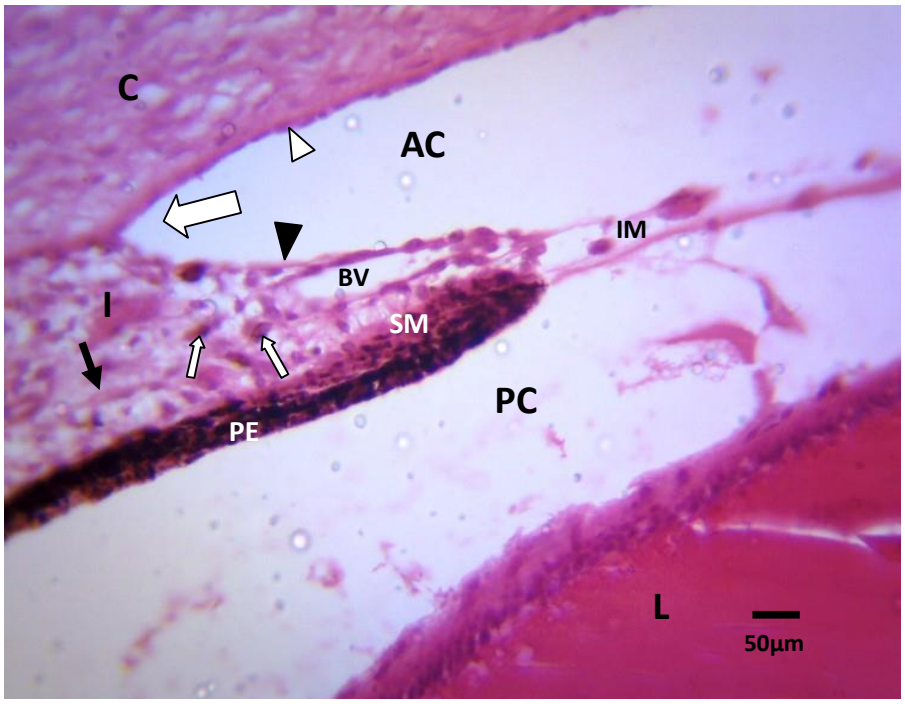

FIG.23 Section of the anterior eye (Day 59 fetus)showing the iris **I**, cornea **C** and lens **L**; the iridal endothelium (black arrow head), corneal endothelium (white arrow head),iridocorneal angle (large white arrow), fibroblast (black arrow), pigment cells (small white arrows), developing smooth muscle cells containing pigment granules **SM** closely related to the posterior pigmented iridal epithelium **PE** at the iridal margin, iridopupilary membrane **IM** continous with iridal blood vessel **BV,** anterior chamber **AC,** posterior chamber **PC.** Note that the iridocorneal angle was still closed. H&E

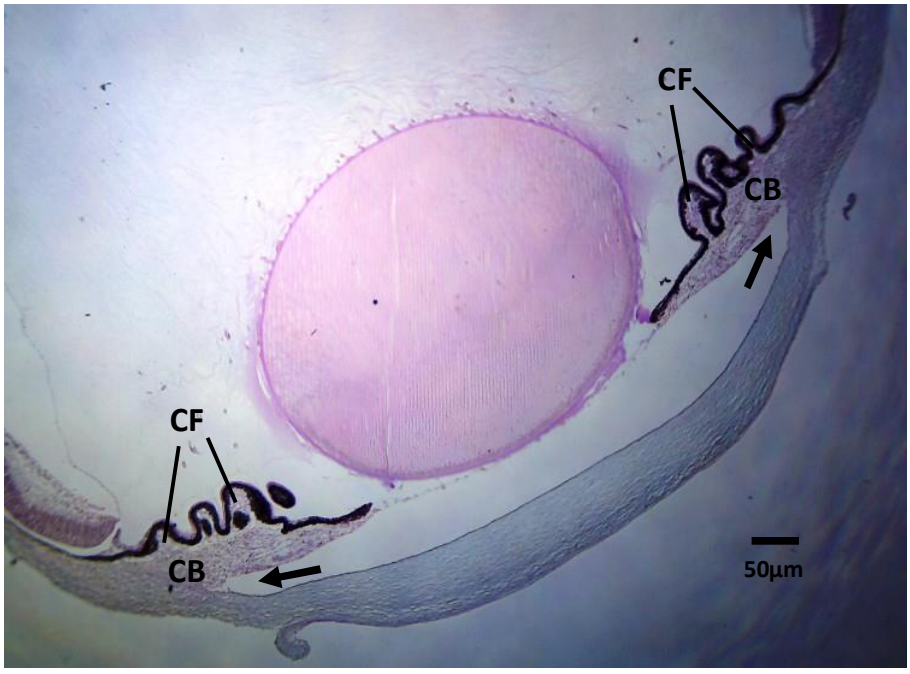

FIG.24 Section of the anterior globe (Day 59 fetus) showing the developing ciliary body **CB,** developing ciliary folds **CF,** iridocorneal angle (arrows). AB-PAS. pH 2.5

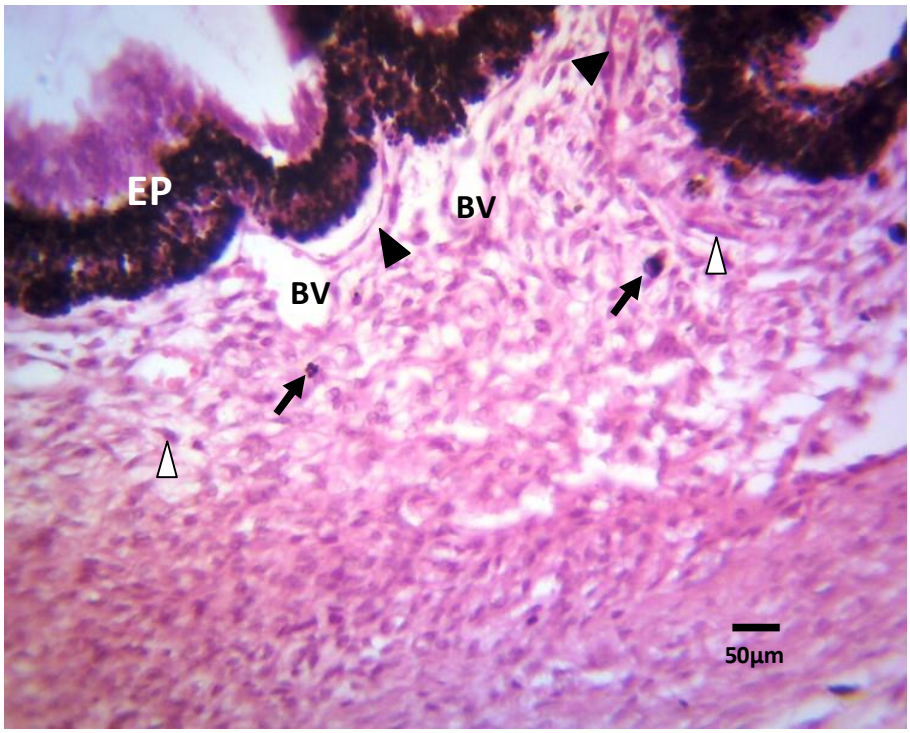

FIG.25 Section of the developing ciliary body (Day 59 fetus) showing melanocytes (black arrows), fibroblasts (white arrow heads), and blood vessels **BV** with branches going into the ciliary folds (black arrow heads), double layered epithelium **EP**. H&E

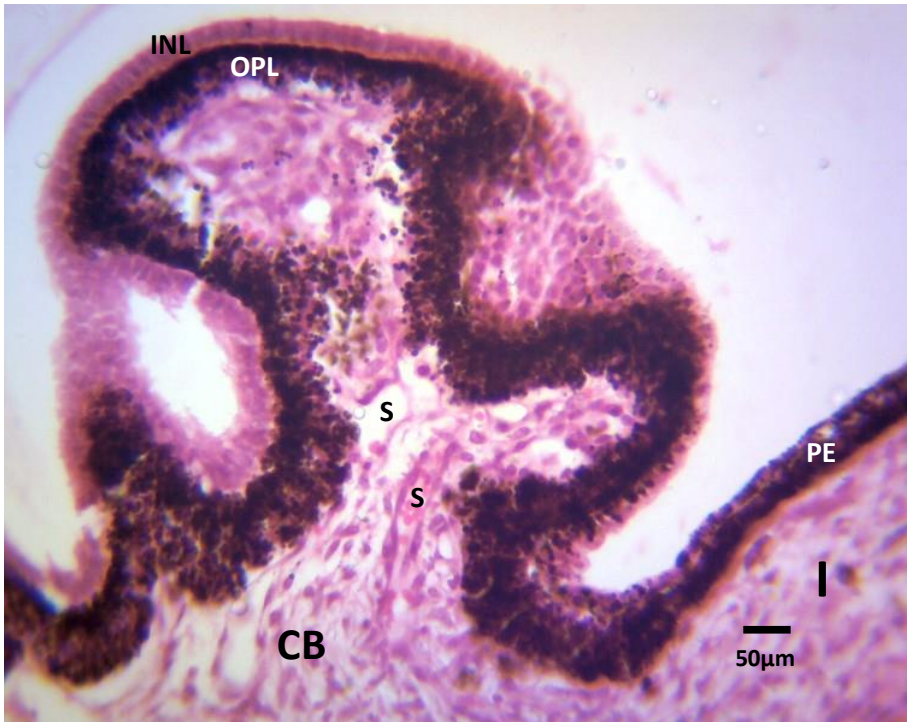

FIG.26 Day 59 fetal section of the ciliary body **CB** and iris **I** showing the inner nonpigmented layer **INL** and outer pigmented layer **OPL** of the ciliary body, sinusoids **S** running into the core of the ciliary fold, the bilayered posterior iridal epithelium **PE.** Note that the ciliary epithelium is continuous with the posterior iridal epithelium. H&E.

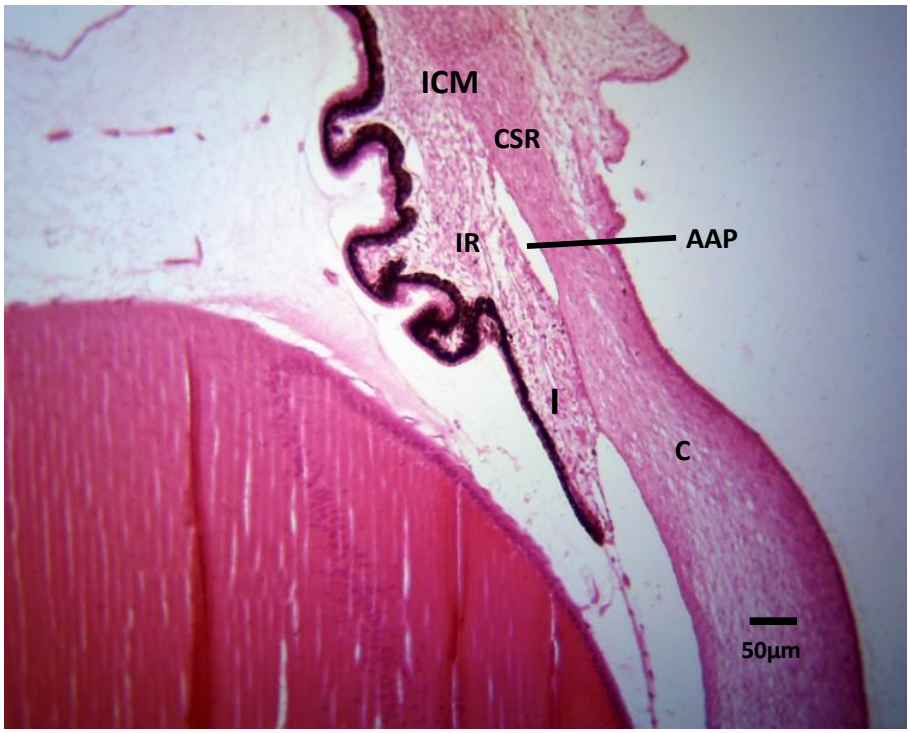

FIG.27 Section of the anterior eye (Day 59 fetus) showing the iris **I,** cornea **C,** the iridal region **IR** and the corneoscleral region **CSR** of the iridocorneal meshwork **ICM;** angular aqueous plexus **AAP.** H&E.

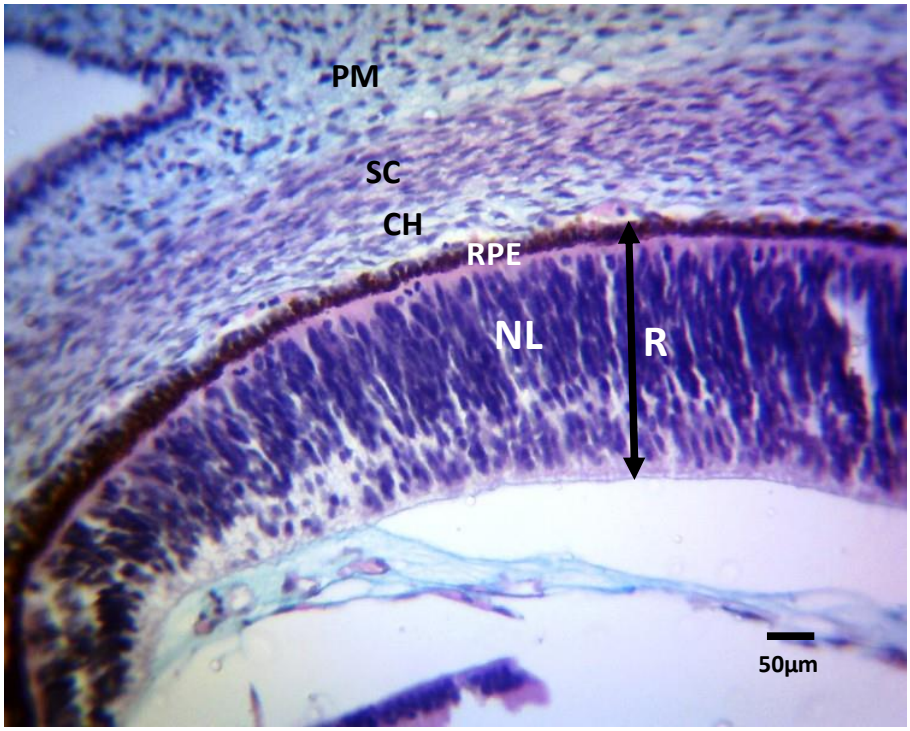

FIG.28 Section of the anterior globe (Day 59 fetus) showing the developing retina **R** composed of the retinal pigment epithelium **RPE** and the neuroblastic layer **NL,** mesenchymal condensations of the choroid **CH,** sclera **SC** and periocular mesenchyme **PM.** AB-PAS. pH 2.5

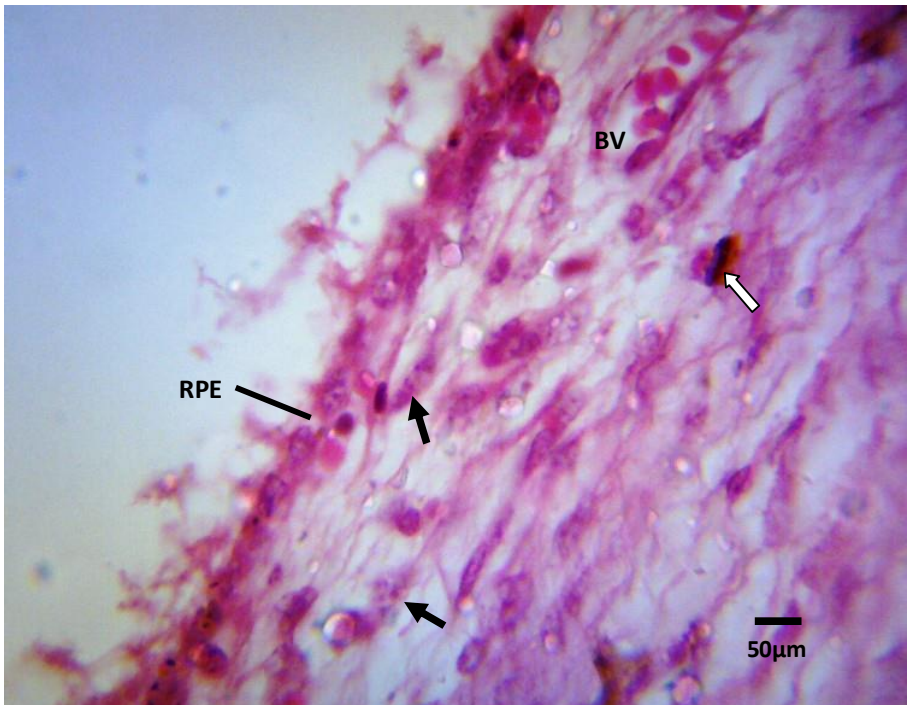

FIG.29 Section of the developing choroid (tapetal region) (Day 59 fetus) showing pigment cells (white arrow), fibroblasts (black arrows), which are more euchromatic than those found in the non tapetal region, choroidal blood vessels **BV** and the retinal pigment epithelium **RPE.** Note that the cells of the retinal pigment epithelium are devoid of pigments. H&E.

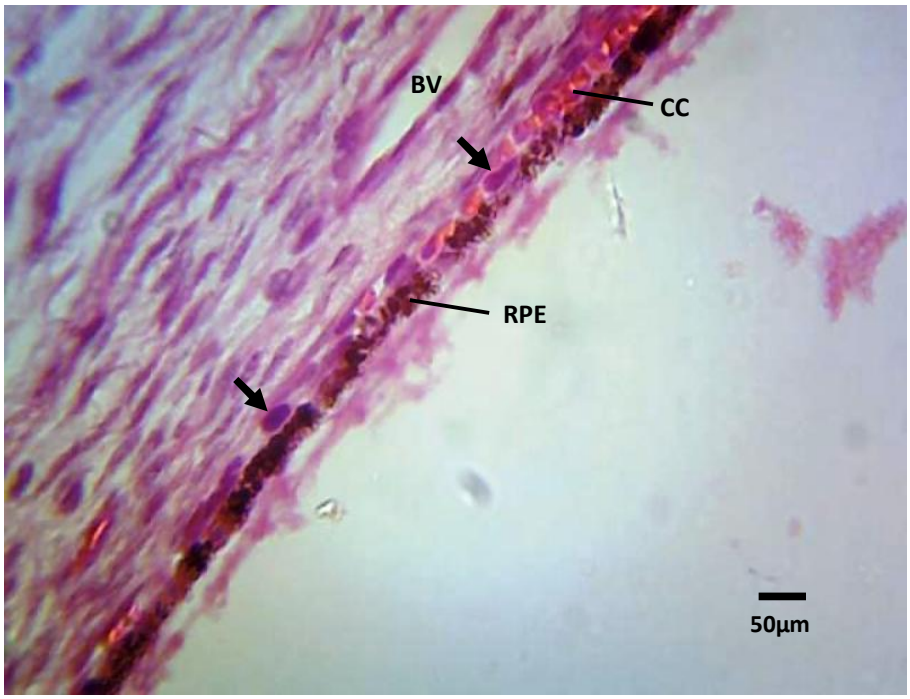

FIG.30 Section of the developing choroid (Day 59 fetus) (transitional region) showing fibroblasts (black arrows), which are less euchromatic than those found in the tapetal region, choroidal blood vessel **BV,** choriocapilaris **CC,** retinal pigment epithelium **RPE.** Note that cells of the retinal pigment epithelium contain sparse pigment granules with some cells devoid of the pigments.H&E

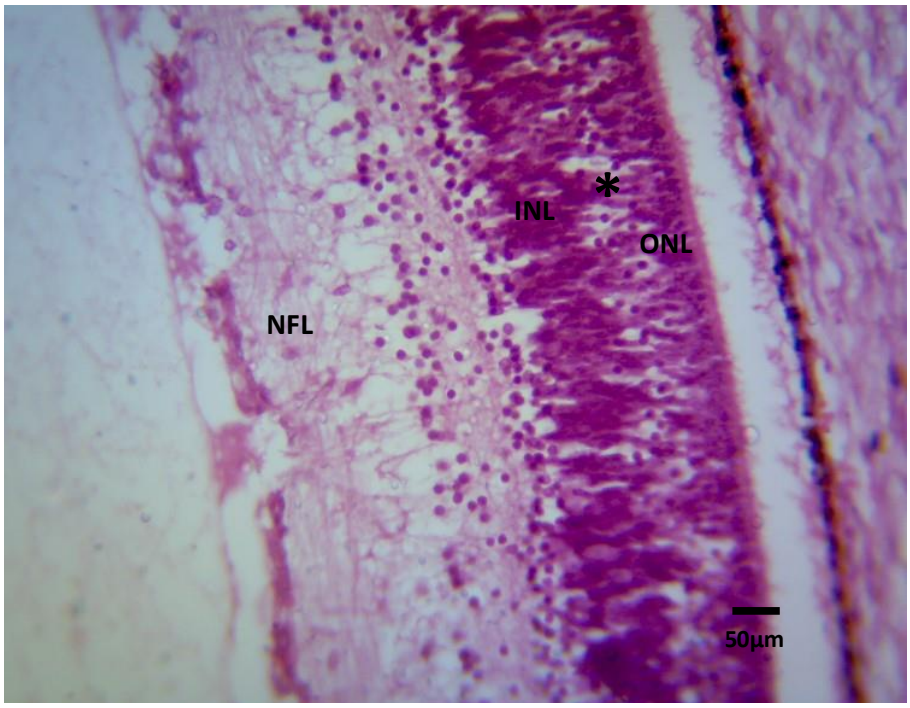

FIG.31 Section of the developing retina (Day 59 fetus) showing the inner nuclear layer **INL,** outer nuclear layer **ONL,** nerve fibre layer **NFL** and the developing transient layer of Chievitz (asterix).H&E

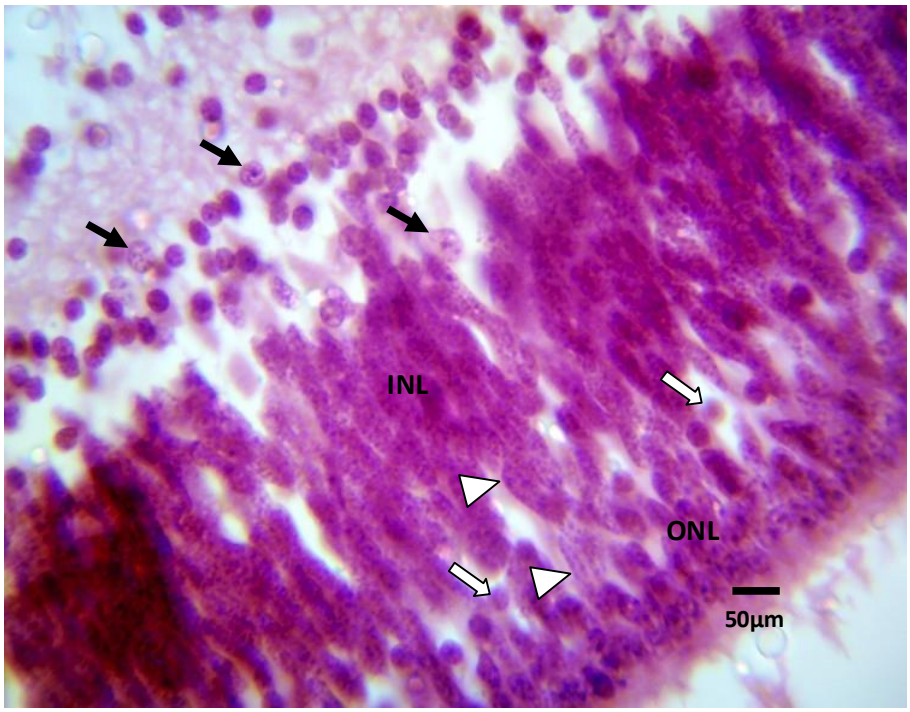

FIG.32 Section of the developing retina (Day 59 fetus) showing the outer neuroblastic layer **ONL**, the inner neuroblastic layer **INL,** the euchromatic spherical cells differentiating from the inner neuroblastic layer (black arrows) and the spherical cells differentiating from the outer neuroblastic layer (white arrows). Note that most of the cells were still columnar in shape (white arrow heads). H&E.

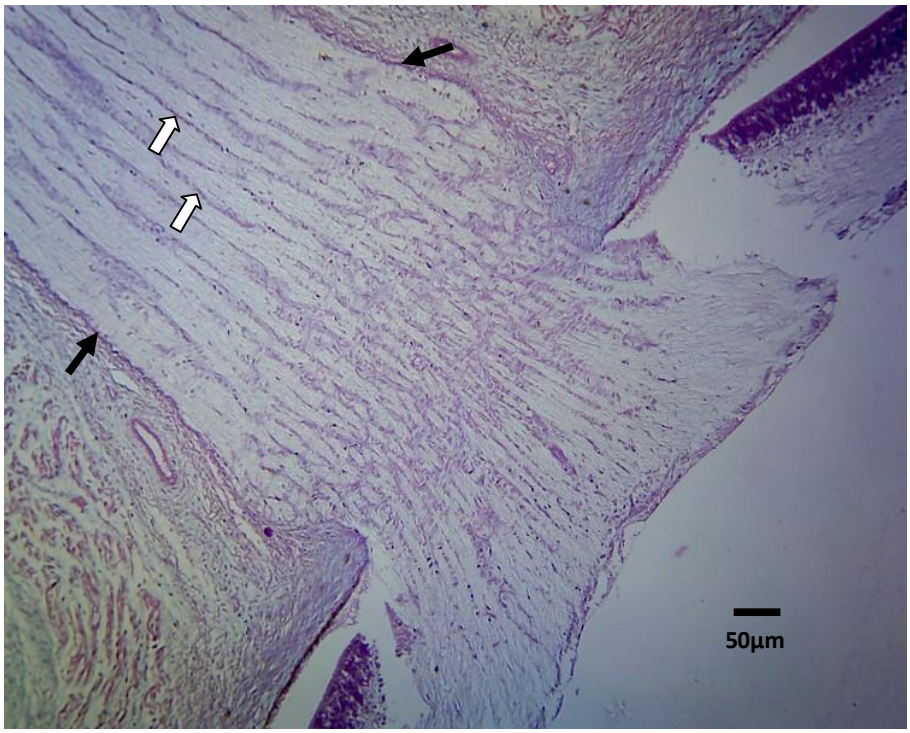

FIG.33 Section of the developing optic nerve (Day 59 fetus) showing the connective tissue sheaths (black arrows) and connective tissue septae (white arrows). AB-PAS. pH 2.5

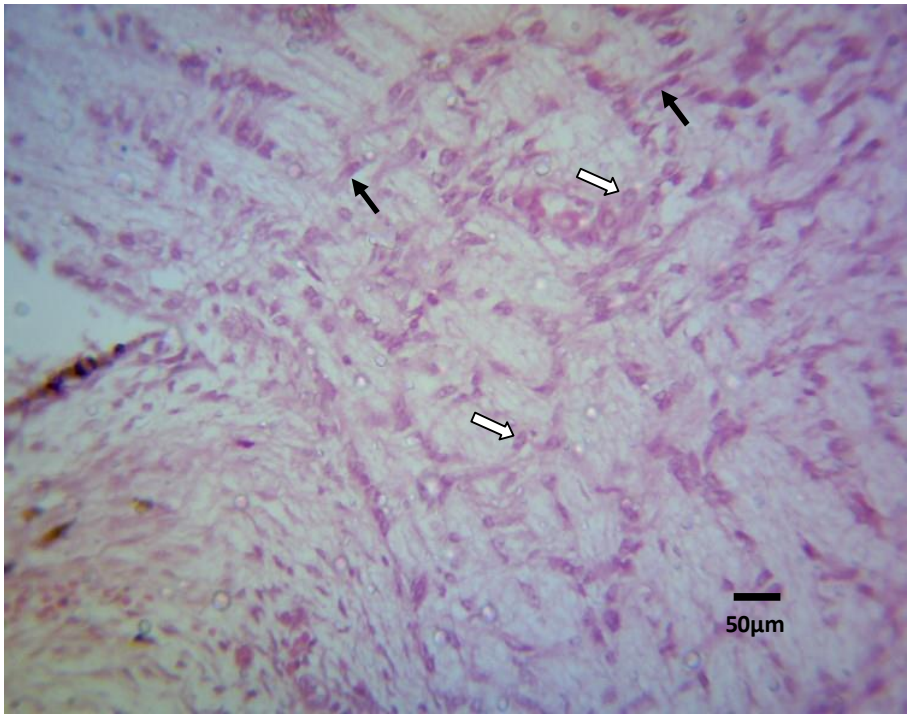

FIG.34 Section of the optic nerve (Day 59 fetus) showing the glial cells (white cells), fibroblasts (black arrows) oriented perpendicularly to the optic nerve fibres at the cribiform plate region. Note that the lamina cribrosa lacks pigmentation. H&E

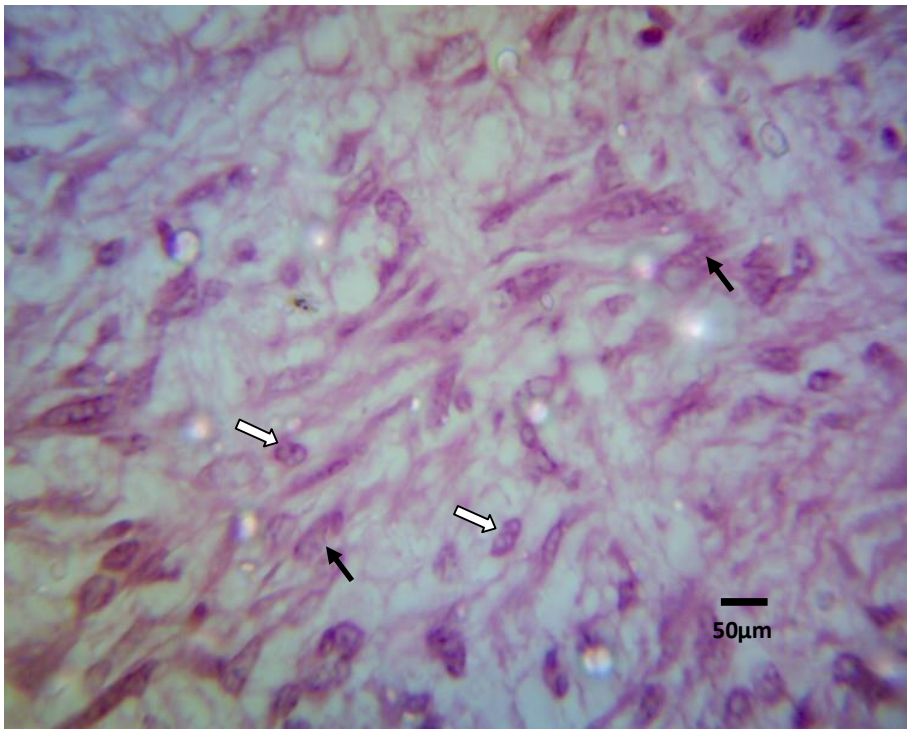

FIG.35 Section of the optic nerve (Day 59 fetus) showing the glial cells (white arrows), fibroblasts (black arrows) oriented perpendicularly to the optic nerve fibres at the cribiform plate region. Note that the lamina cribrosa lacks pigmentation. H&E

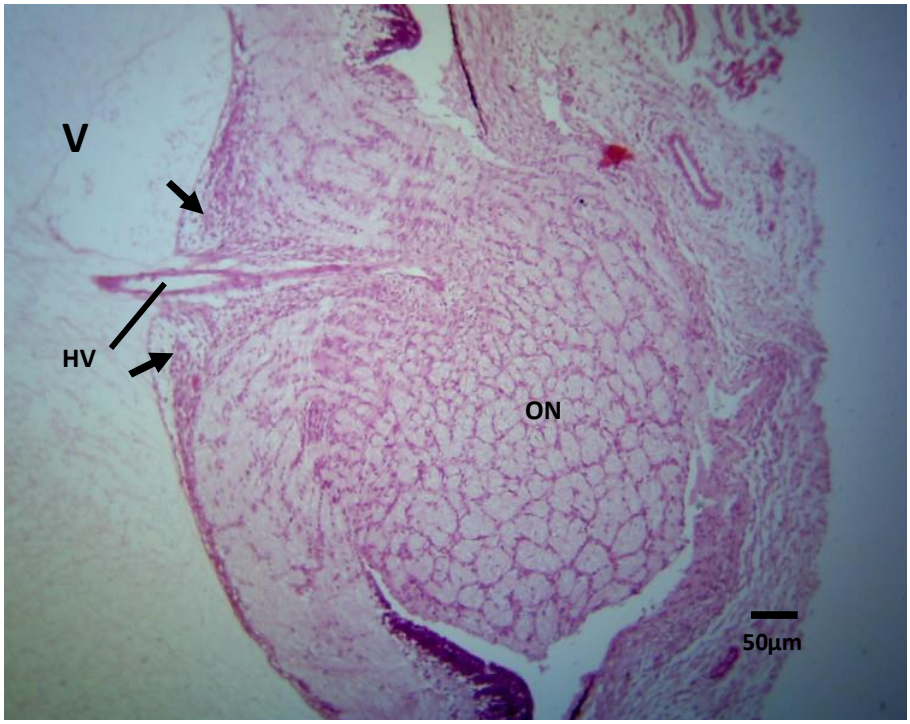

FIG.36 Section of the developing optic nerve (Day 59 fetus) showing the hyaloid vessel **HV,** optic nerve **ON,** vitreous **V** and optic stalk cells displaced inwards (black arrows).H&E.

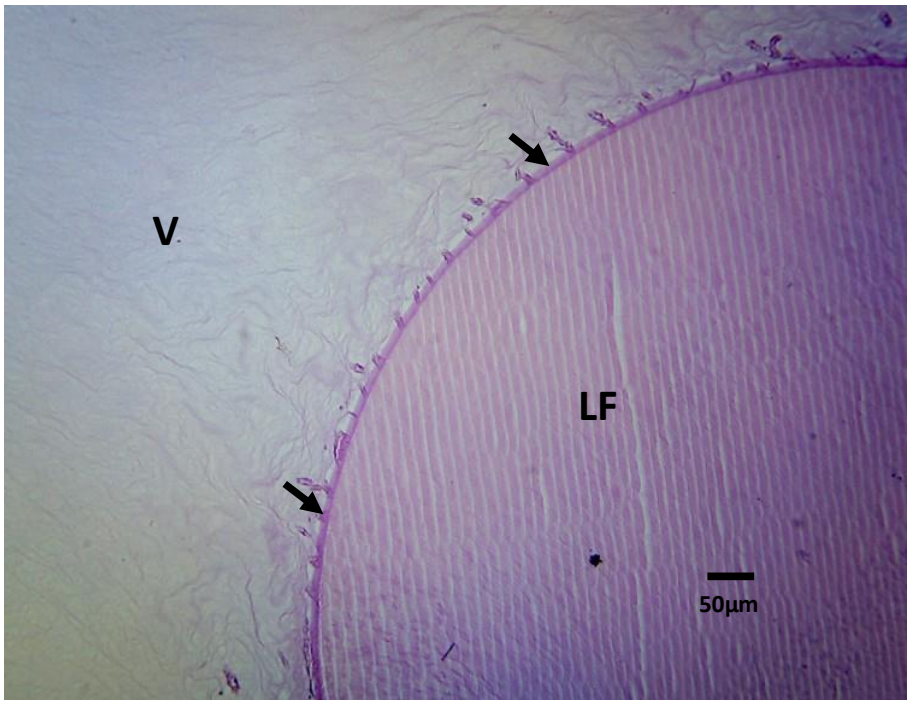

FIG.37 Section of the developing lens (Day 59 fetus) showing the lens fibres **LF,** PAS positive posterior lens capsule (black arrows); the vitreous **V** surrounds the lens posteriorly and helps keep it in place. AB-PAS. pH 2.5

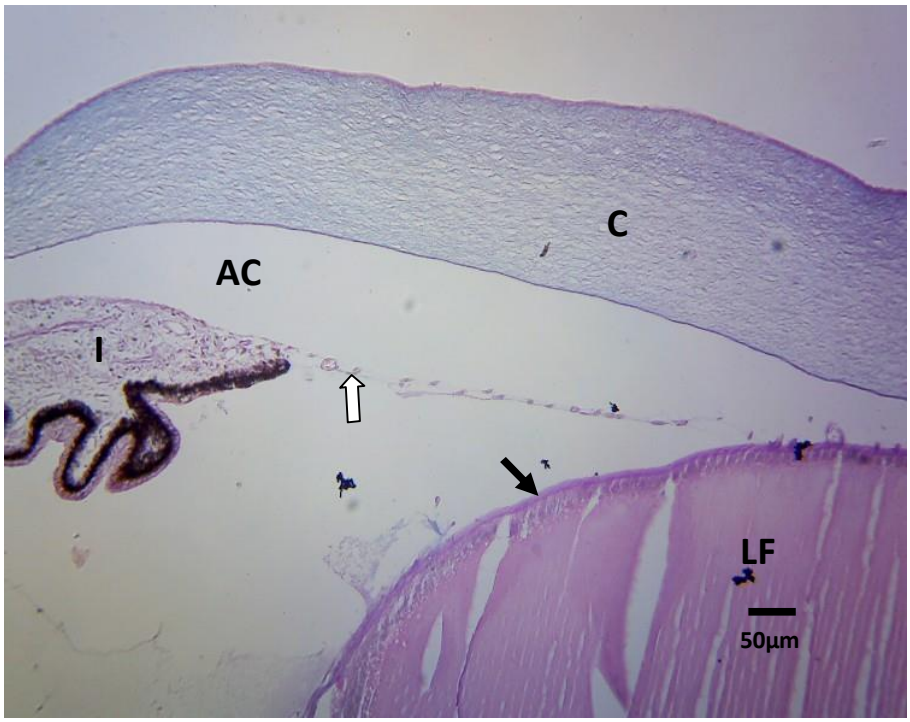

FIG.38 Section of the developing anterior eye (Day 59 fetus) showing the lens fibres **LF,** PAS positive anterior lens capsule (black arrow); anterior chamber **AC,** iris **I,** iridopupilary membrane (white arrow), cornea **C.** AB-PAS. pH 2.5

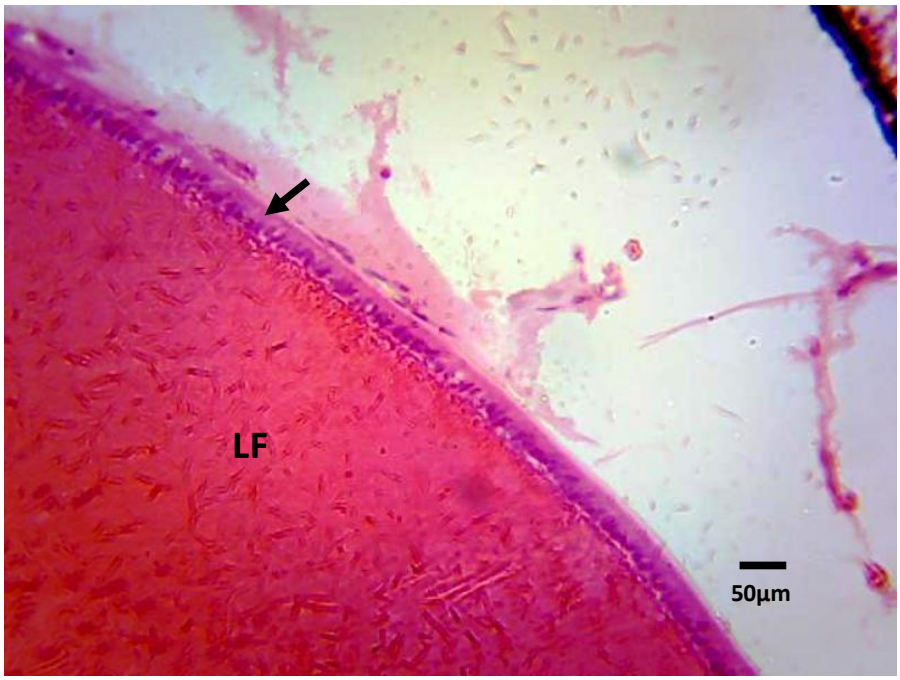

FIG.39 Section of the developing lens (Day 59 fetus) showing the lens fibres **LF** and the columnar anterior lens epithelium (black arrow). H&E

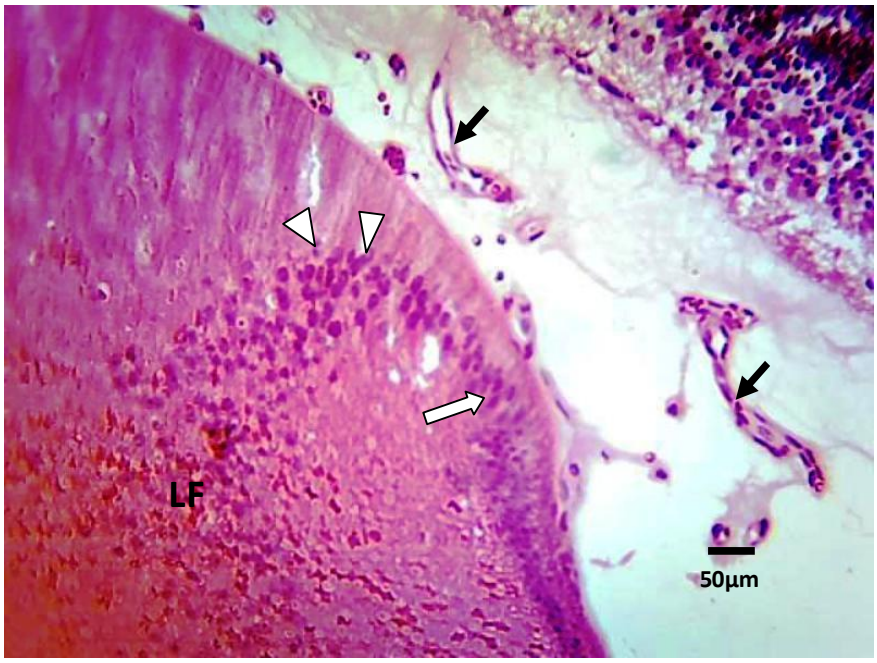

FIG.40 Section of the developing lens (Day 59 fetus) (equator) showing the lens fibres **LF**, the pseudostratified columnar anterior lens epithelium (white arrow) at the equatorial region, lens bow (arrow heads) and hyaloid vessels surrounding the lens. H&E

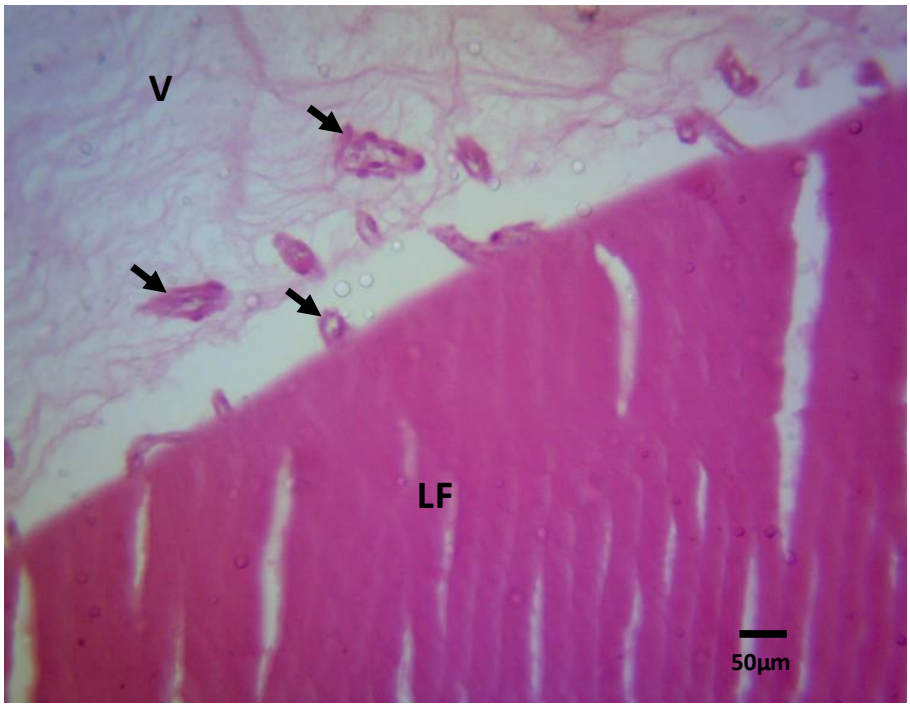

FIG.41 Section of the developing lens (Day 59 fetus) showing the hyaloid vessels (arrows) on the posterior surface of the lens, forming the tunica vasculosa lentis, lens fibres **LF** and the vitreous **V.** H&E

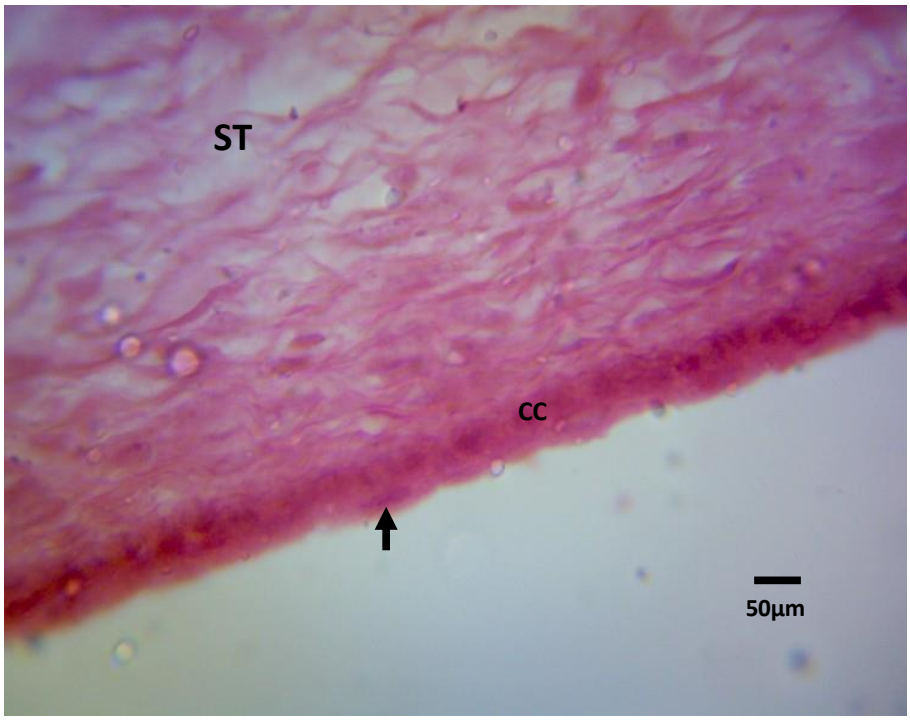

FIG.42 Section of a day 75 fetal cornea showing the basal cuboidal cells **CC** and the superficial flattened cells (arrow) making up the anterior corneal epithelium; corneal stroma **ST.**H&E.

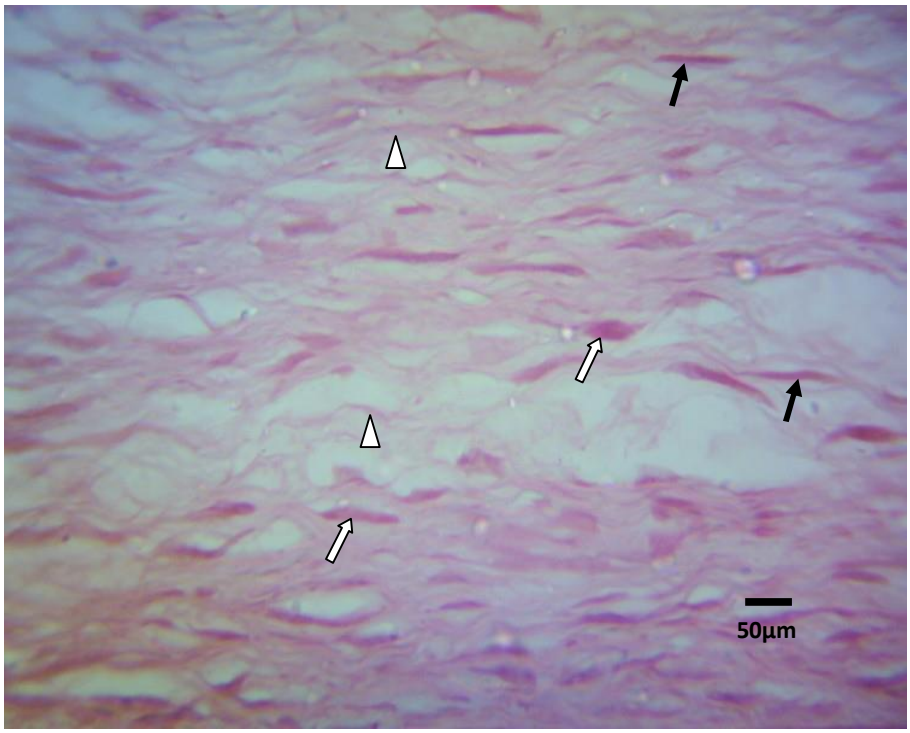

FIG.43 Section of day 75 fetal corneal stroma, containing fibroblasts (white arrows), fibrocytes (black arrows) and collagen fibres (arrow heads).H&E

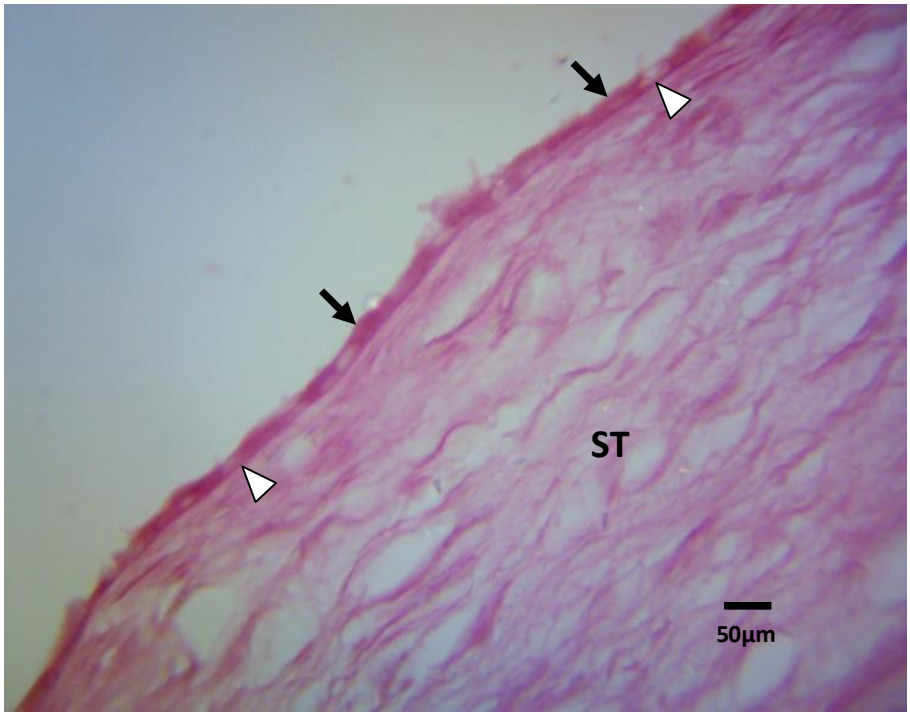

FIG.44 Section of a posterior cornea (Day 75 fetus) showing the corneal stroma **ST,** the low cuboidal cells making up the corneal endothelium (black arrow) and the thin Descemet's membrane (arrow heads).H&E

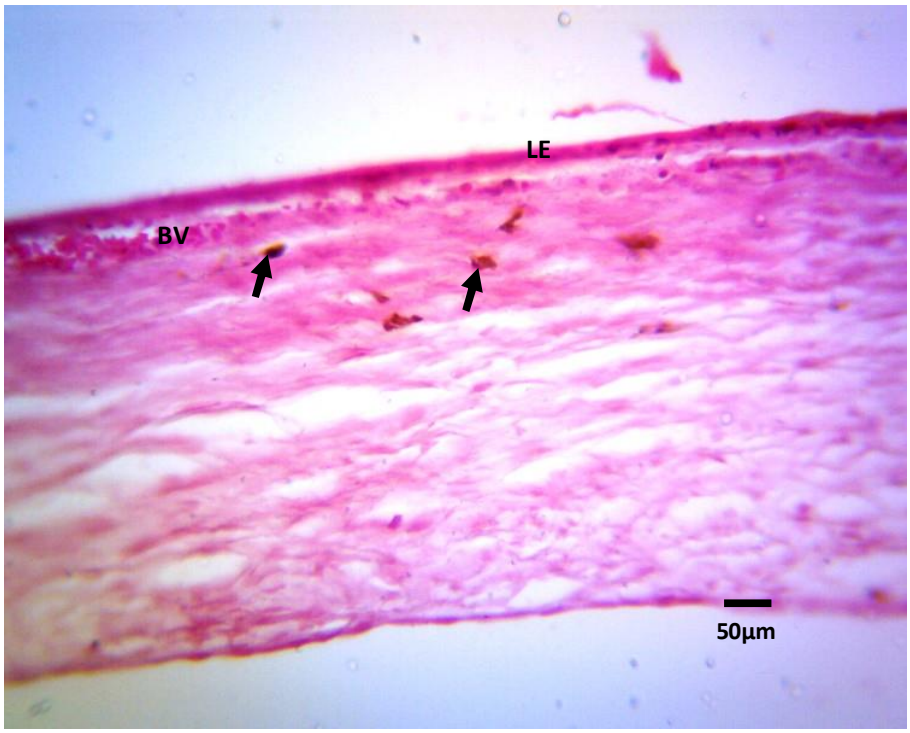

FIG.45 Section of the limbus (Day 75 fetus) showing melanocytes (black arrow), blood vessel **BV** and the anterior epithelium **LE** at the limbal region of the cornea. Note the irregularly arranged collagen fibres. H&E

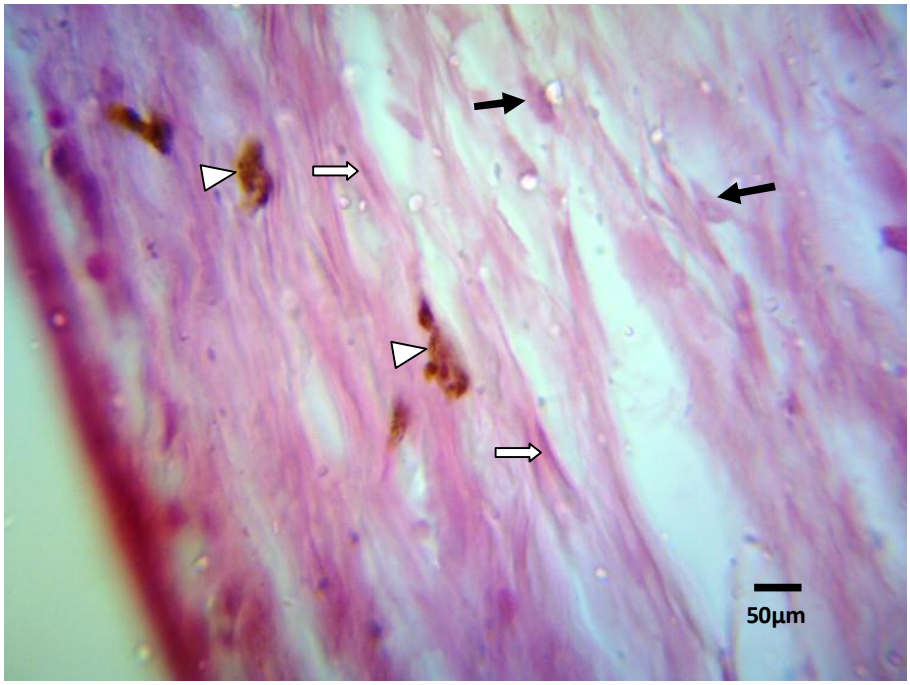

FIG.46 Section of the limbus (Day 75 fetus) showing fibroblasts (black arrows), fibrocytes (white arrows), melanocytes (arrow heads). H&E.

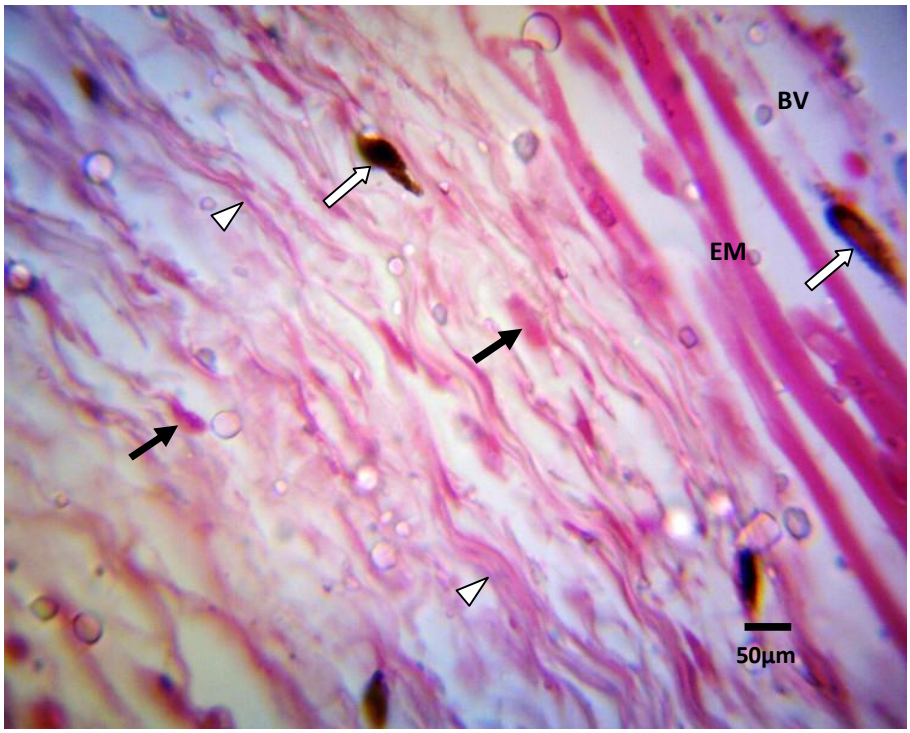

FIG.47 Section of the sclera (Day 75 fetus) showing spindle shaped melanocytes (white arrows), fibroblasts (black arrows), collagen fibres (arrow heads), extraocular muscle fibres **EM,** blood vessel **BV.** H&E

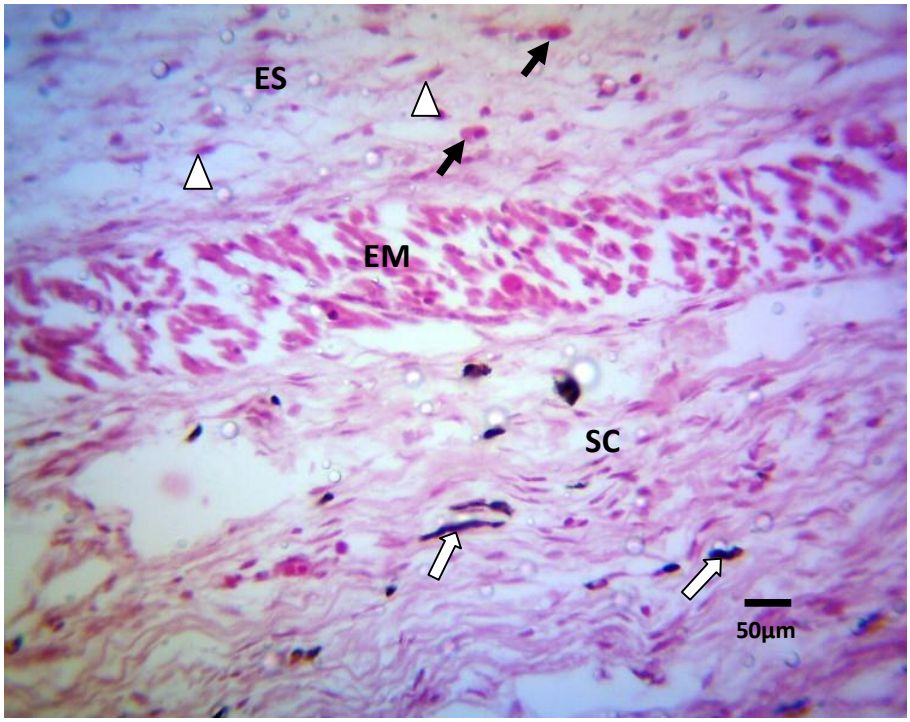

FIG.48 Section of the sclera (Day 75 fetus) showing melanocytes (white arrows) within the sclera proper **SC**, extraocular muscle **EM,** episclera **ES** made up of loose connective tissue containing few differentiating mesenchymal cells (black arrow) and fibroblasts (arrow heads). H&E.

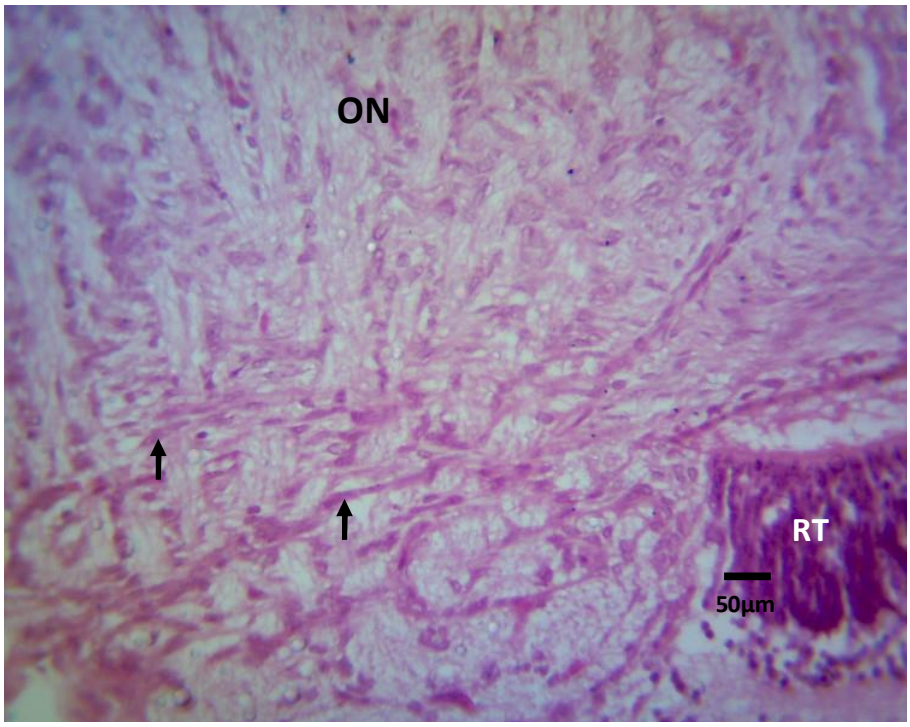

FIG.49 Section of day 75 fetal optic nerve head showing the retina **RT**, optic nerve **ON** and the developing lamina cribrosa where fibroblasts (black arrow) are crossing the optic nerve meridionally. H&E.

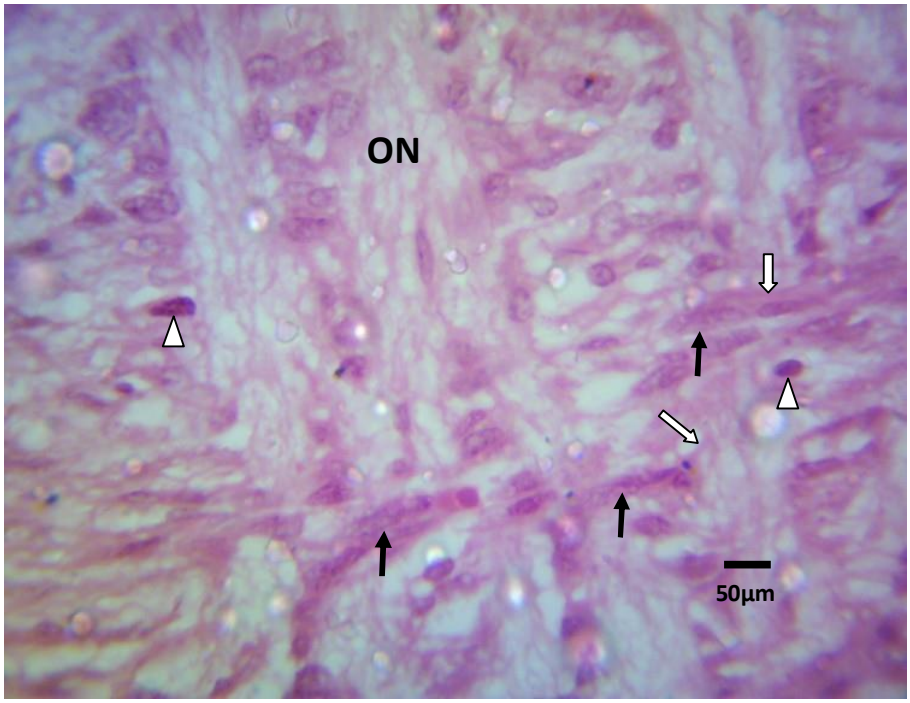

FIG.50 Section of the developing lamina cribrosa (Day 75 fetus) showing the fibroblasts (arrows) crossing the optic nerve fibres **ON** meridionaly; collagen fibres laid down by the fibroblasts (white arrows) and melanocytes (arrow heads).H&E.

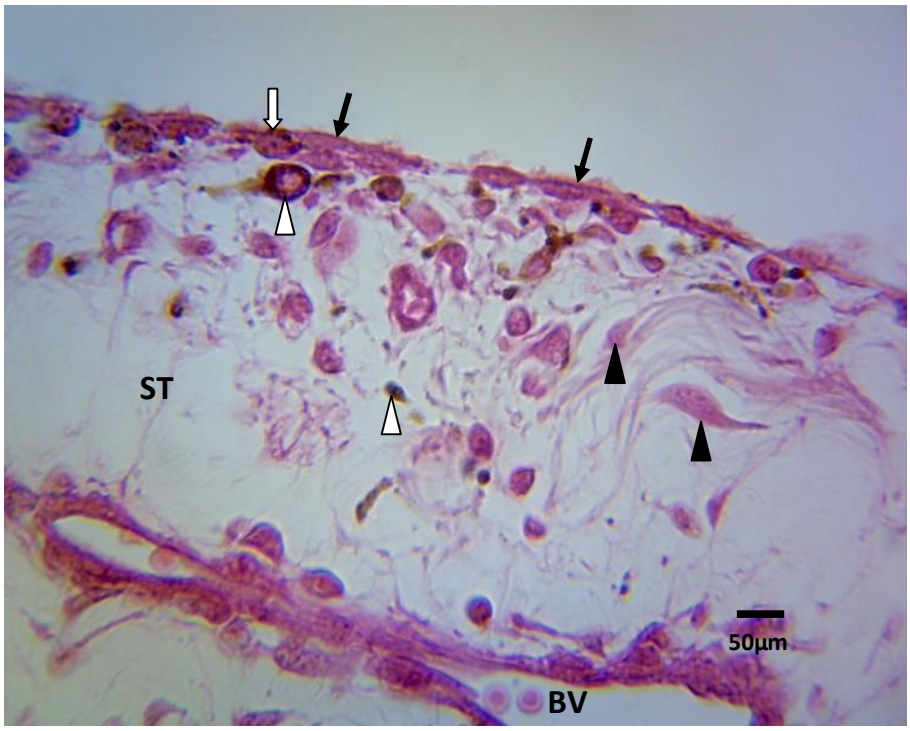

FIG.51 Section of the developing iris (Day 75 fetus), showing fibroblasts (black arrows) and melanocytes (white arrow) making up the anterior epithelium; the loose connective tissue stroma **ST** containing fibroblasts (black arrow heads), melanocytes (white arrow heads) and blood vessel **BV.**H&E.

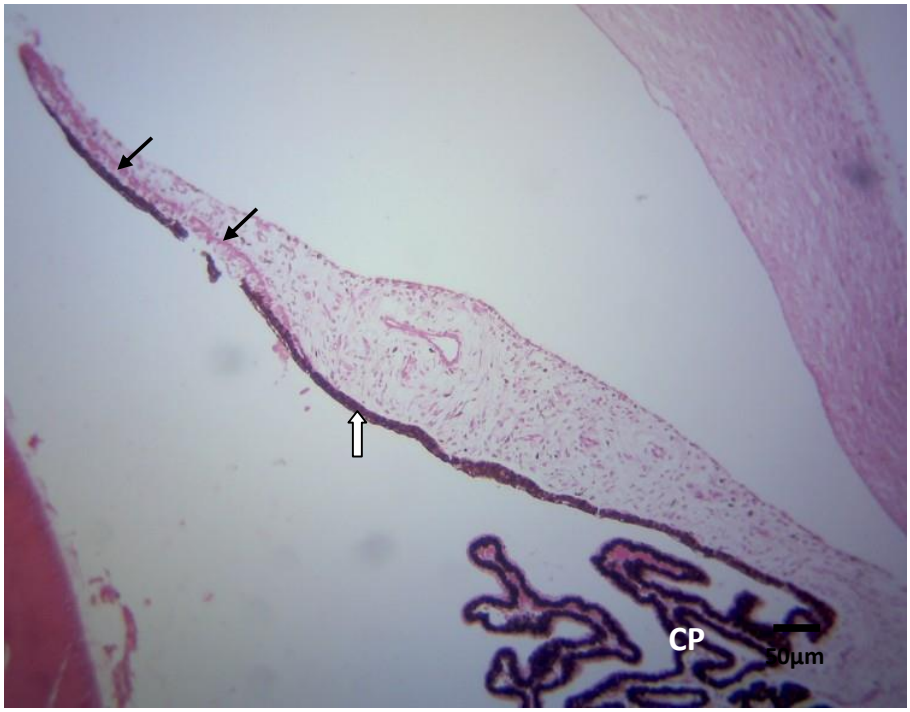

FIG.52 Section of the developing iris (Day 75 fetus) showing the constrictor pupillary muscle (arrow), posterior epithelium of the iris (white arrow) and ciliary processes **CP.** H&E.

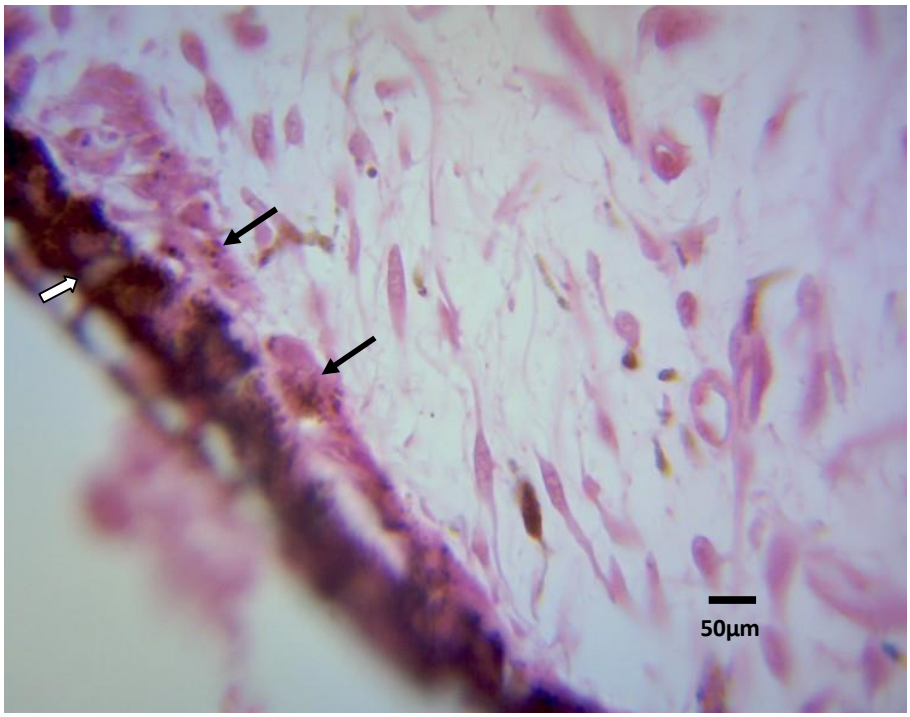

FIG.53 Section of the developing iris (Day 75 fetus) showing the constrictor pupillary muscle cells (arrows), posterior epithelium of the iris (white arrow)**.** Note that the muscle cells contain pigments. H&E

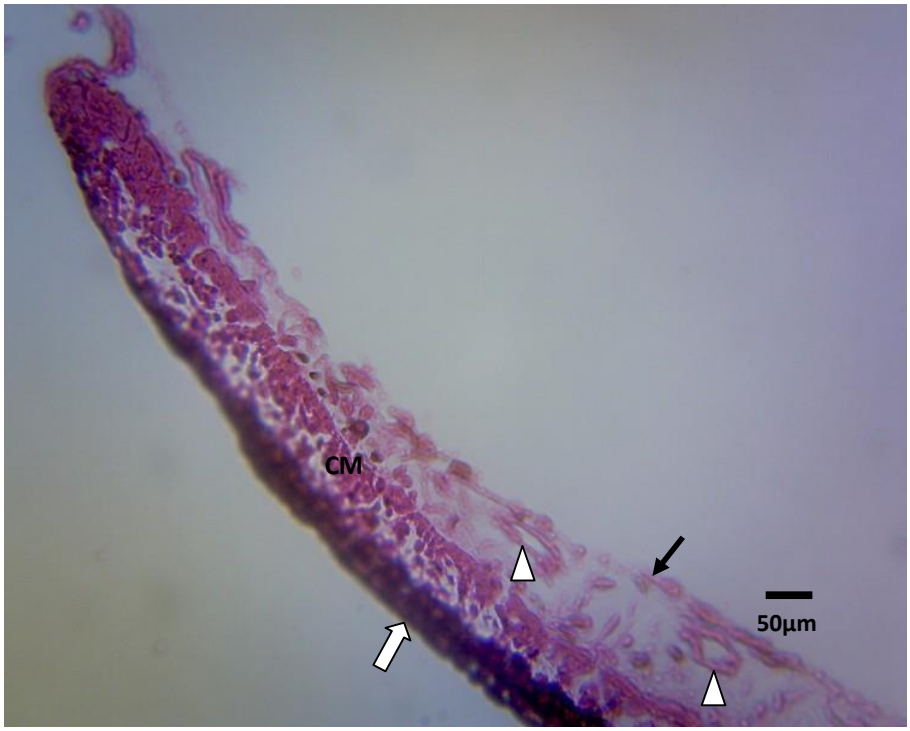

FIG.54 Section of the developing iris (Day 75 fetus) showing the anterior epithelium (black arrow), blood vessels (arrow heads), constrictor pupillae muscle **CM** and the double layered pigmented posterior epithelium (white arrow).H&E.

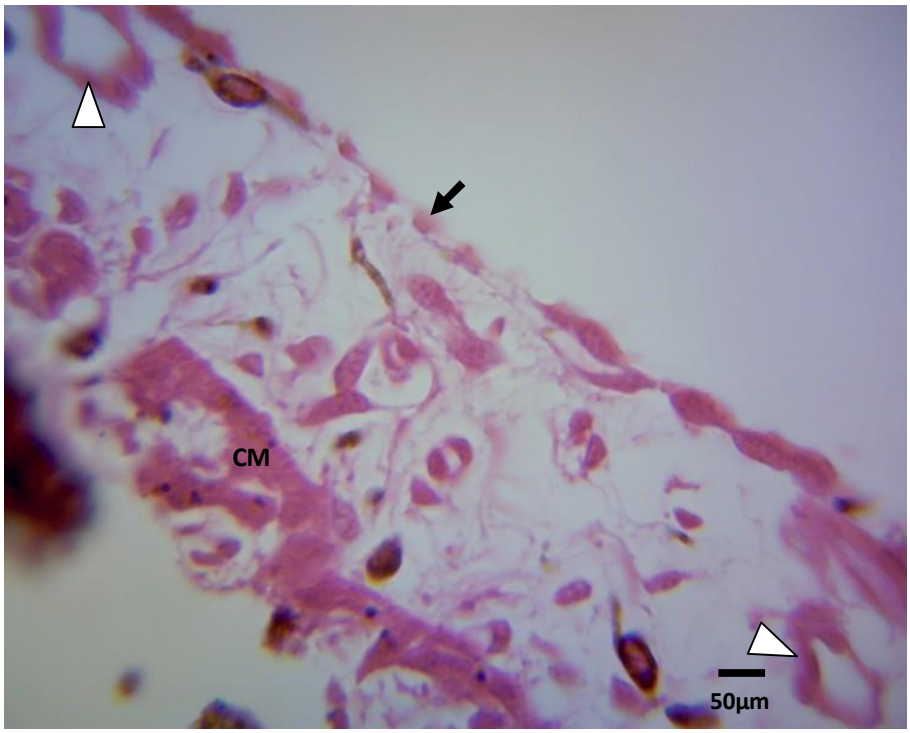

FIG.55 Section of the developing iris (Day 75 fetus) showing the anterior epithelium (arrow), blood vessels (arrow heads) and constrictor pupilae muscle **CM.**H&E.

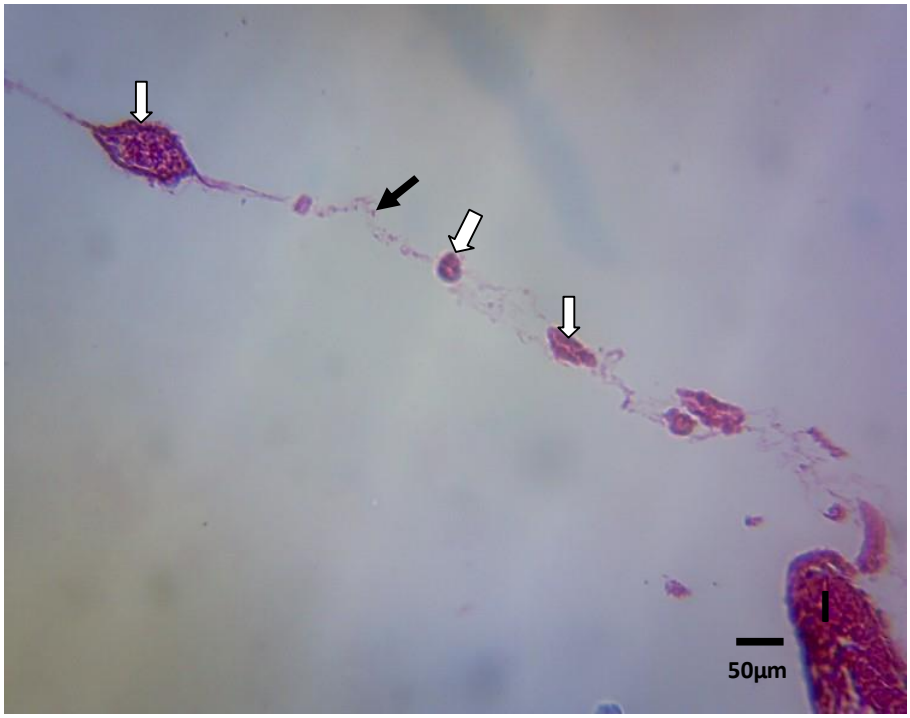

FIG.56 Section of the anterior globe (Day 75 fetus) showing the tip of the iris **I,** and the blood vessels (white arrows) of the fibrous iridopupilary membrane (black arrow). H&E.

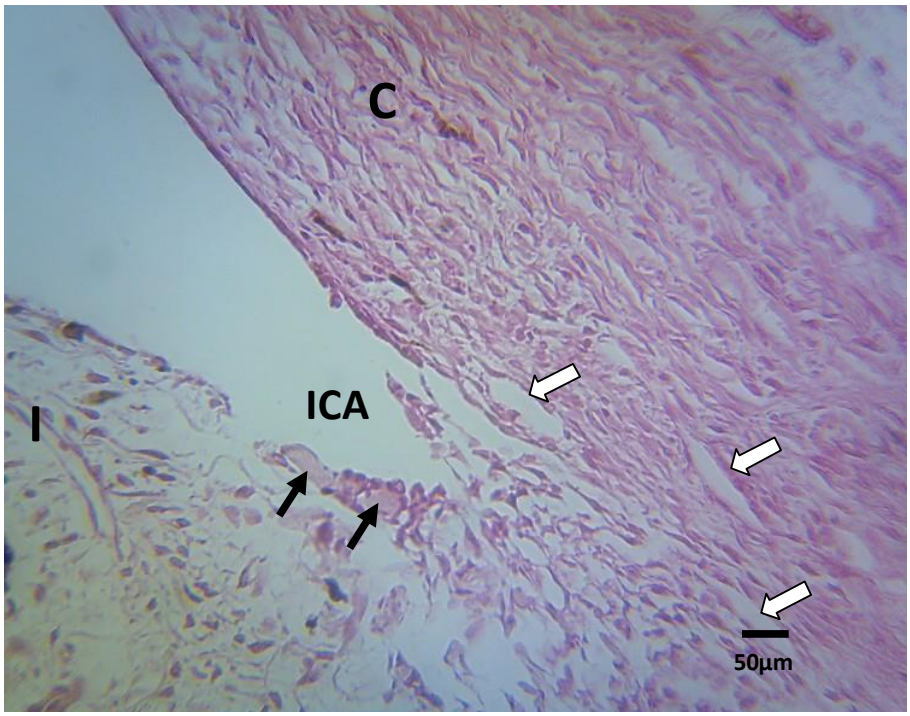

FIG.57 Section of the anterior globe (Day 75 fetus) showing the iris **I,** the cornea **C**, the iridocorneal angle **ICA,** aqueous angular plexus (white arrows) and the pectinate ligament (black arrows) at the base of the iris. H&E.

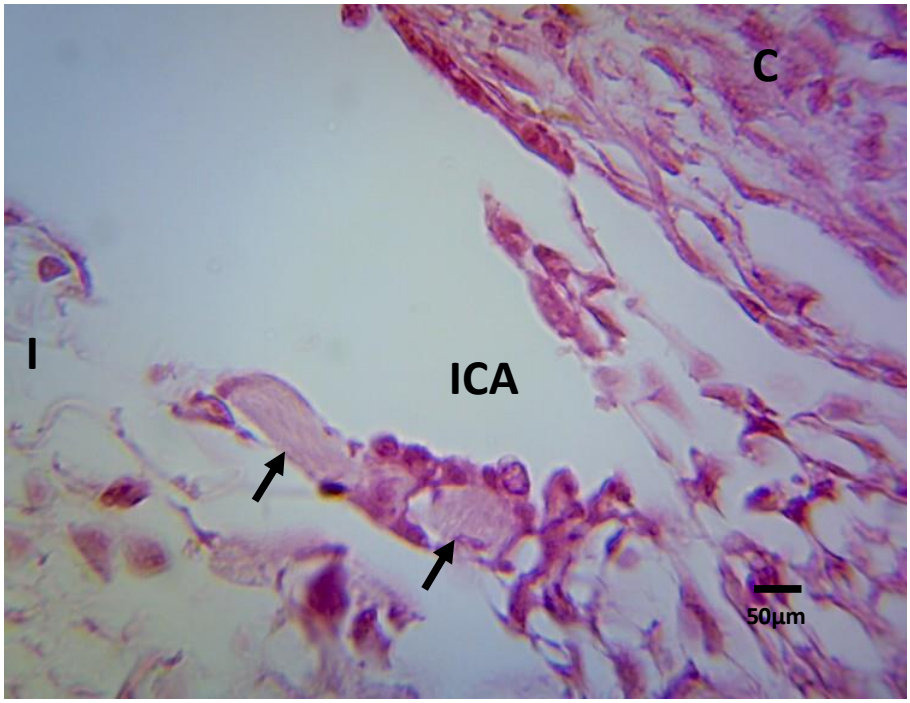

FIG.58 Section of the anterior globe (Day 75 fetus) showing the base of the iris **I,** the peripheral cornea **C**, the iridocorneal angle **ICA** and the pectinate ligament (black arrows) at the base of the iris. H&E.

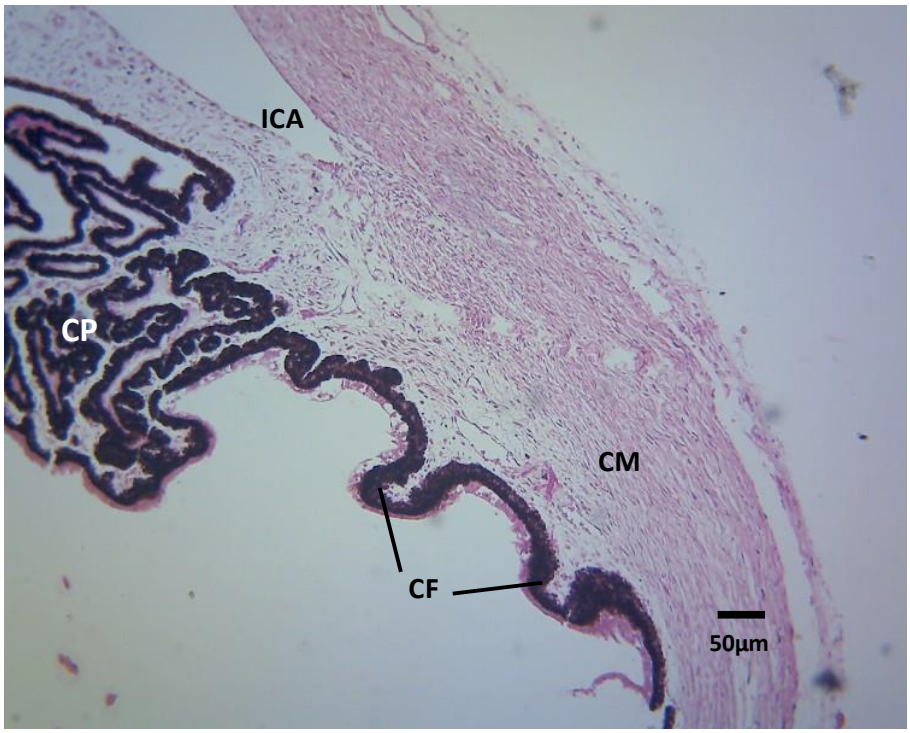

FIG.59 Section of the developing ciliary body (Day 75 fetus) showing the ciliary processes **CP** at the base of the iris**,** ciliary folds **CF** caudal to them, iridocorneal angle **ICA** and the ciliary body musculature **CM.**H&E.

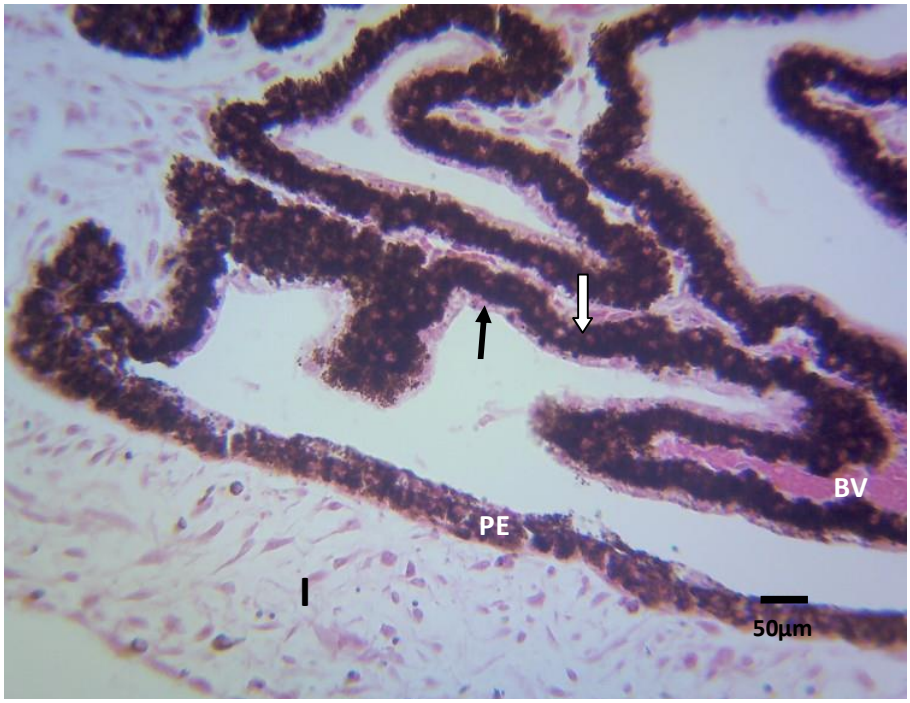

FIG.60 Section of the developing ciliary processes and iris (Day 75 fetus) showing ciliary body epithelium composed of the inner non pigmented cells (black arrow) and the outer pigmented cells (white arrow), blood vessel **BV** within the core of the processes, iris **I,** posterior iris epithelium **PE**. Note that the non pigmented cells are cuboidal over the processes; also the posterior iris epithelium is continuous with the ciliary epithelium even when it is composed of two layers of pigmented cells.H&E

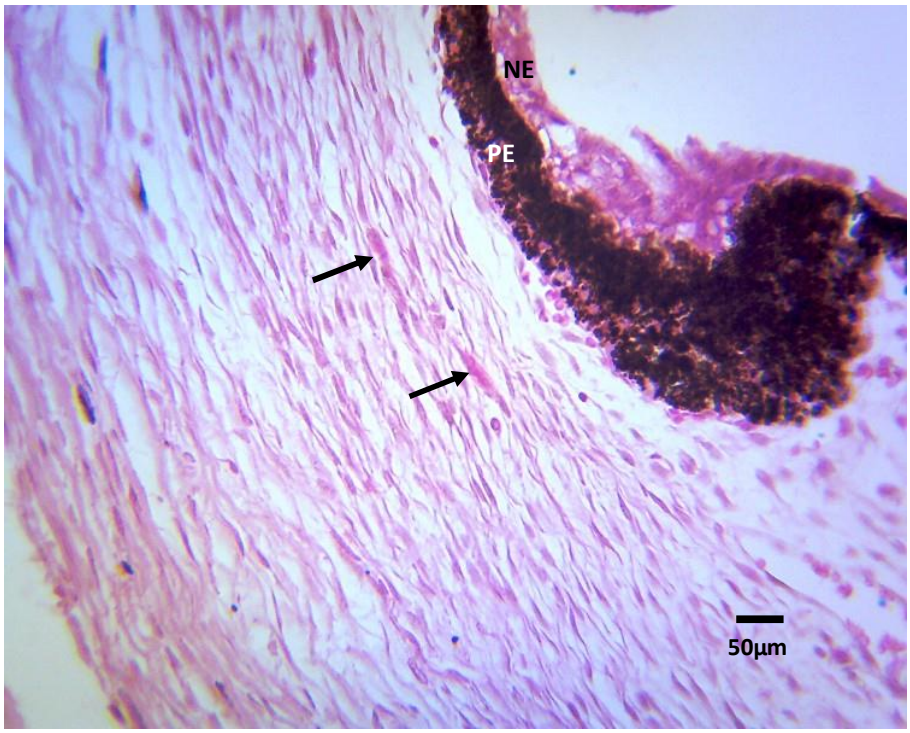

FIG.61 Section of the developing ciliary body (Day 75 fetus) (posterior region) showing the outer pigmented cell layer and the inner non pigmented cell layer of the ciliary epithelium; differentiating ciliary body smooth muscle cells (black arrows). H&E

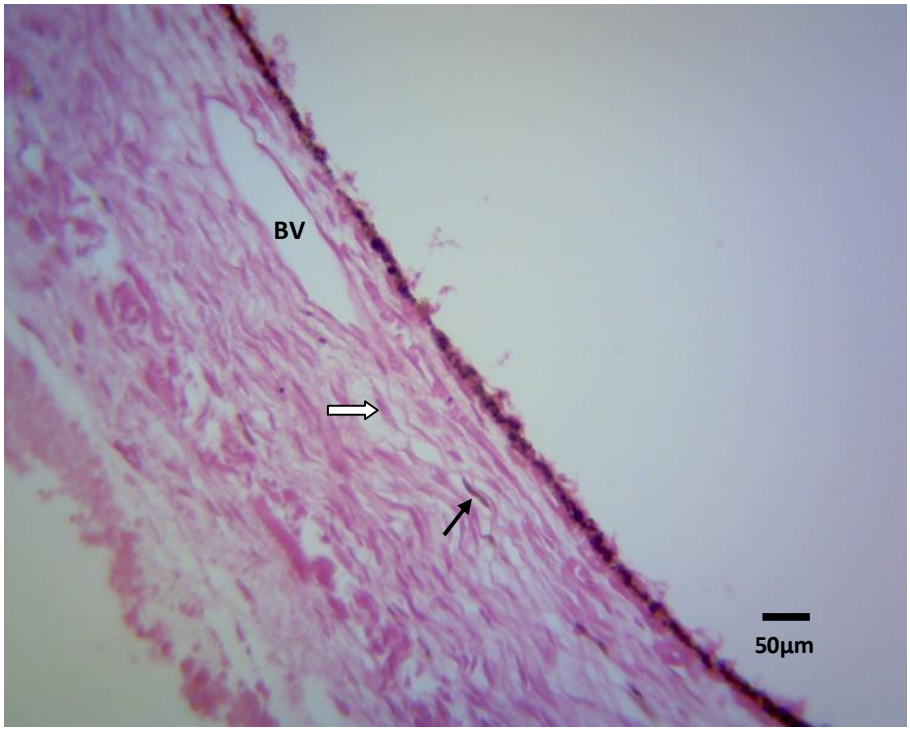

FIG.62 Section of the developing choroid (Day 75 fetus) (non tapetal region) showing large choroidal blood vessels **BV**, spindle shaped melanocytes (black arrow) and fibroblast (white arrow).H&E.

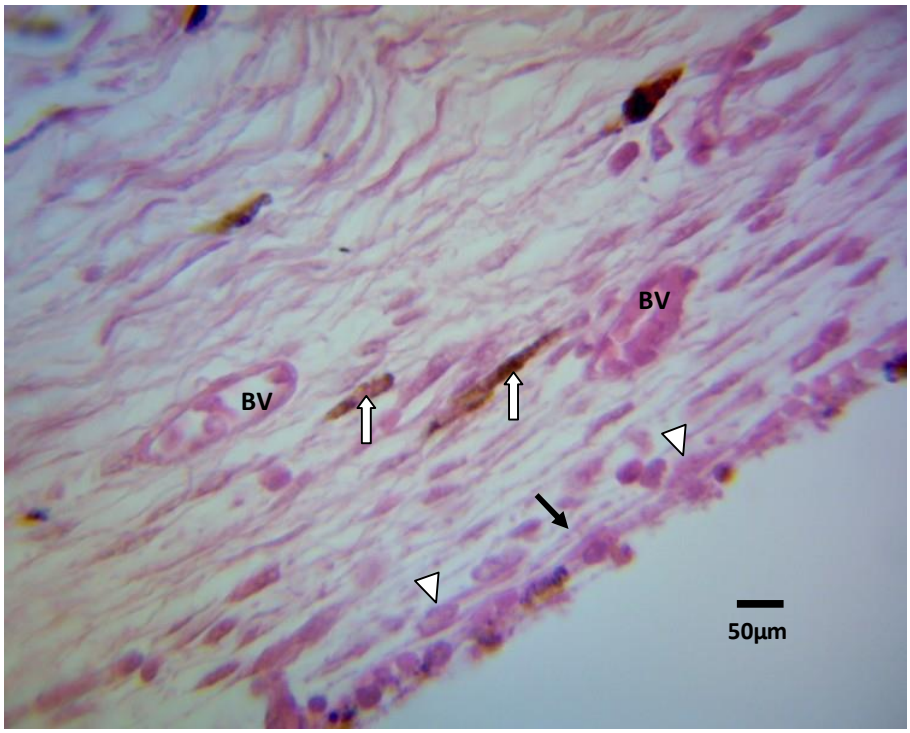

FIG.63 Section of the developing choroid (Day 75 fetus) (tapetal region) showing fibrocyte (black arrow) and fibroblasts (arrow heads) stacking up outside the choriocapilaris, blood vessels **BV**, spindle shaped melanocytes (white arrows).H&E.

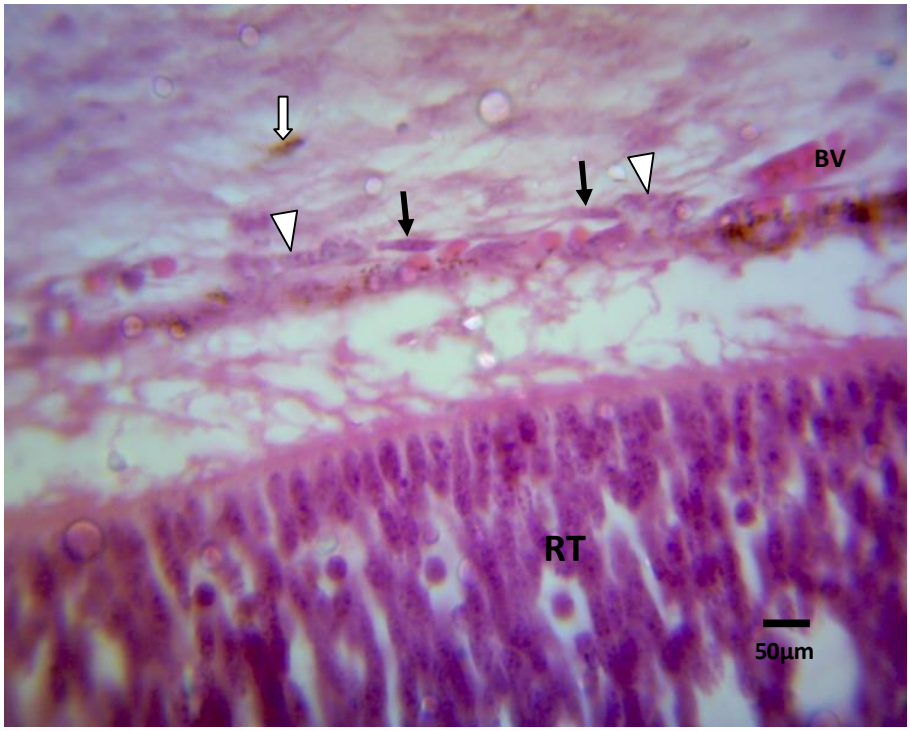

FIG.64 Section of the developing posterior globe (Day 75 fetus) (tapetal region) showing fibrocytes (black arrows) and fibroblasts (arrow heads) stacking up outside the choriocapilaris; blood vessel **BV**, melanocyte (white arrow) and retina **RT**. Note that the space between the retina and the choroid is artifactual.H&E

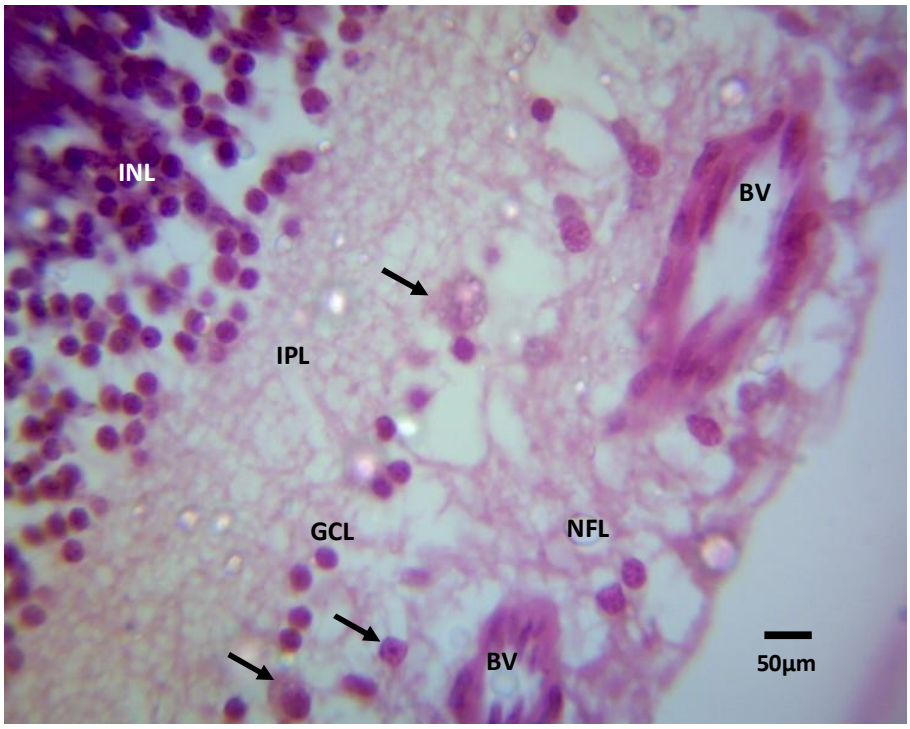

FIG.65 Section of the inner region of the developing retina (Day 75 fetus) showing nerve fibre layer **NFL**, blood vessels **BV,** within the NFL**,** ganglion cell layer **GCL** containing differentiating ganglion cells (black arrows), inner neuroblastic layer **INL** containing differentiating spherical cells, and the inner plexiform layer **IPL** separating the GCL and the INL.H&E.

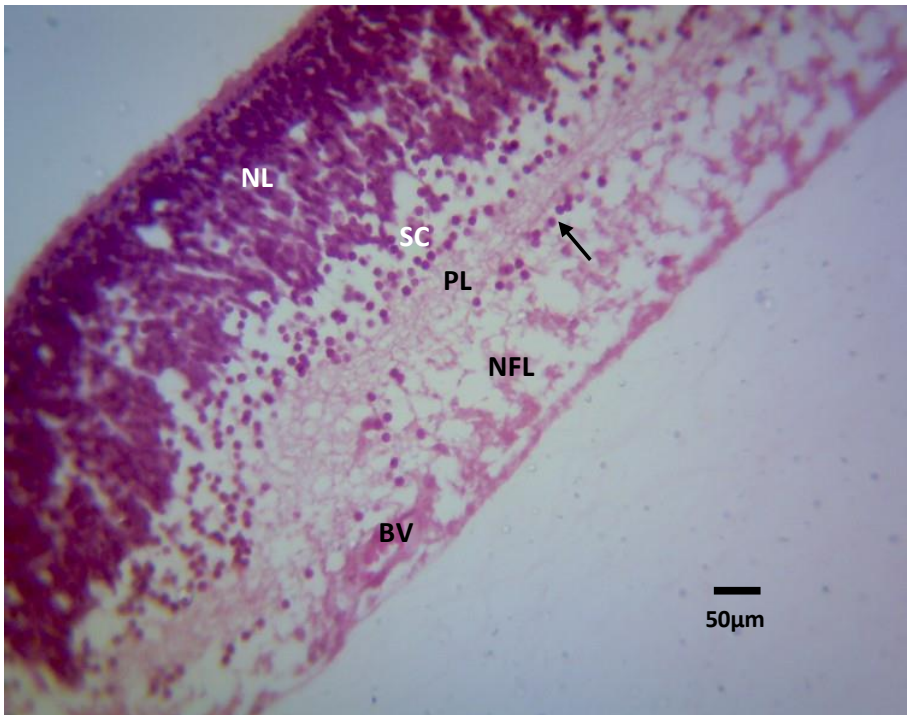

FIG.66 Section of the peripheral retina (Day 75 fetus) showing the ganglion cell layer made up of ganglion cells (arrow), plexiform layer **PL** between ganglion cell layer and neuroblastic layer, differentiating spherical cells **SC** moving away from the neuroblastic layer **NL,** blood vessel **BV** and nerve fibre layer **NFL.**H&E

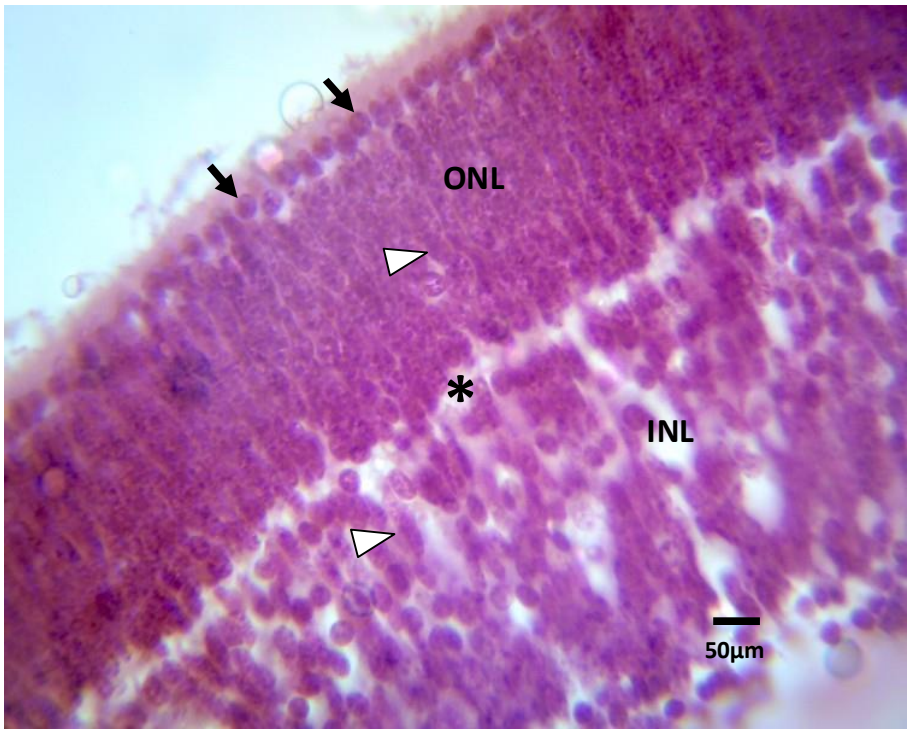

FIG.67 Section of the central retina (Day 75 fetus) showing the newly formed inner nuclear layer **INL** and outer nuclear layer **ONL;** spherical cells on the outer region of the ONL (black arrows), columnar cells in both layers (arrow heads) and plexiform layer (asterix). Note that the INL had separated from the ONL at this stage with a plexiform layer between them. H&E

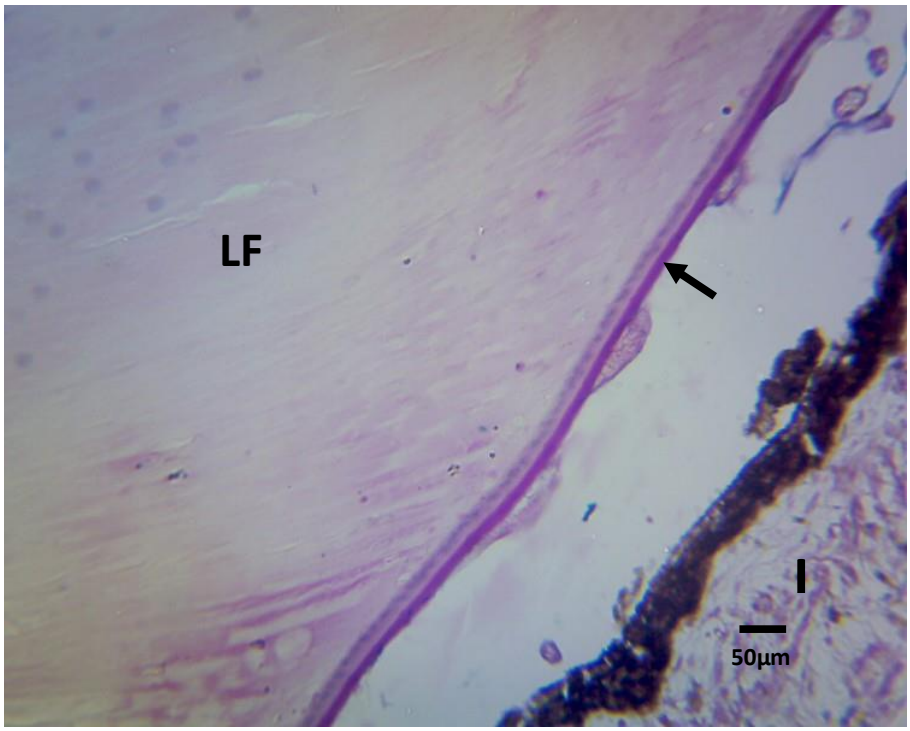

FIG.68 Section of the developing lens and iris (Day 75 fetus) showing the iris stroma **I,** lens fibres **LF** and the PAS positive lens capsule (arrow).AB-PAS. pH 2.5

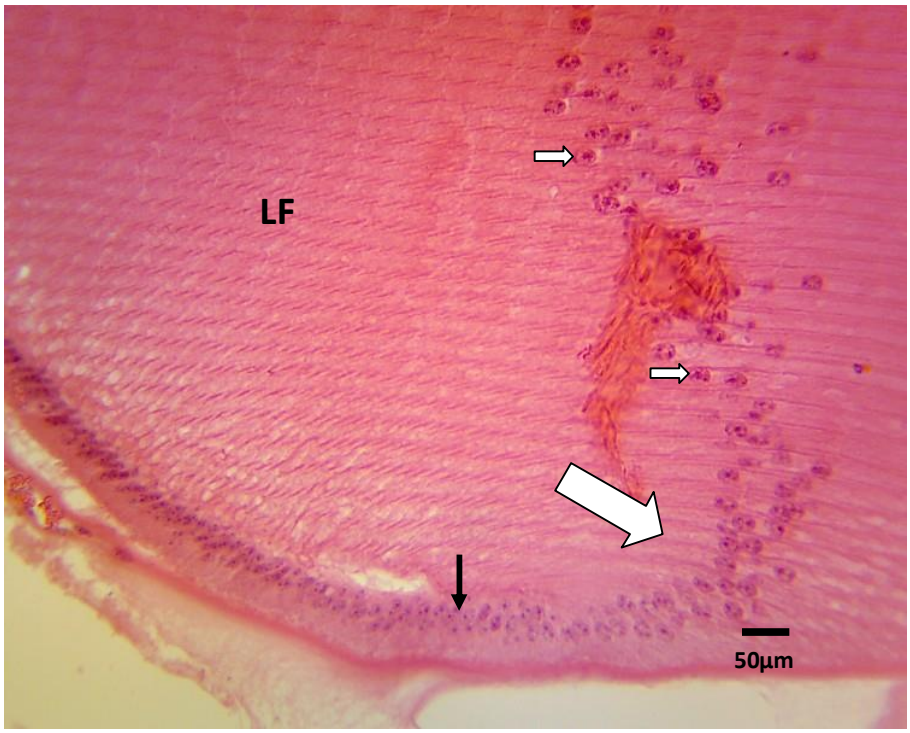

FIG.69 Section of the developing lens, at the equator, (Day 75 fetus) showing the lens fibres **LF** and their nuclei (white arrows), lens bow (large arrow) and the equatorial lens epithelium (black arrow).H&E

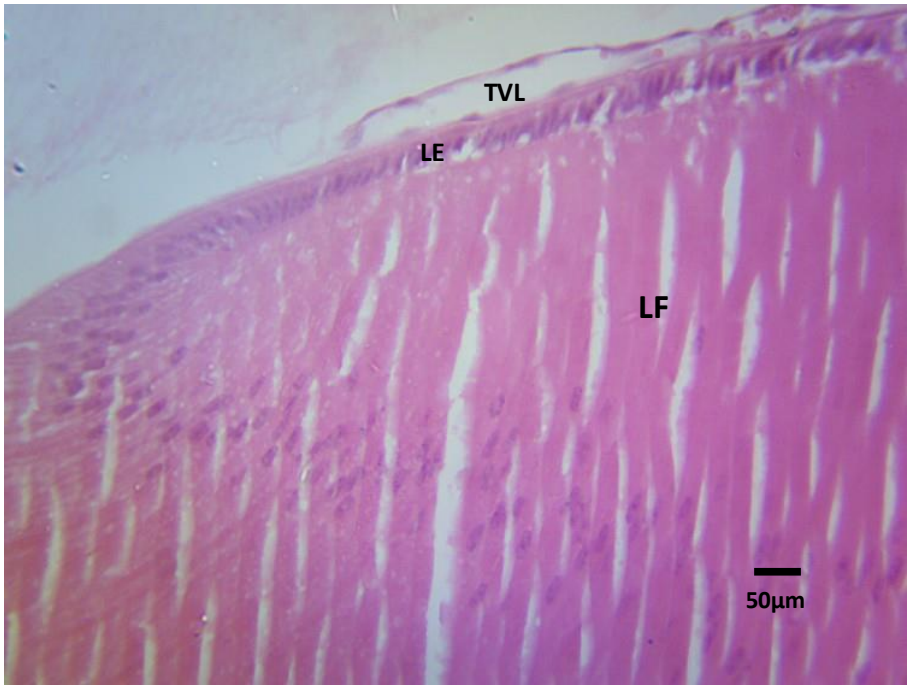

FIG.70 Section of the developing lens (anterior region) (Day 75 fetus) showing the lens fibres **LF** lens epithelium **LE** and a vessel of the tunica vasculosa lentis **TVL**.H&E

FIG.71 Section of developing lens (posterior region) (Day 75 fetus) showing the lens fibres **LF** lens and vessels of the tunica vasculosa lentis (arrows).H&E

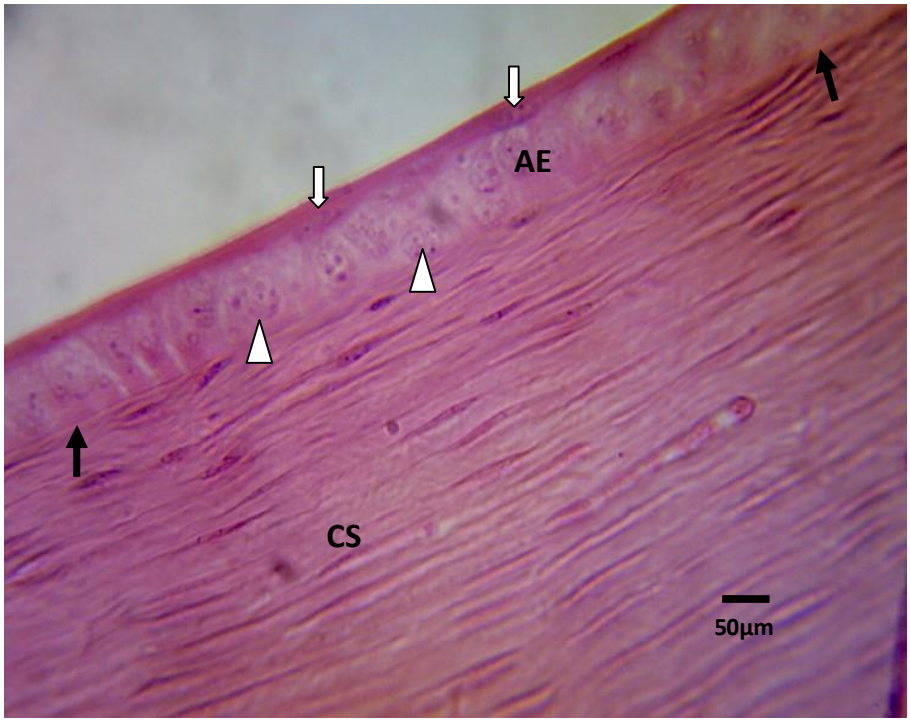

FIG.72 Section of the developing cornea (Day 105 fetus) showing the corneal stroma **CS,** corneal anterior epithelium **AE** composed of the basal cuboidal cells (arrow heads) and superficial spindle shaped cells (white arrow), basement membrane of the anterior epithelium (black arrow).H&E

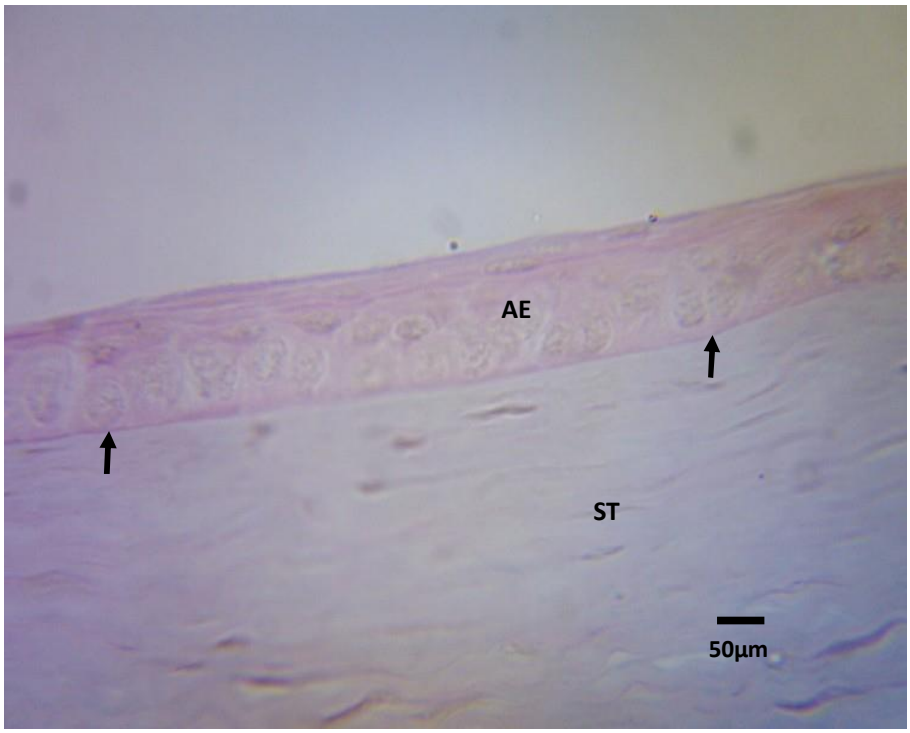

FIG.73 Section of the developing cornea (Day 105 fetus) showing the PAS positive basement membrane of the anterior epithelium - Bowman's membrane (black arrows); corneal anterior epithelium **AE** and the corneal stroma **ST**. Note that the epithelium is PAS positive while the stroma is alcian blue positive AB-PAS. pH 2.5

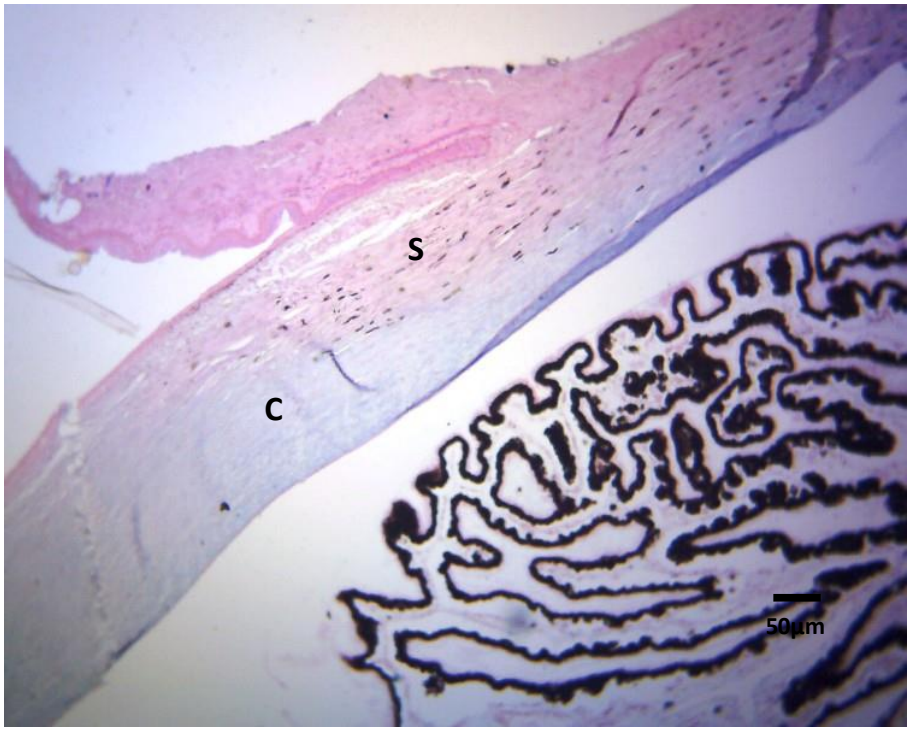

FIG.74 Section of the corneoscleral region (limbus) (Day 105 fetus) showing the PAS positive scleral region **S** and the alcian blue positive corneal region **C**. X100 AB-PAS. pH 2.5

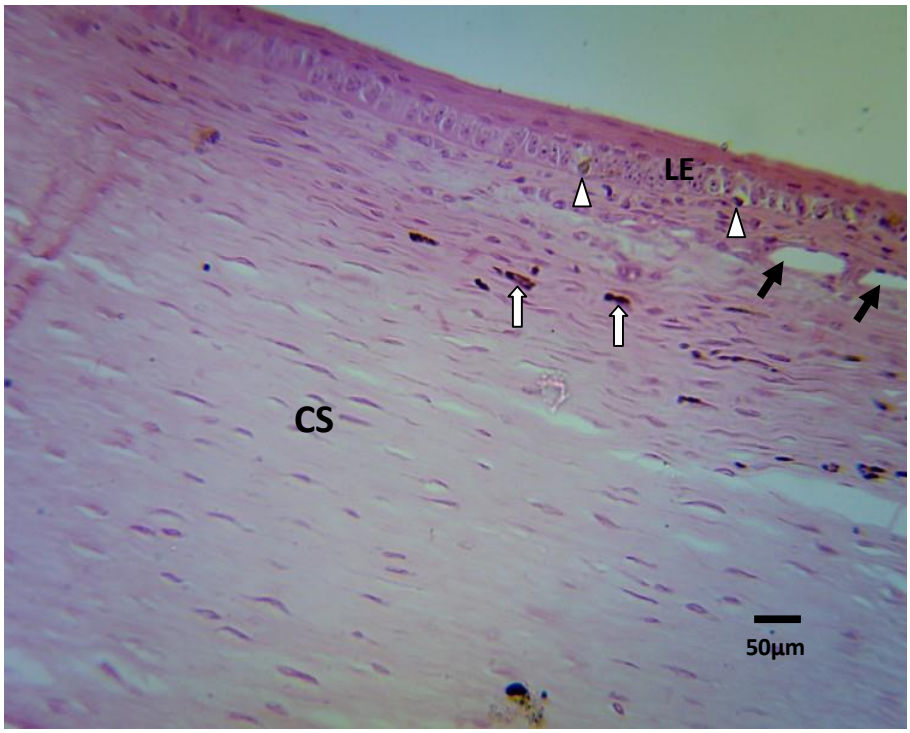

FIG.75 Section of the limbus (Day 105 fetus) showing the scleral stroma **CS,** thickened epithelium of the limbus **LE,** with some cells containing pigments (arrow heads)**;** melanocytes (white arrows) and blood vessels (black arrows). Note the irregular arrangement of the stroma as it goes into the scleral stroma (upper right). H&E

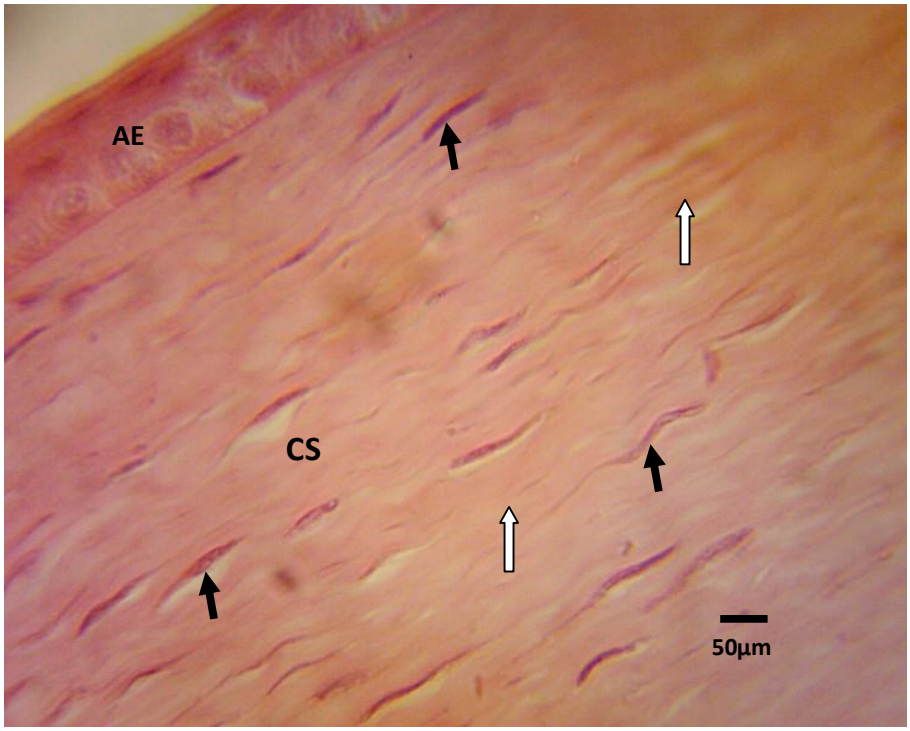

FIG.76 Section of the developing cornea (Day 105 fetus) showing the scleral stroma **CS** made up of fibroblasts (black arrows) sandwiched in between regularly arranged collagen fibres (white arrows); corneal anterior epithelium **AE.** H&E.

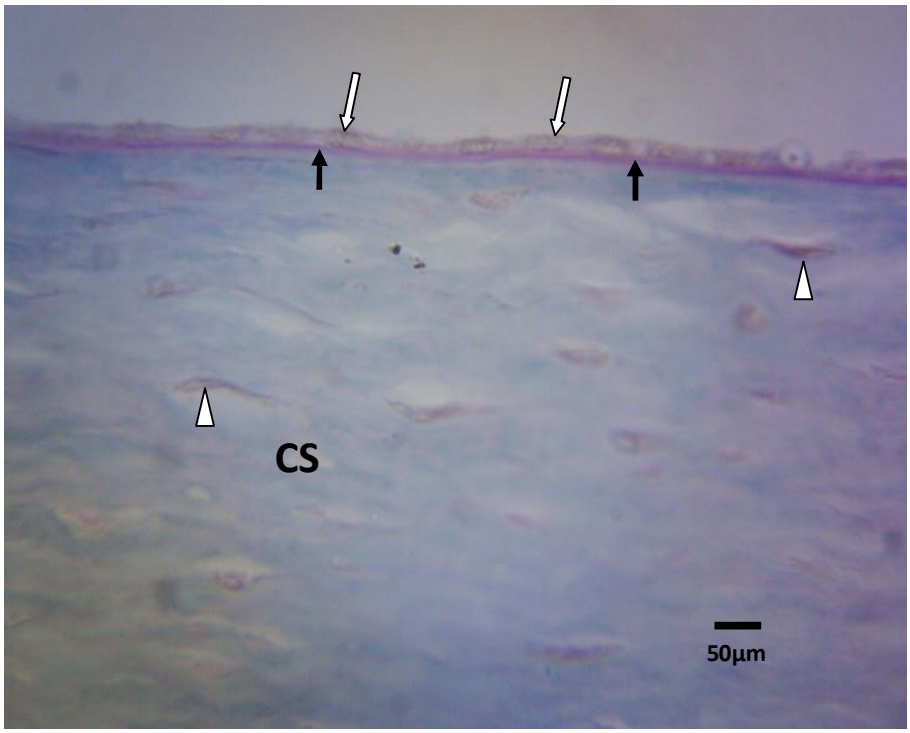

FIG.77 Section of the developing cornea (posterior region) (D 105 fetus) showing the AB positive corneal stroma **CS** made up of fibroblasts (arrow heads) sandwiched in between regularly arranged collagen fibres, cuboidal cells of the corneal posterior epithelium- the endothelium (white arrows) sitting on a PAS positive basement membrane (Descemet's membrane)**.** AB-PAS. pH 2.5

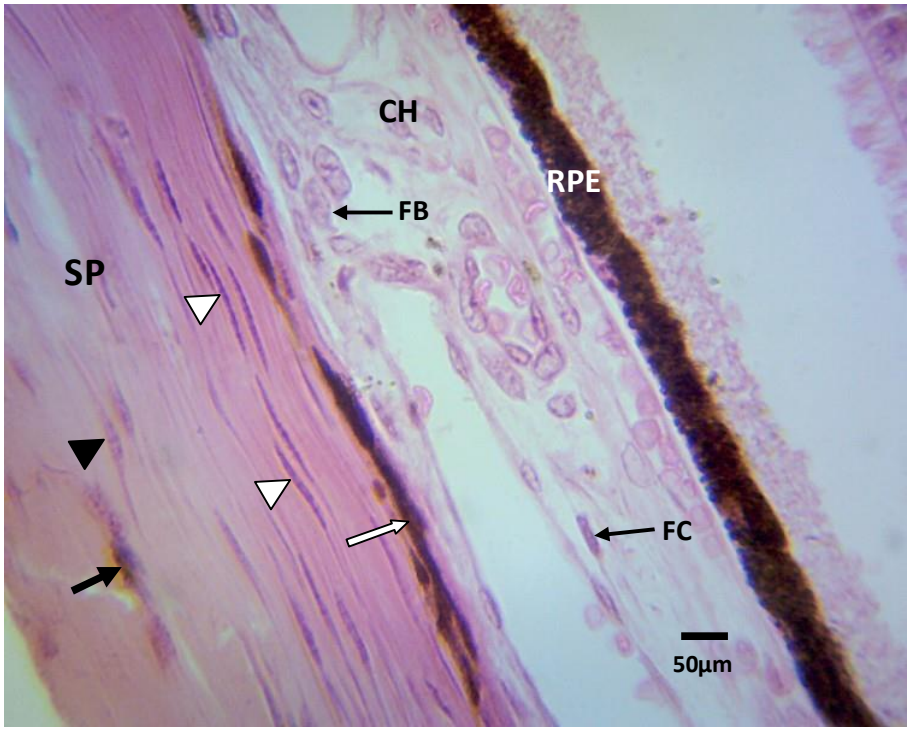

FIG.78 Section of the developing eye (D 105 fetus)showing the lamina fusca (white arrow) of the sclera, the sclera proper **SP** containing fibrocytes (arrow heads), fibroblast (black arrow head) and melanocytes (black arrow); choroid **CH** containing fibrocytes **FC** and fibroblasts **FB;** retinal pigment epithelium **RPE.**H&E

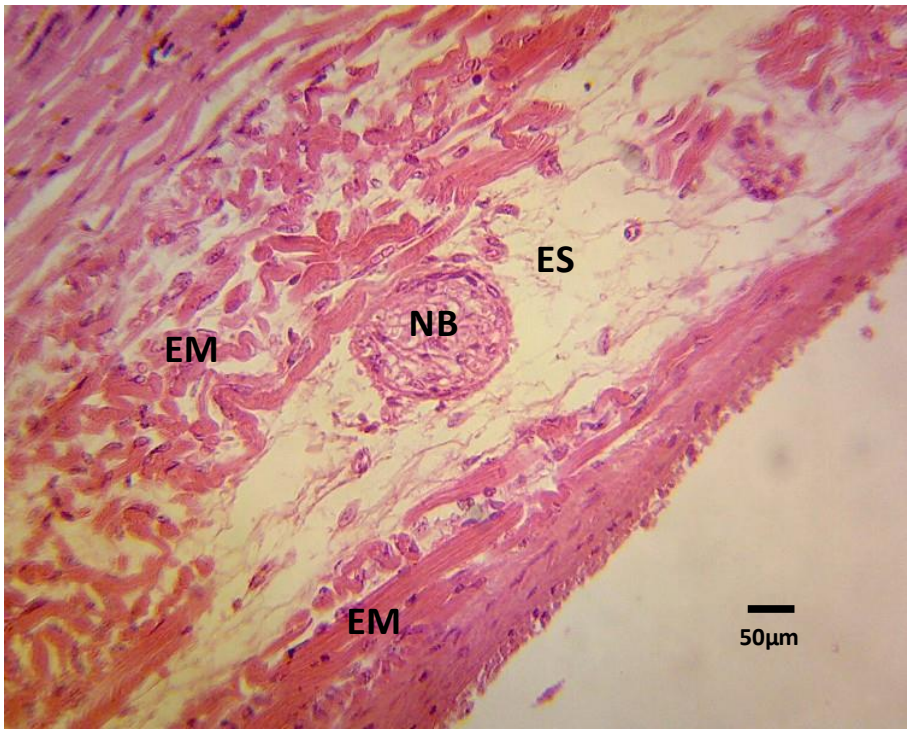

FIG.79 Section of the sclera (D 105) showing the episclera **ES** made up of loose connective tissue; extraocular muscle **EM,** nerve bundle **NB.** H&E

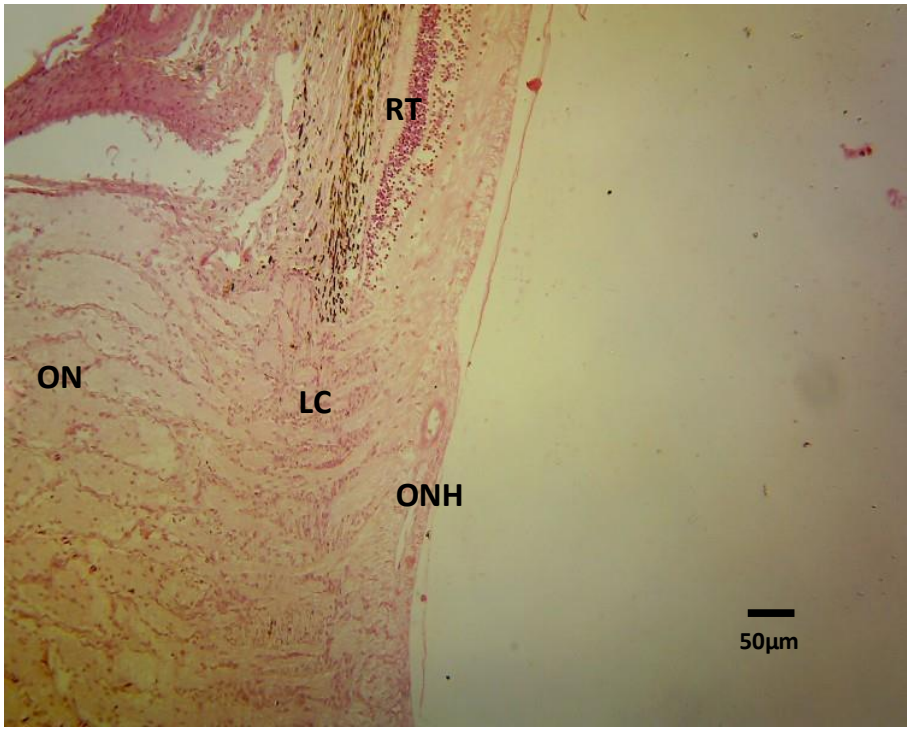

FIG.80 Section of the posterior globe (D 105 fetus) showing the retina **RT**, optic nerve **ON,** optic nerve head **ONH,** lamina cribrosa **LC.**H&E

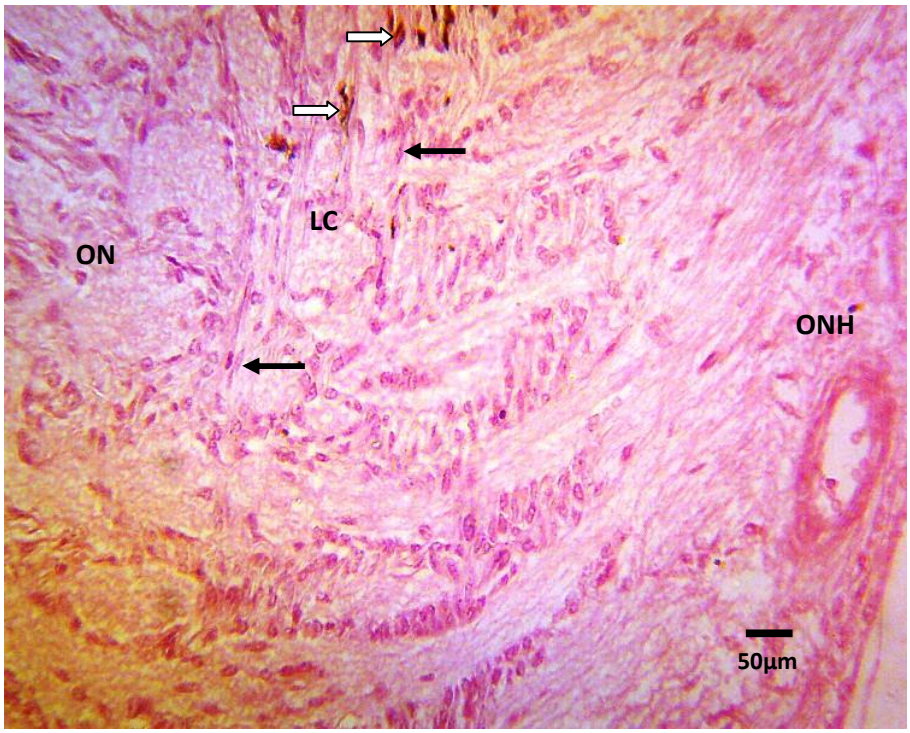

FIG.81 Section of the posterior globe(Day 105 fetus) showing the optic nerve **ON,** optic nerve head **ONH,** lamina cribrosa **LC,** containing melanocytes (white arrows) and fibrocytes (black arrows)**.** H&E.

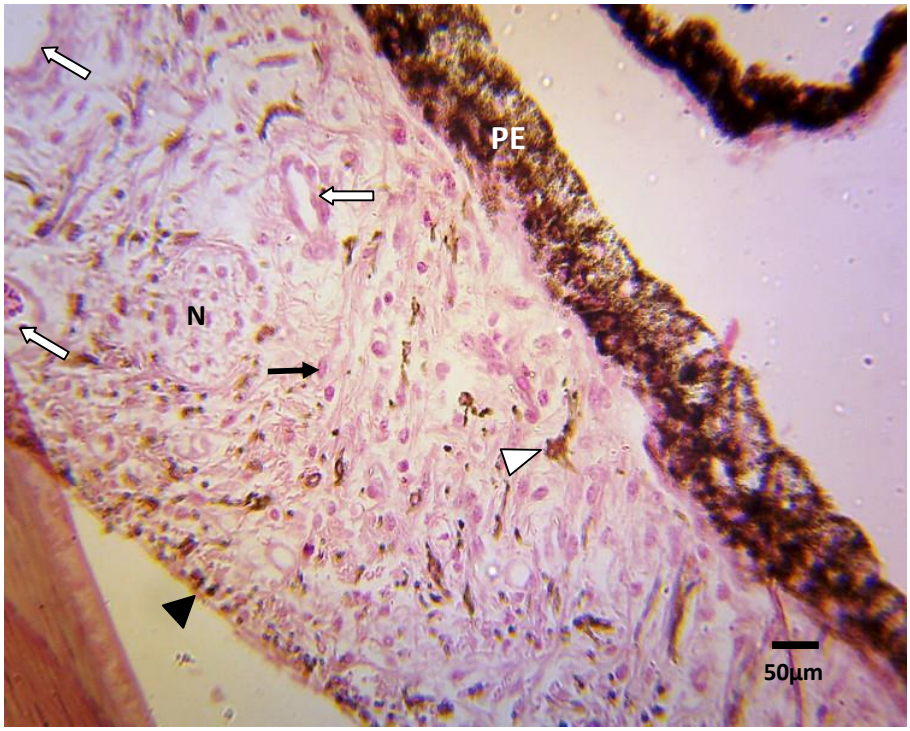

FIG.82 Section of the iris (Day 105 fetus) showing the posterior epithelium **PE,** blood vessels (white arrows), fibroblast (black arrow), nerve **N,** melanocytes (white arrow head) and anterior epithelium (black arrow head)**.**H&E.

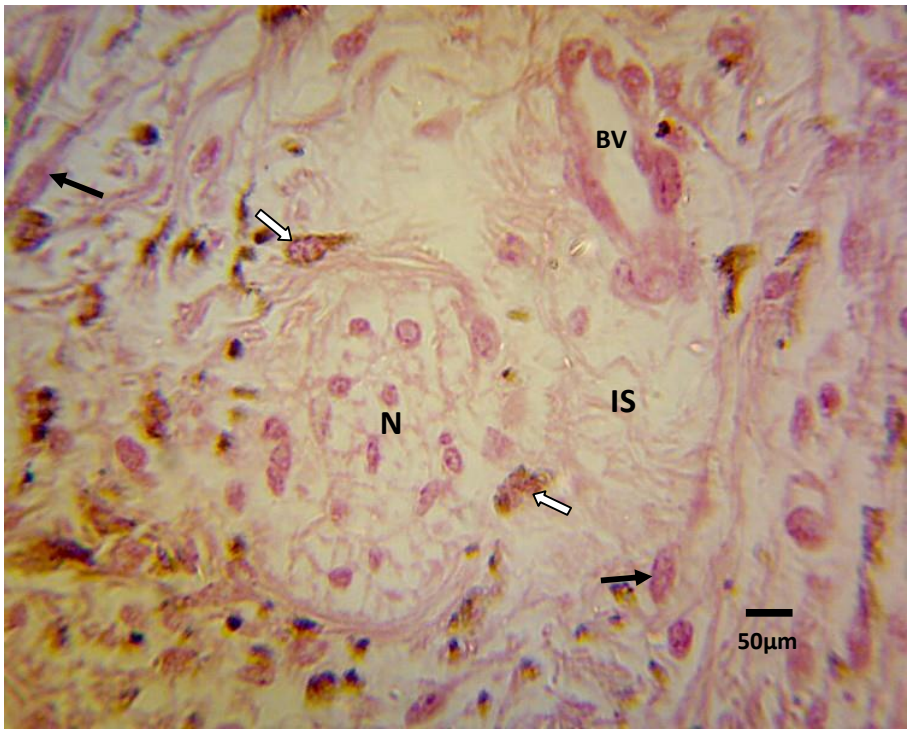

FIG.83 Section of Day 105 fetus iridal stroma **IS** showing a blood vessel **BV**, fibroblasts (black arrows), nerve **N,** melanocytes (white arrows)**.** Note that the stroma is made up of loose connective tissue. H&E

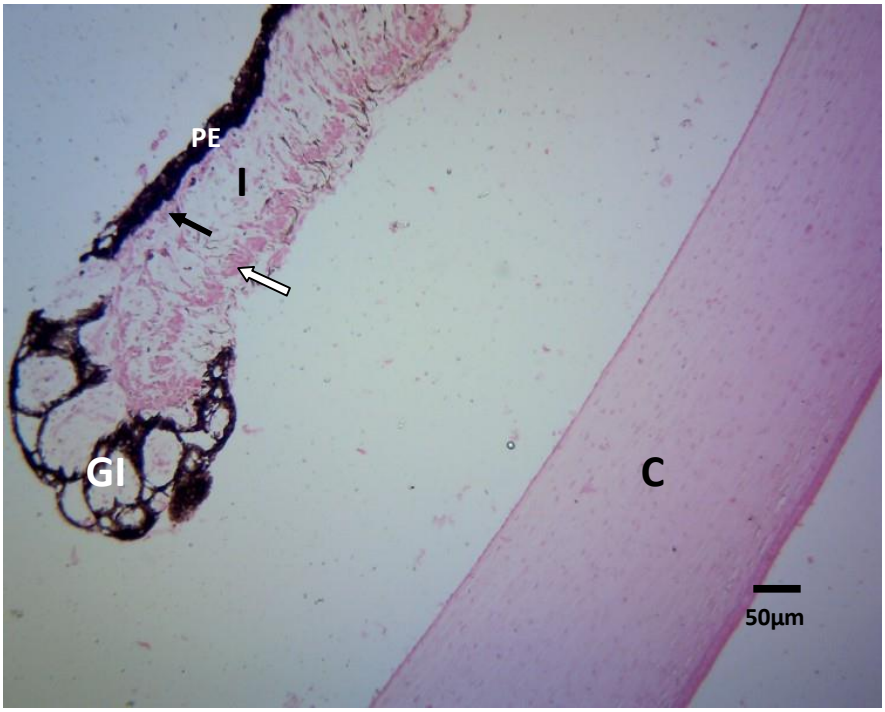

FIG.84 Section of the anterior eye (Day 105 fetus) showing the cornea **C;** the iris **I** showing the dilator pupilae muscle (black arrow)**,** constrictor pupilae muscle (white arrow)**,** granula iridica **GI,** posterior epithelium **PE.**H&E

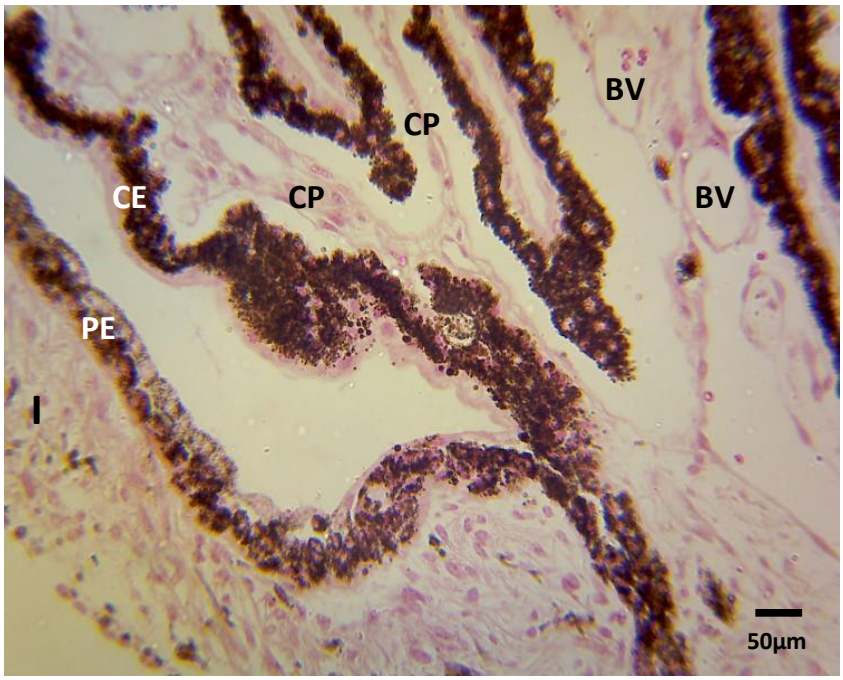

FIG.85 Section of the anterior eye (Day 105 fetus) showing the base of the iris **I,** ciliary processes **CP,** double layered ciliary epithelium **CE,** double layered iridal posterior pigmented epithelium **PE** and blood vessels **BV** wihin the ciliary processes.Note that both cell layers of the iridal posterior epithelium are pigmented while the layers of the ciliary epithelium, which is continuous with it, have an outer pigmented and inner nonpigmented cell layers.H&E

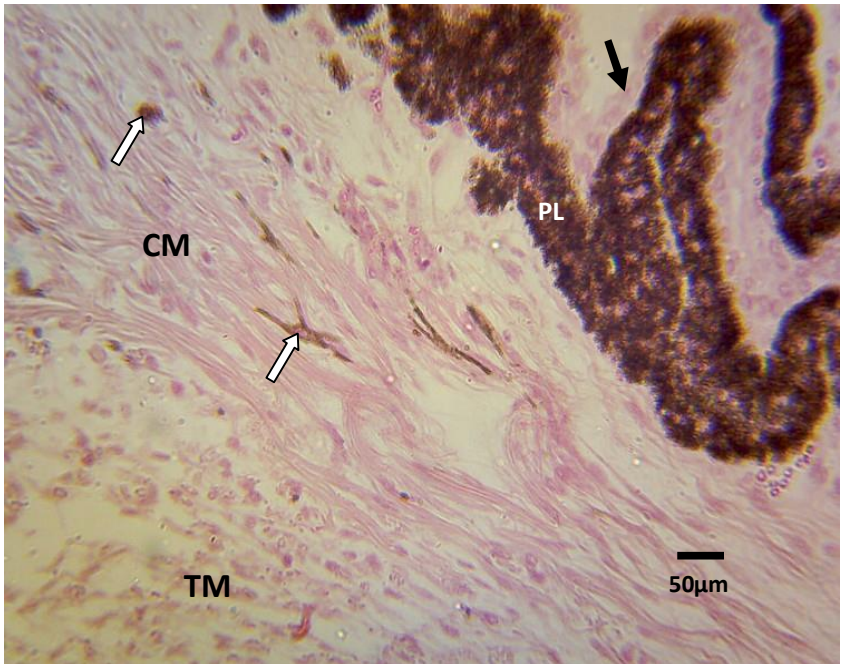

FIG.86 Section of the ciliary body (Day 105 fetus) showing its epithelium composed of an inner nonpigmented cell layer (black arrow) and an outer pigmented cell layer **PL**; ciliary body musculature **CM;** melanocytes (white arrows), trabecular meshwork **TM.** Note that cells of the nonpigmented layer are cuboidal over the processes.H&E

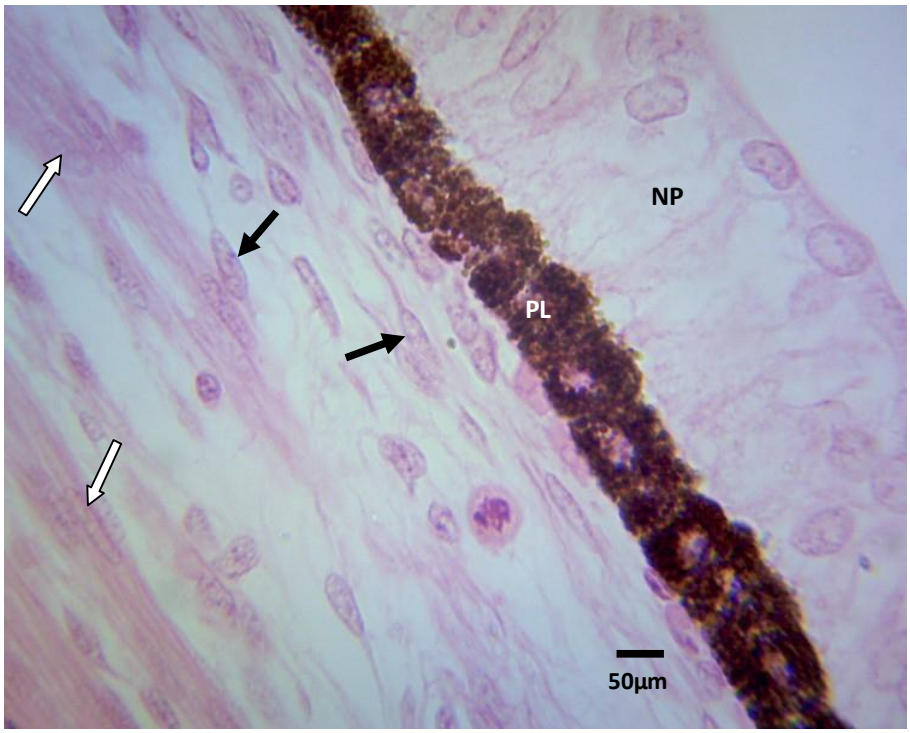

FIG.87 Section of the ciliary body (pars plana region) (Day 105 fetus) showing its epithelium composed of an inner nonpigmented cell layer **NP** and an outer pigmented cell layer **PL**; smooth muscle cells of the ciliary body musculature (white arrows)**;** fibroblasts (black arrows). Note that the **NP** is composed of pseudostratified columnar cells. H&E

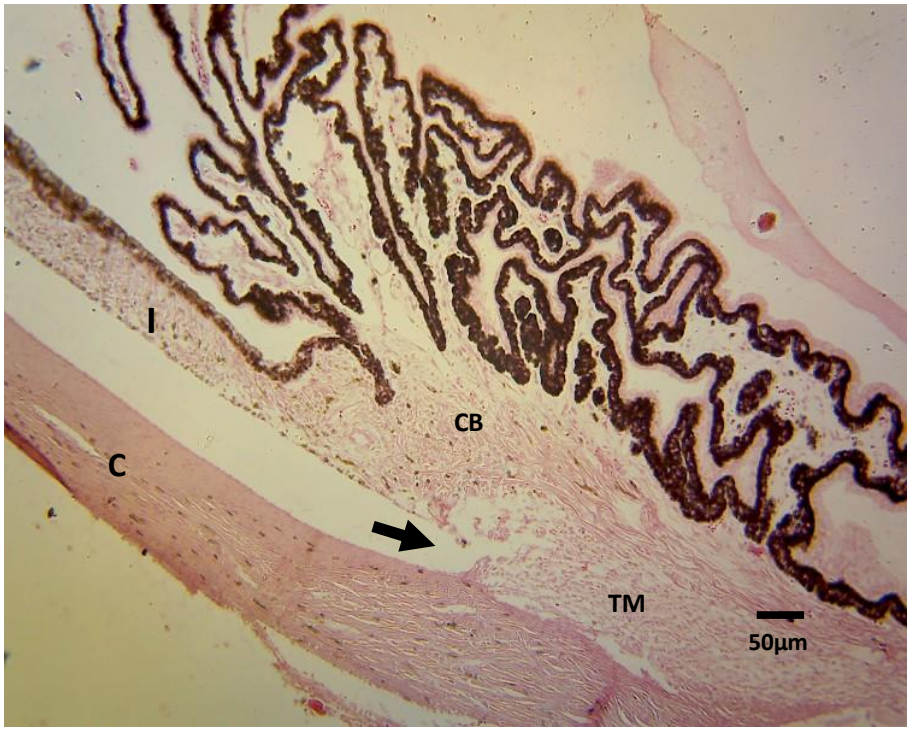

FIG.88 Section of the anterior eye (Day 105 fetus) showing the open iridocorneal angle (black arrow), iris **I**, cornea **C**, trabecular meshwork **TM** and ciliary body **CB**. H&E.

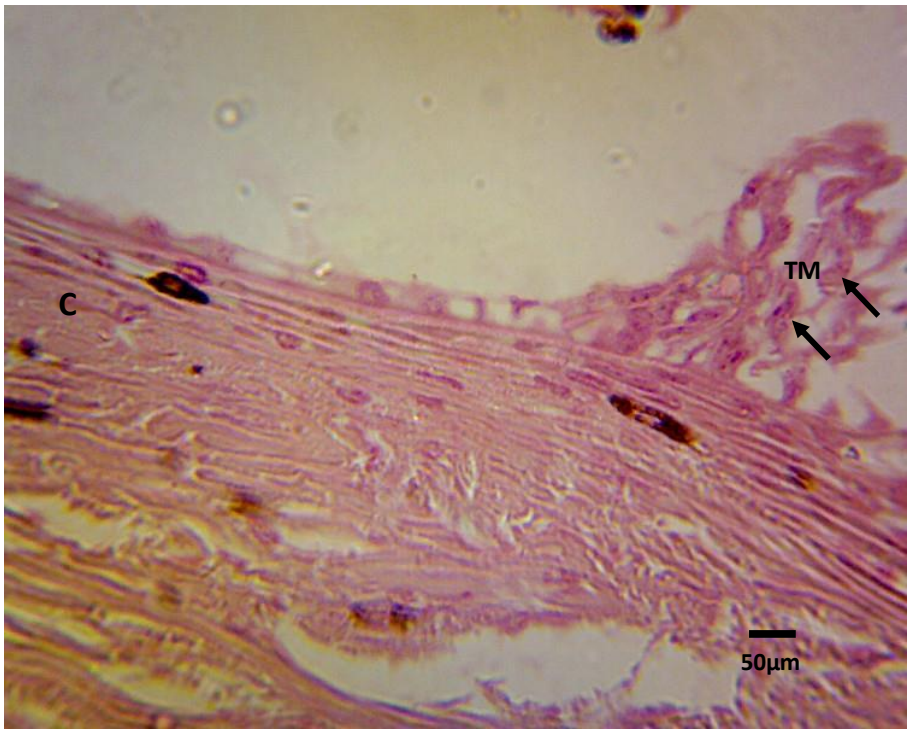

FIG.89 Section of the anterior eye (Day 105 fetus) showing the spaces of the trabecular meshwork **TM,** lined by trabecular cells (black arrows).H&E.

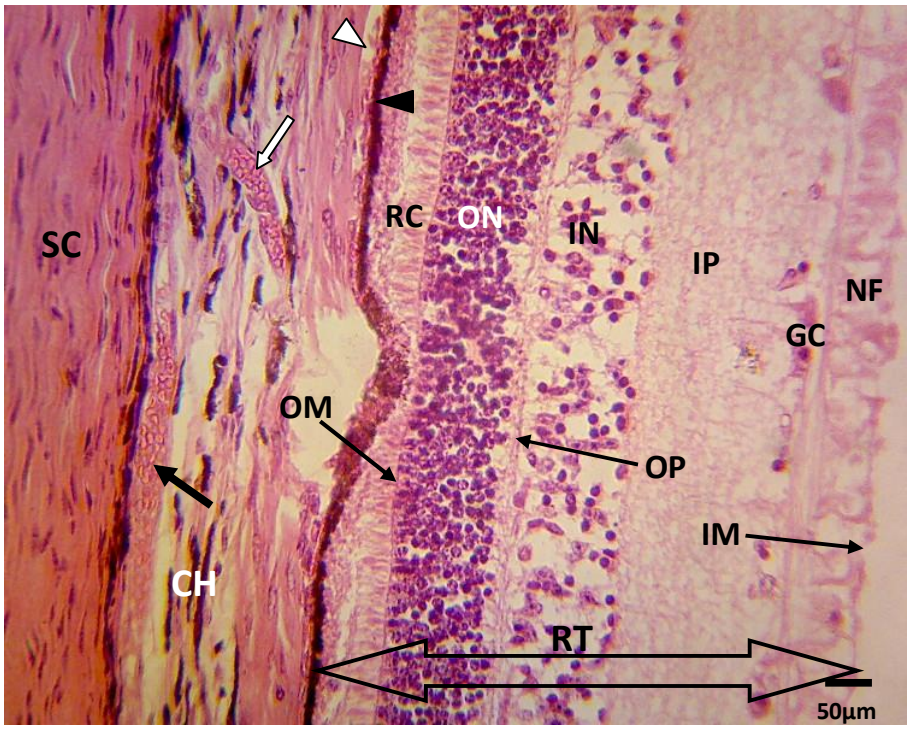

FIG.90 Section of the globe (day 105 fetus) showing the choroid **CH** sandwiched between the sclera **SC** and the retina **RT** consisting of the retinal pigment epithelium (black arrow head), rods and cones **RC**, outer limiting membrane **OM**, outer nuclear layer **ON**, outer plexiform layer **OP**, inner nuclear layer **IN**, inner plexiform layer **IP**, ganglion cell layer **GC**, nerve fibre layer **NF,** inner limiting membrane **IM,** choriocapilaris (white arrow head), large blood vessel (black arrow), smaller vessel (white arrow) running perpendicularly connecting the choriocapilaris and the large vessel layer. H&E.

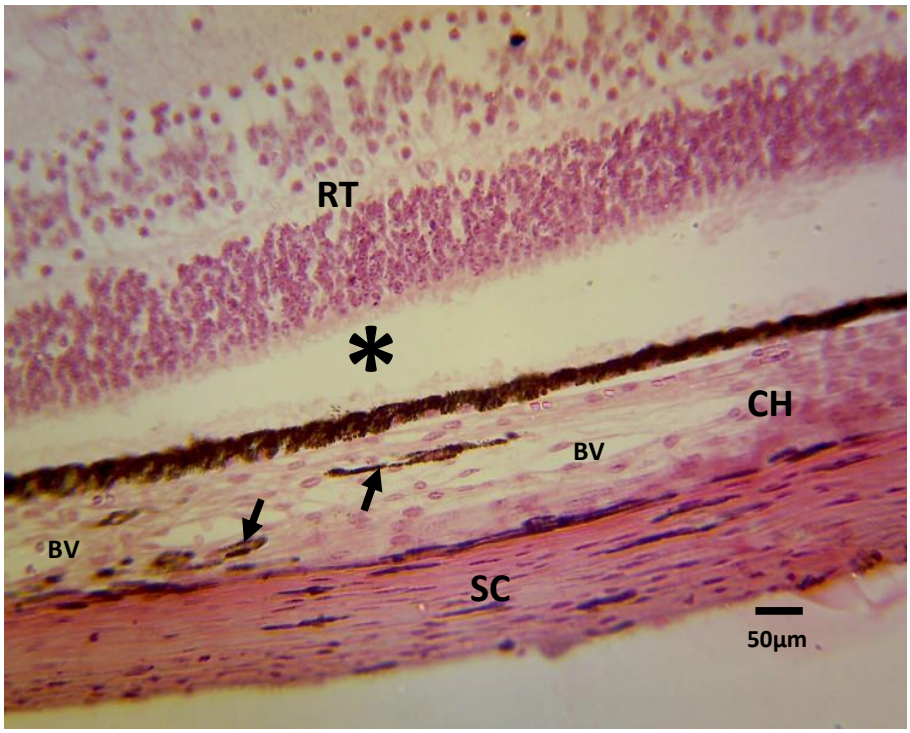

FIG.91 Section of the globe (Day 105 fetus) showing the choroid **CH** sandwiched between the sclera **SC** and the retina **RT**; spindle shaped melanocytes (arrows), blood vessels **BV**. Note that the separation (asterix) within the retina is artifactual. H&E

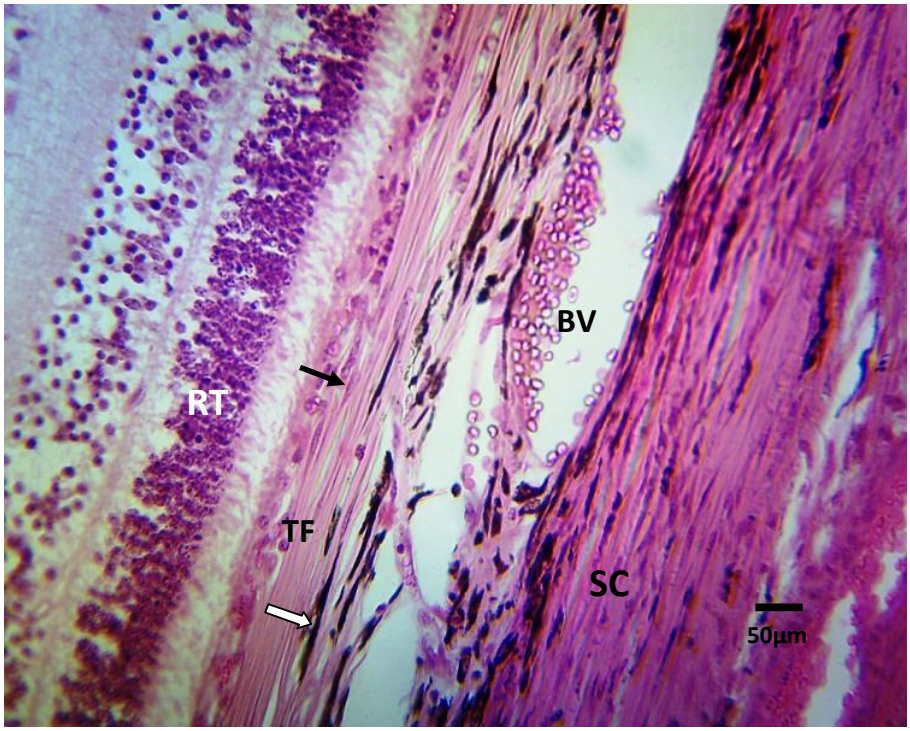

FIG.92 Section of the dorsal posterior choroid (Day 105 fetus) showing the tapetum fibrosum **TF**, melanocytes (white arrow), fibroblast (black arrow), blood vessel **BV**, sclera **SC** and retina **RT**. H&E.

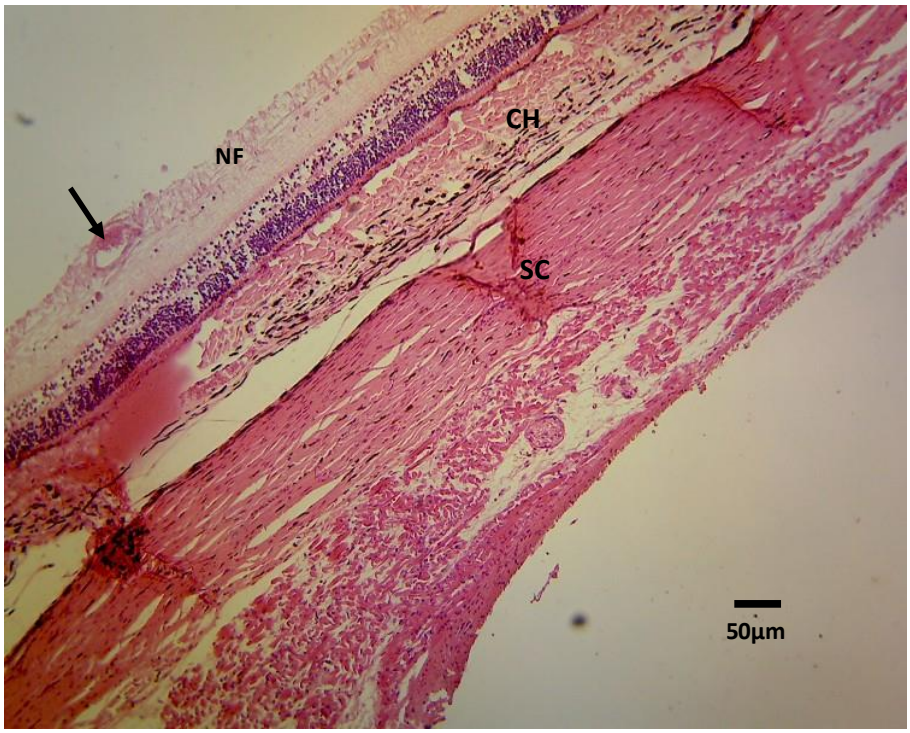

FIG.93 Section of the posterior globe (Day 105 fetus) showing a large blood vessel (arrow) within the nerve fibre layer **NF** of the retina; choroid **CH**, sclera **SC**.H&E

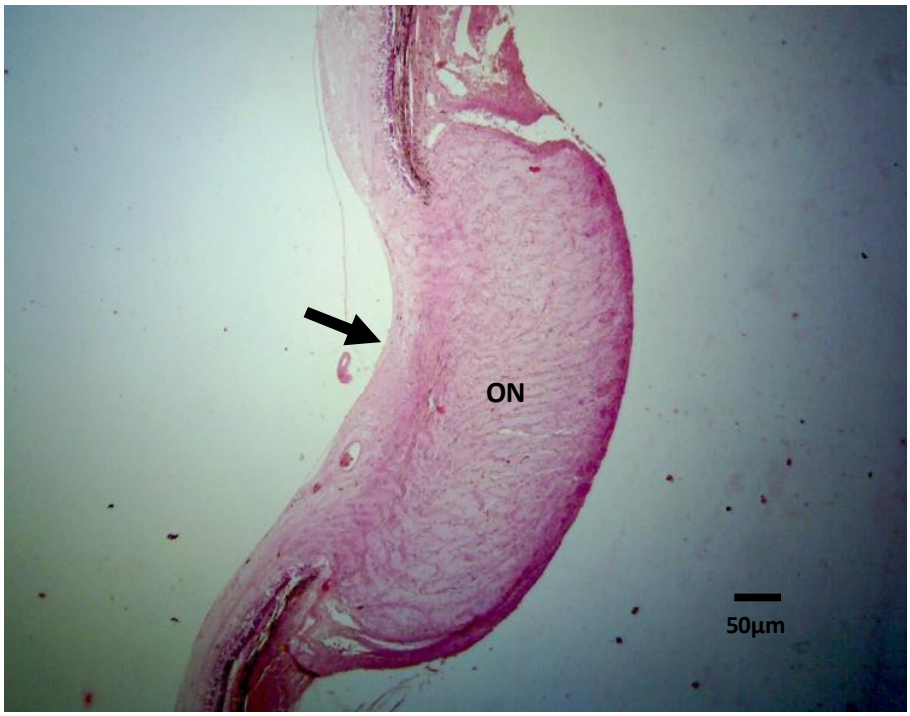

FIG.94 Section of the posterior globe (Day 105 fetus) showing the physiologic cup (arrow) at the optic nerve head, optic nerve **ON**. H&E.

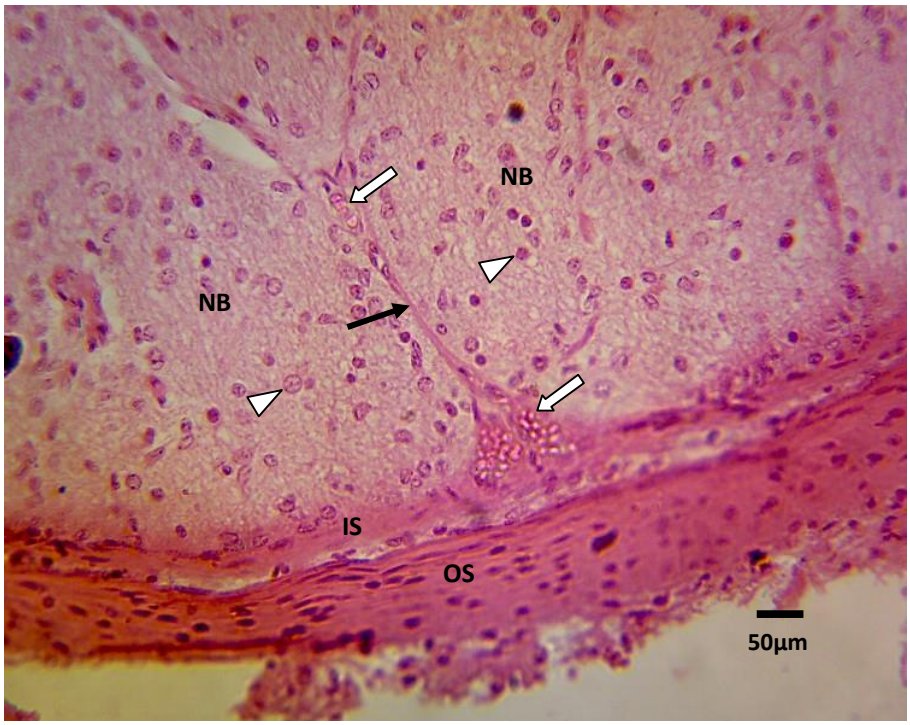

FIG.95 Section of the optic nerve (Day 105 fetus) showing the internal connective tissue sheath **IS** (corresponding to pia mater), outer connective tissue sheath **OS** (corresponding to dura mater),blood vessels (white arrows) within the connective tissue septa (black arrow) separating the axons into nerve bundles **NB,** neuroglial cells (arrow heads).H&E

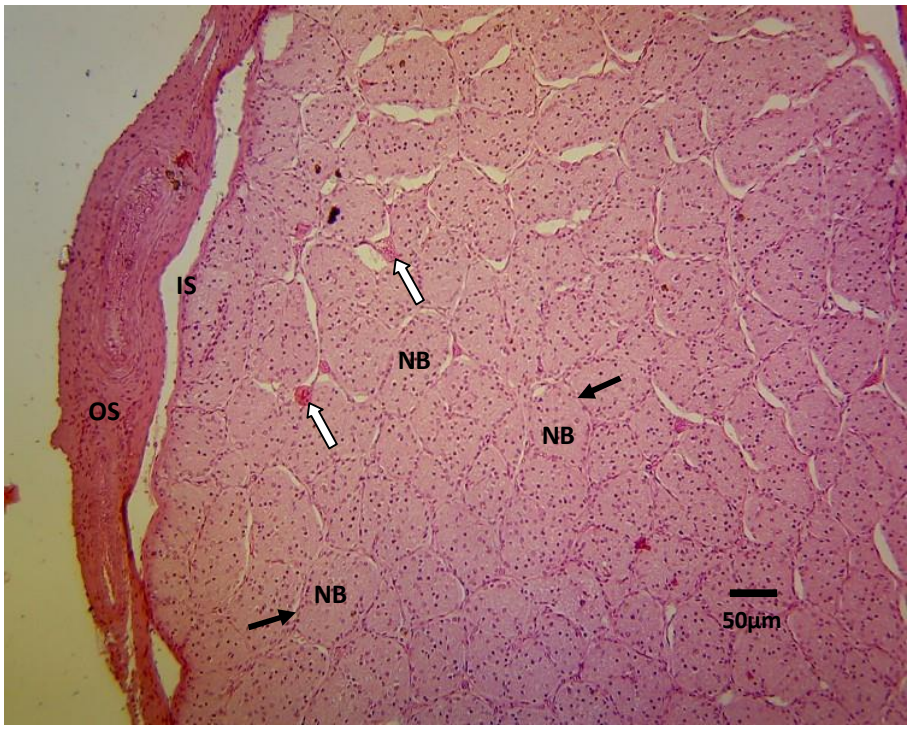

FIG.96 Section of the optic nerve (Day 105 fetus) showing the internal connective tissue sheath **IS**, outer connective tissue sheath **OS**,blood vessels (white arrows),connective tissue septa (black arrow) separating the axons into nerve bundles **NB**.H&E

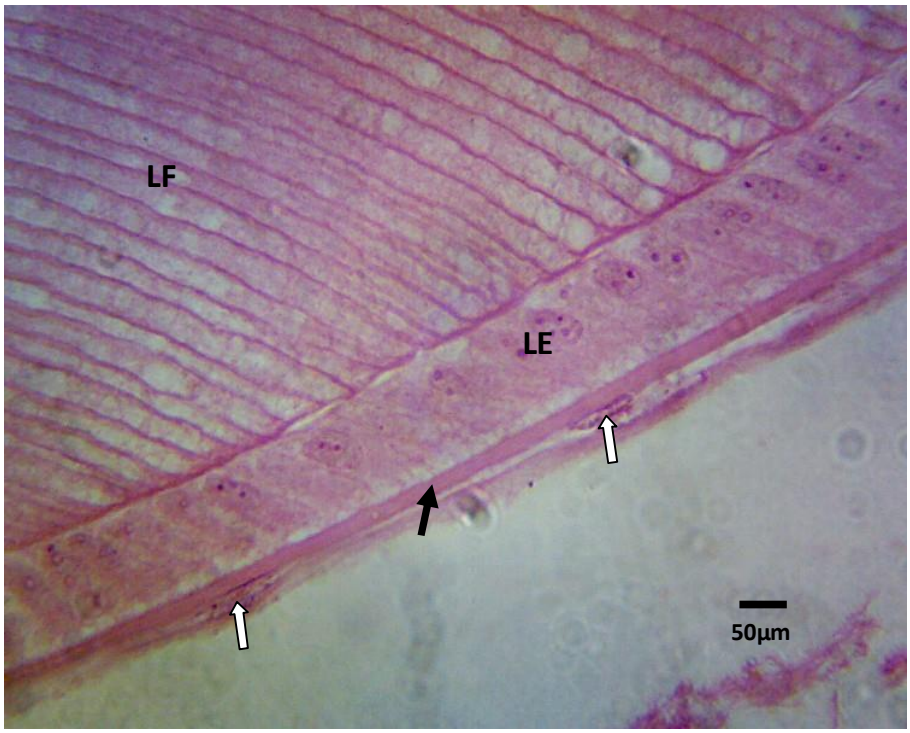

FIG.97 Section of the anterior surface of the lens (equatorial region) (Day 105 fetus) showing the lens capsule (black arrow), columnar cells of the lens epithelium **LE**, lens fibres **LF** and vessels of the tunica vasculosa lentis (white arrow).H&E

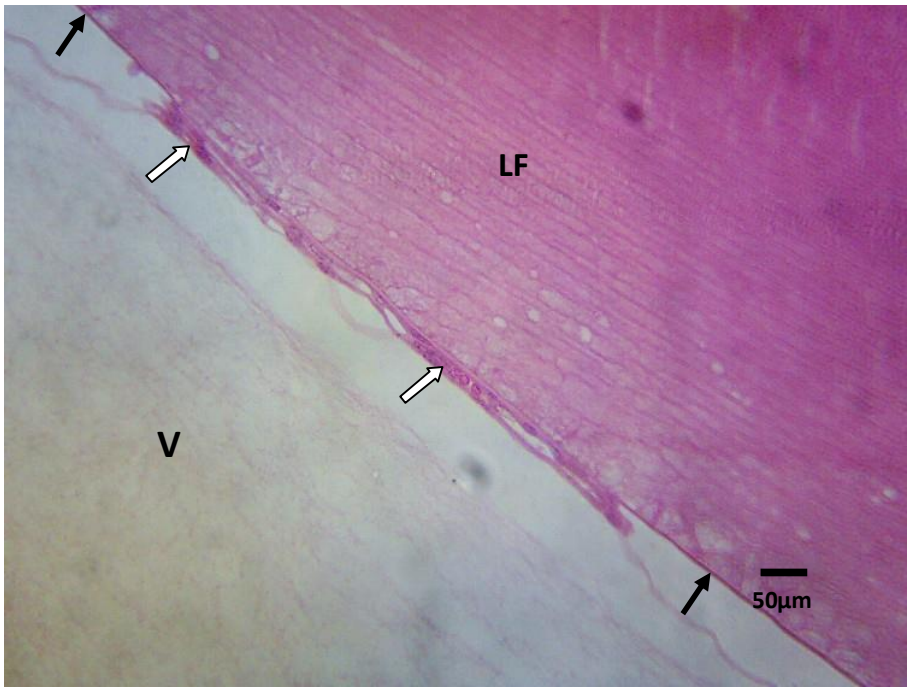

FIG.98 Section of the posterior surface of the lens (Day 105 fetus) showing the thin lens capsule (black arrows), lens fibres **LF**, vessel of the tunica vasculosa lentis (white arrows), and vitreous **V**. Note that the posterior lens surface was not lined by any epithelium. H&E

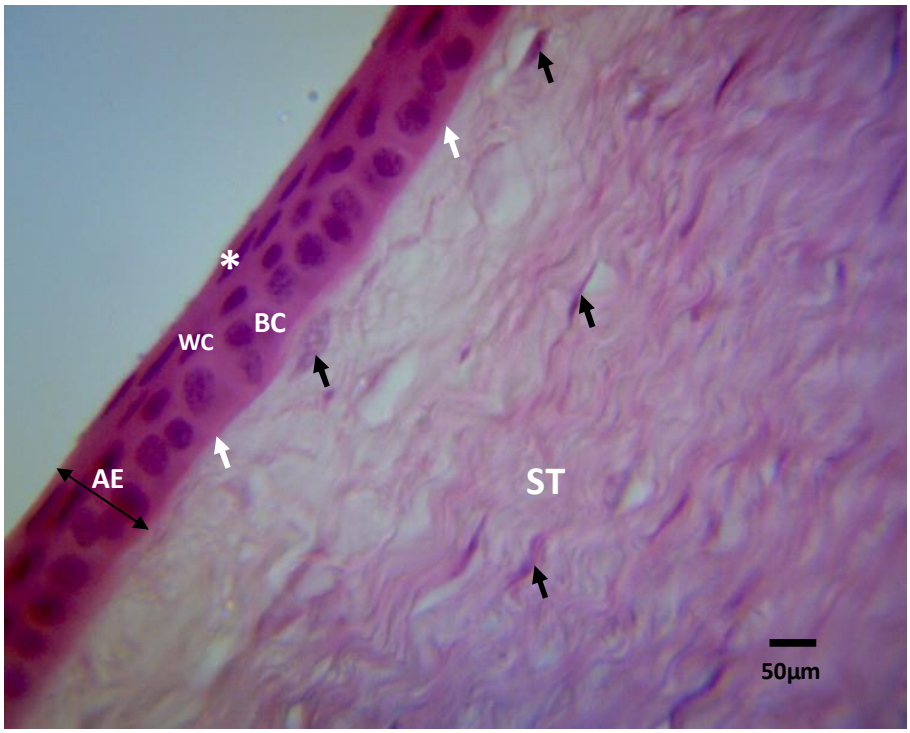

FIG. 99 Section of neonate cornea showing the corneal stroma **ST**; anterior epithelium **AE;** basal cell layer **BC;** wing cells **WC;** superficial cells (asterix); bowman's membrane (white arrows); keratocytes (black arrows).H&E.

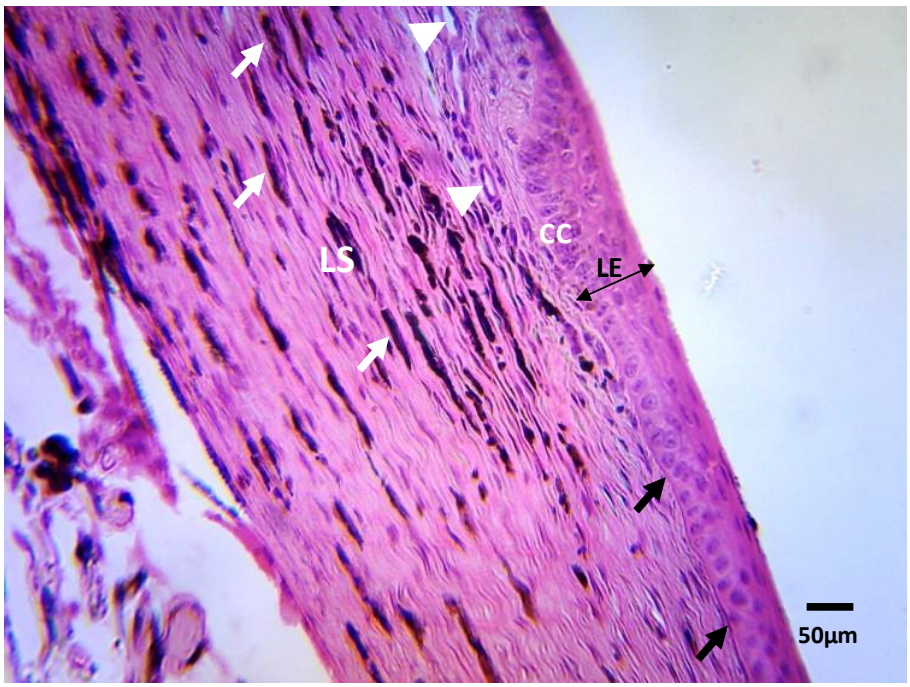

FIG. 100 A section of the neonate limbus showing the limbal stroma **LS**; Limbal epithelium **LE;** columnar basal cells of the limbal epithelium **CC;** blood vessels (arrow heads); melanocytes (white arrows); Bowman's Layer (black arrows).H&E.

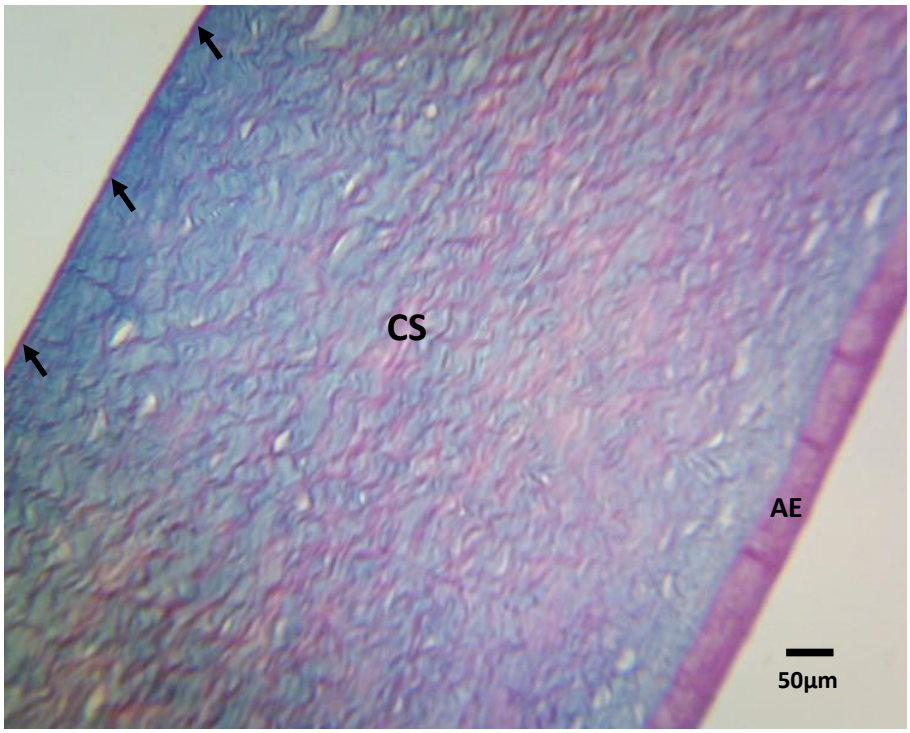

FIG.101 Section of the neonate cornea showing the anterior epithelium **AE;** corneal stroma **CS;** posterior limiting membrane-Descemet's membrane (arrows). Note that the stroma reacted positively to both the PAS and the AB techniques. AB-PAS. pH 2.5

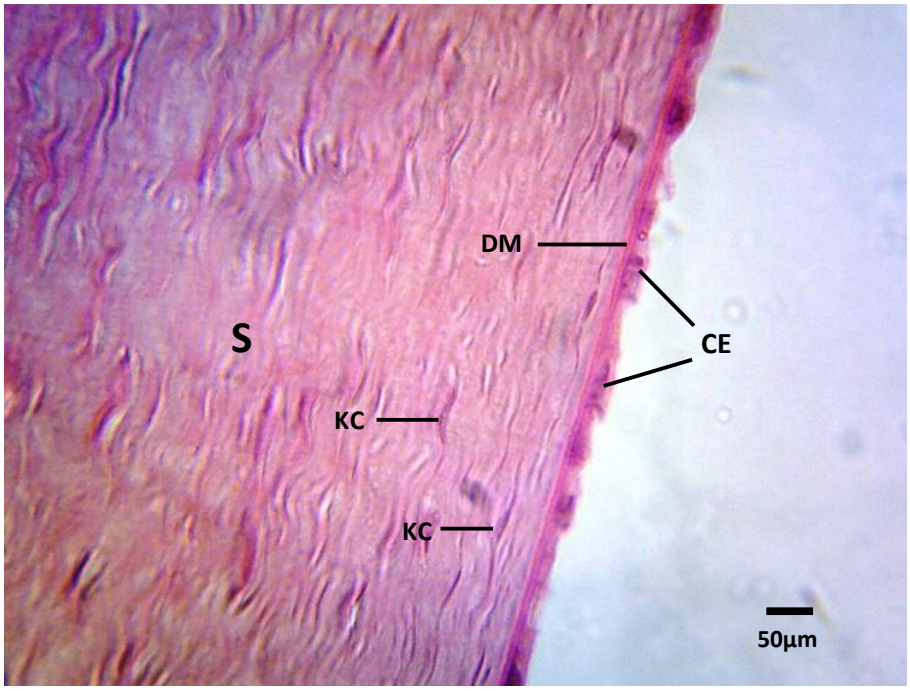

FIG.102 Section of the neonate posterior cornea showing stroma **S;** descemet's membrane **DM;** corneal endothelium **CE;** keratocytes **KC.** H&E

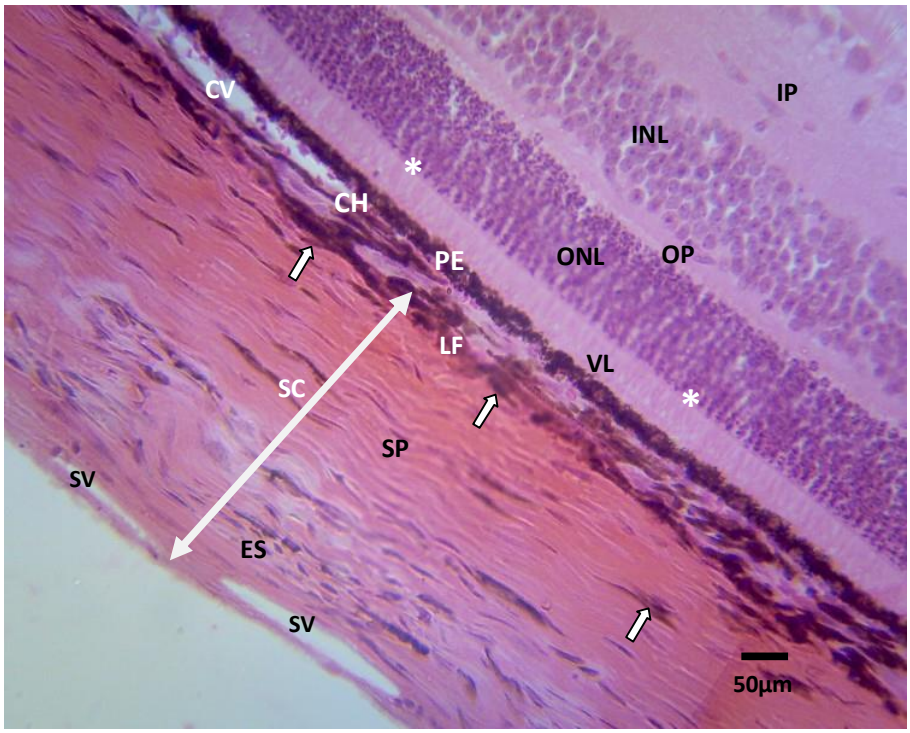

FIG.103 Section of the neonate eye showing the sclera **SC;** episclera **ES** containing blood vessels **SV;** sclera proper **SP;** lamina fusca **LF;** melanocytes (arrows);choroid **CH** containing blood vessels **CV;** retinal pigment epithelium **PE;** visual cell layer **VL;** external limiting membrane (asterix); outer nuclear layer **ONL;** outer plexiform layer **OP;** inner nuclear layer **INL;** inner plexiform layer **IP.**H&E.

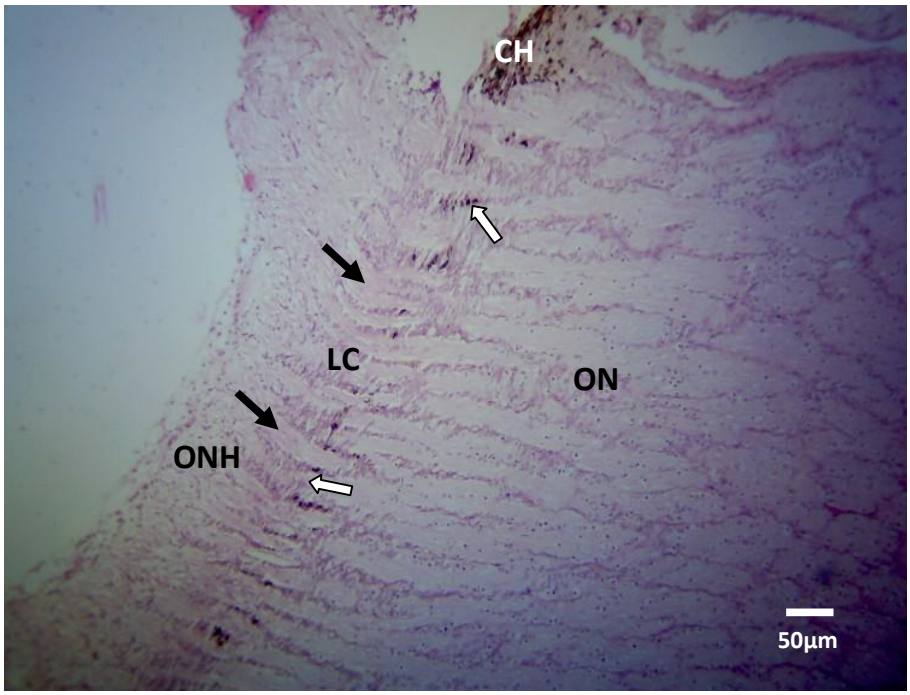

FIG.104 Section of the posterior eye ball (neonate) showing the optic nerve head **ONH;** lamina cribrosa **LC;** astrocyte channels guiding the ganglion cell axons (black arrows); meridionally oriented collagenous fibres (white arrows); optic nerve **ON.** Note the sparse pigmentation of the collagenous fibres.H&E.

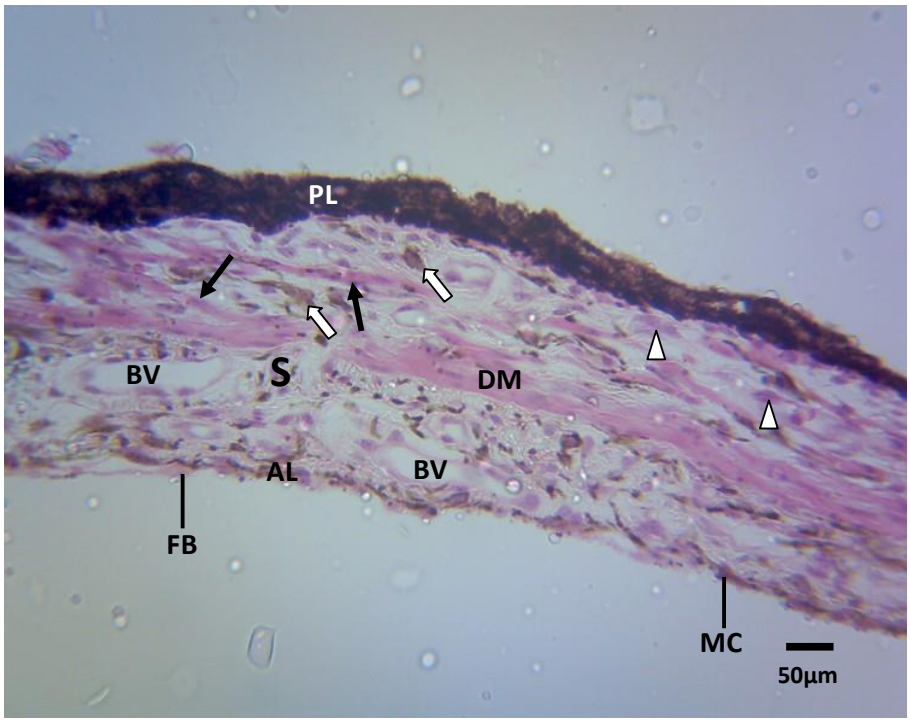

FIG. 105 Section of the neonate iris showing the pigmented double layered epithelium **PL;** the stroma **S** containing the dilator pupillae muscle **DM;** stromal fibroblasts (arrow heads); stromal melanocytes (white arrows); blood vessels **BV.** The anterior layer (endothelium) **AL** comprising of fibroblasts **FB** and melanocytes **MC.** Note the strands of smooth muscle cells containing pigments running from the dilator pupillae muscle to the anterior layer of the posterior pigment epithelium (black arrows).H&E

FIG.106 Section of the neonate iris showing the granula iridica **GI;** constrictor pupilae muscle **CM;** remnant of the iridopupilary membrane (arrow); cornea **C.**H&E

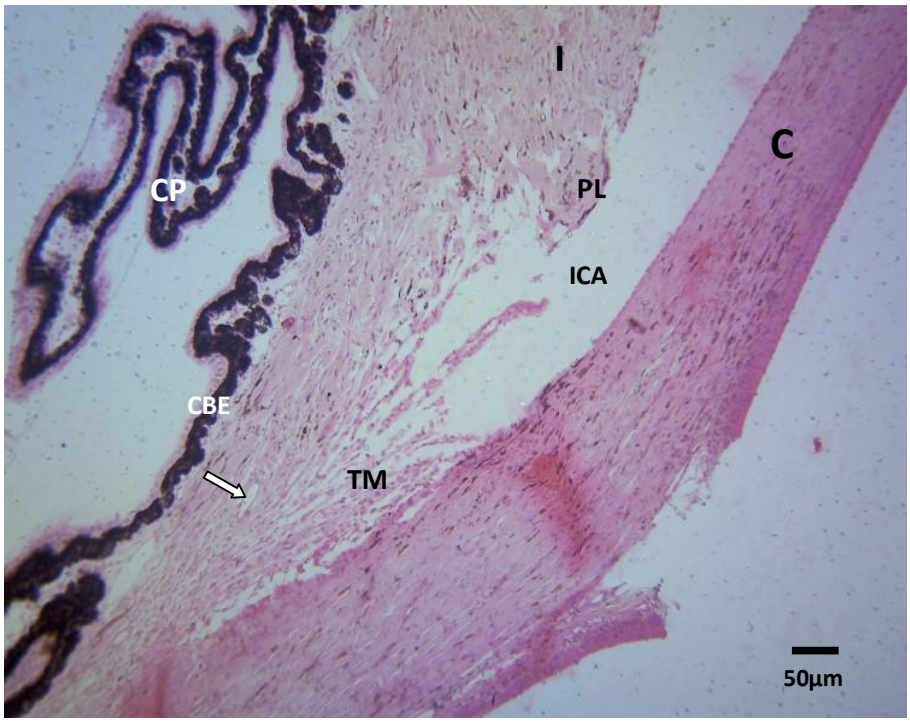

FIG.107 Section of the neonate anterior eye showing the trabecular meshwork **TM;** Iridocorneal angle **ICA;** Cornea **C;** base of iris **I;** ciliary processes **CP;** blood vessel (arrow); ciliary body epithelium **CBE;** Pectinate Ligament **PL**.H&E.

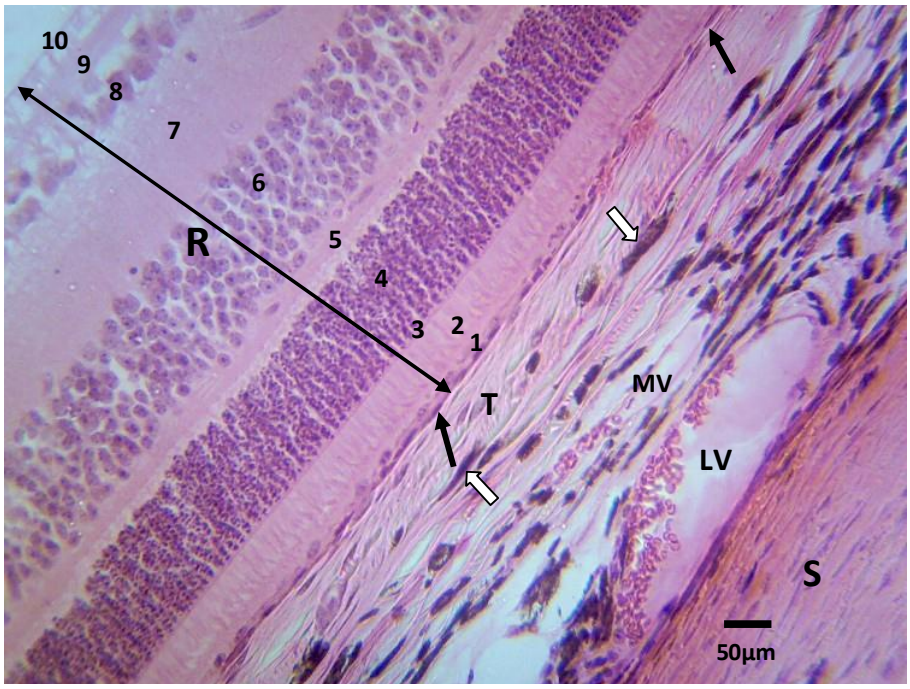

FIG. 108 Section of the neonate globe (tapetal region) showing the sclera **S,** the choroid containing the large vessel **LV** and the medium vessel **MV** within the vessel layer; tapetum fibrosum **T;** melanocytes (white arrows); choriocapilaris (black arrows); retina **R; 1**, Retinal pigment epithelium**; 2**, rod and cone layer; **3**, external limiting membrane; **4**, Outer nuclear layer; **5**, outer plexiform layer; **6**,inner nuclear layer; **7**, inner plexiform layer; **8**, ganglion cell layer; **9**, nerve fibre layer; **10**, inner limiting membrane. Note that the retinal pigment epithelium is devoid of pigments. H&E

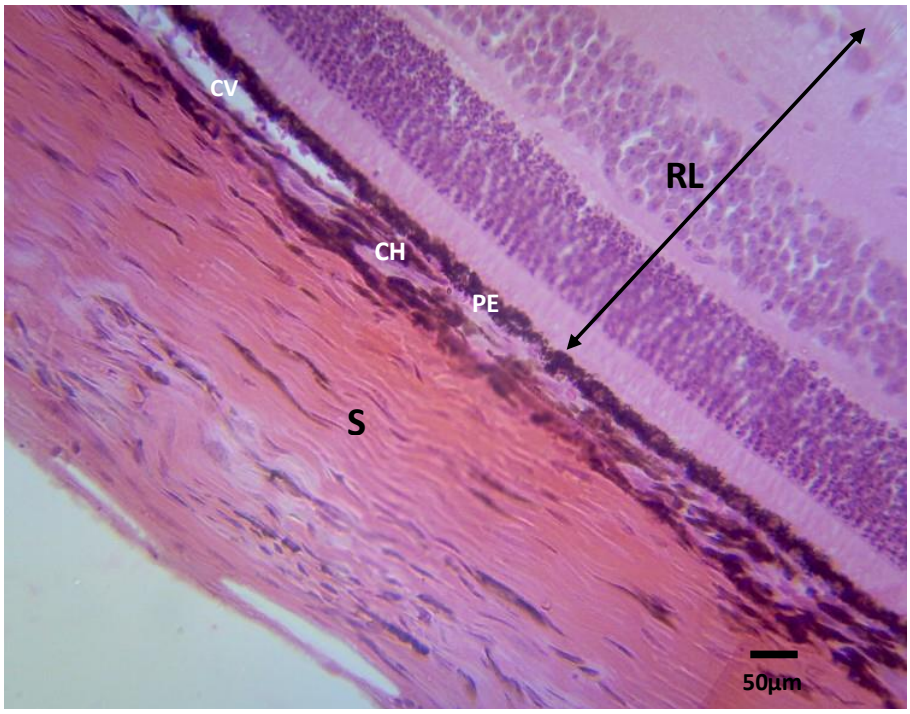

FIG. 109 Section of the neonate globe (non-tapetal region) showing the retinal pigment epithelium **PE;** Sclera **S;** Choroid **CH** containing choroidal blood vessel **CV;** retinal layers **RL**. Note that the retinal pigment epithelium cells are packed with pigment granules. H&E

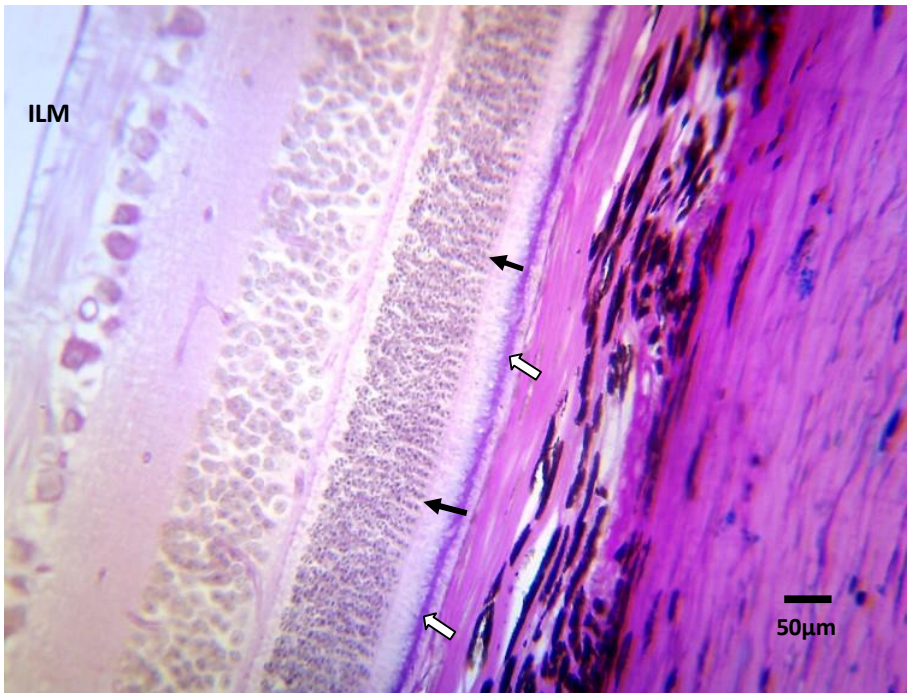

FIG.110 Section of the neonate globe showing Alcian blue positive region of interdigitation (white arrows) between the apical part of the retinal pigment epithelium and the outer segments of the photoreceptors; the PAS positive thin outer limiting membrane (black arrows)**;** the Alcian blue positive inner limiting membrane **ILM.** AB-PAS. pH 2.5

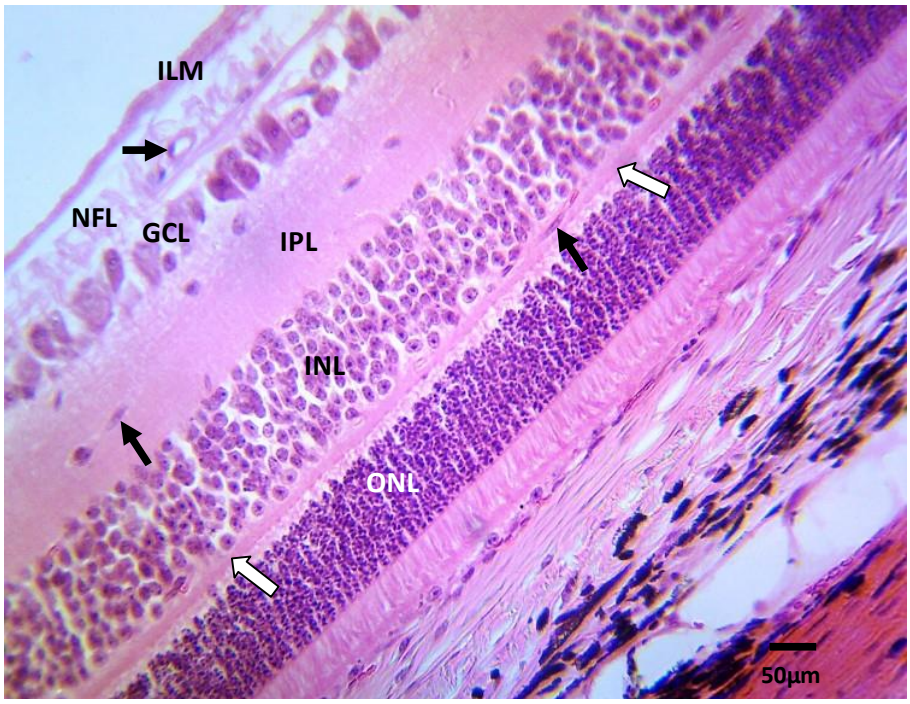

FIG.111 Section of the neonate globe showing the outer nuclear layer **ONL**; outer plexiform layer (white arrows)**;** inner nuclear layer **INL;** inner plexiform layer **IPL;** ganglion cell layer **GCL;** nerve fibre layer **NFL;** inner limiting membrane **ILM;** blood vessels (black arrows). H&E.

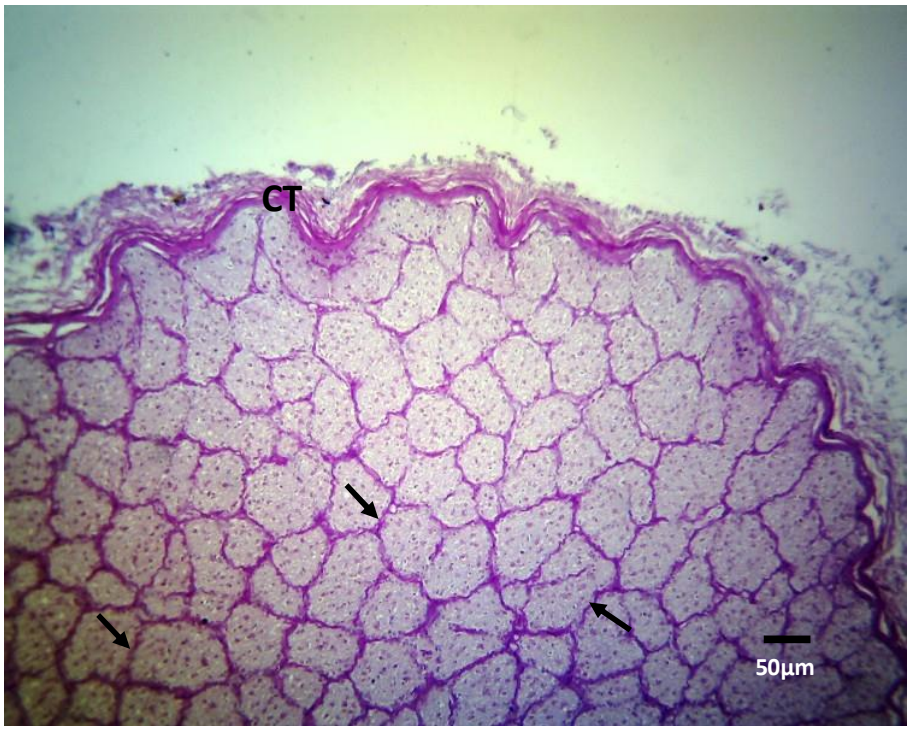

FIG. 112 Section of the neonate optic nerve showing the connective tissue cover of the optic nerve **CT**; connective tissue septae dividing the optic nerve into bundles (arrows). PAS. pH 2.5

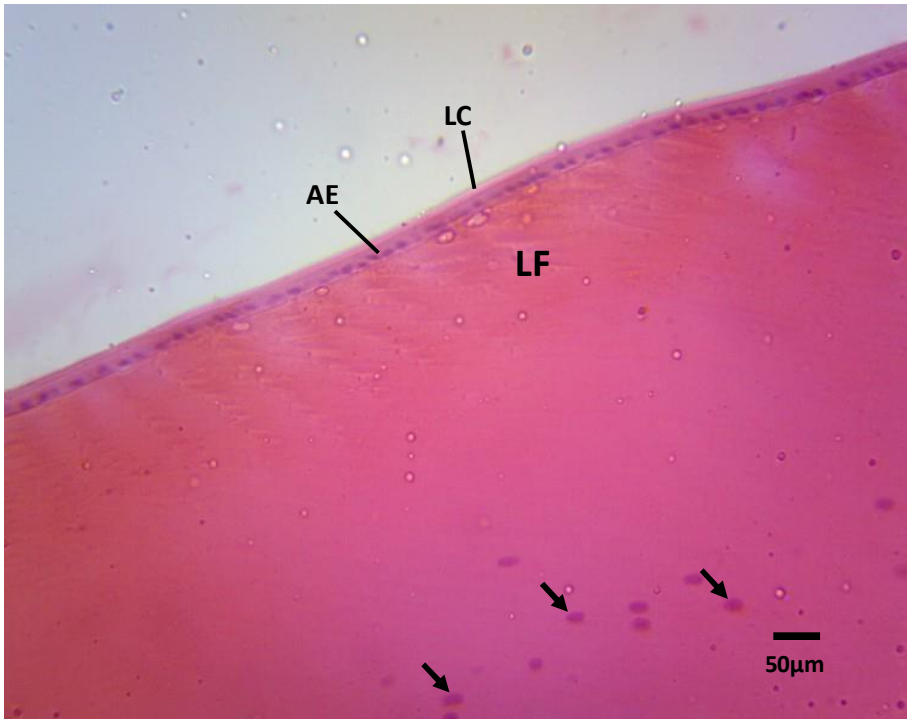

FIG. 113 Section of the neonate lens (central anterior portion) showing the anterior lens capsule **LC;** cuboidal anterior epithelium **AE;** lens fibres **LF;** lens fibre nuclei (arrows).H&E

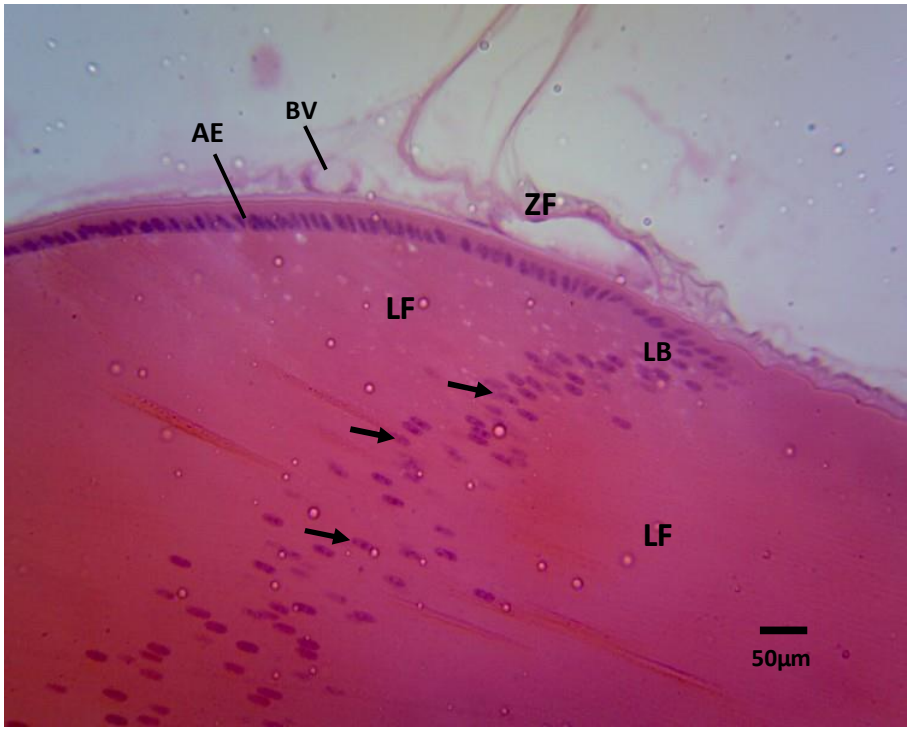

FIG. 114 Section of the neonate lens (equator) showing the columnar anterior lens epithelium **AE;** lens fibre nuclei (arrows) forming the lens bow **LB;** zonular fibres **ZF;** blood vessel **BV;** lens fibres **LF.** H&E

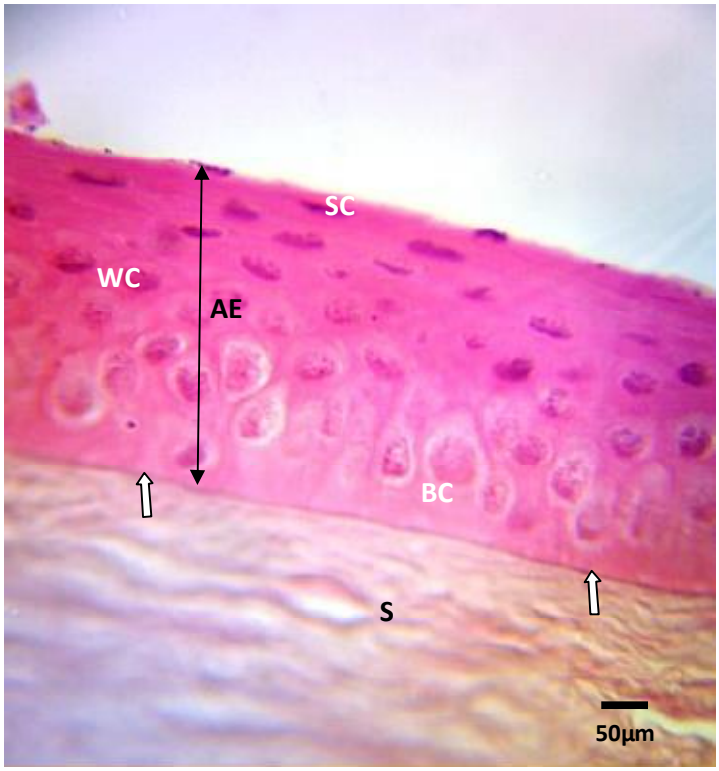

FIG.115 Section of the adult cornea showing anterior epithelium **AE** and the stroma **S**. basal cell layer **BC;** wing cells **WC;** superficial cells **SC**; Bowman's membrane (arrows). Note the perinuclear halo in the basal cells.H&E

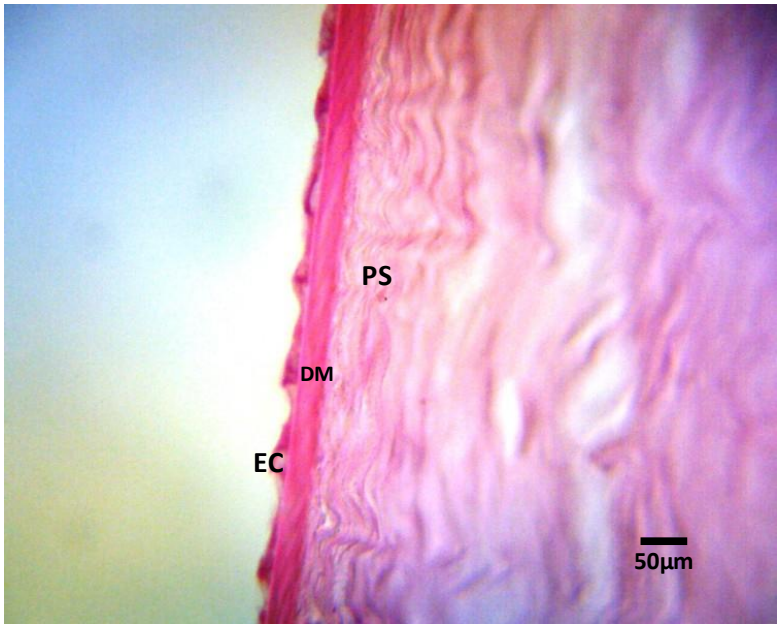

FIG.116 Section of the adult cornea showing the Posterior stroma **PS**; the Descemet's membrane **DM**; the endothelial cell **EC**.H&E

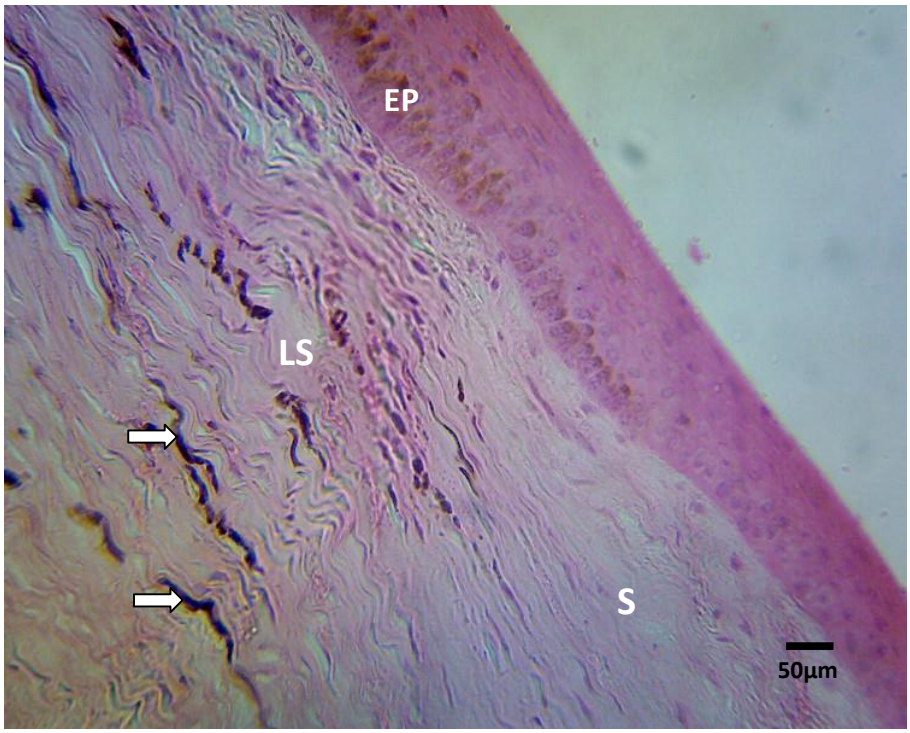

FIG. 117 Section of the adult limbus showing pigment cells (arrows); Pigment containing epithelial cells **EP**; Limbal stroma **LS**; Corneal stroma **S**. Note the thickness of the limbal epithelium and the irregular arrangement of the connective tissue fibres of its stroma.H&E

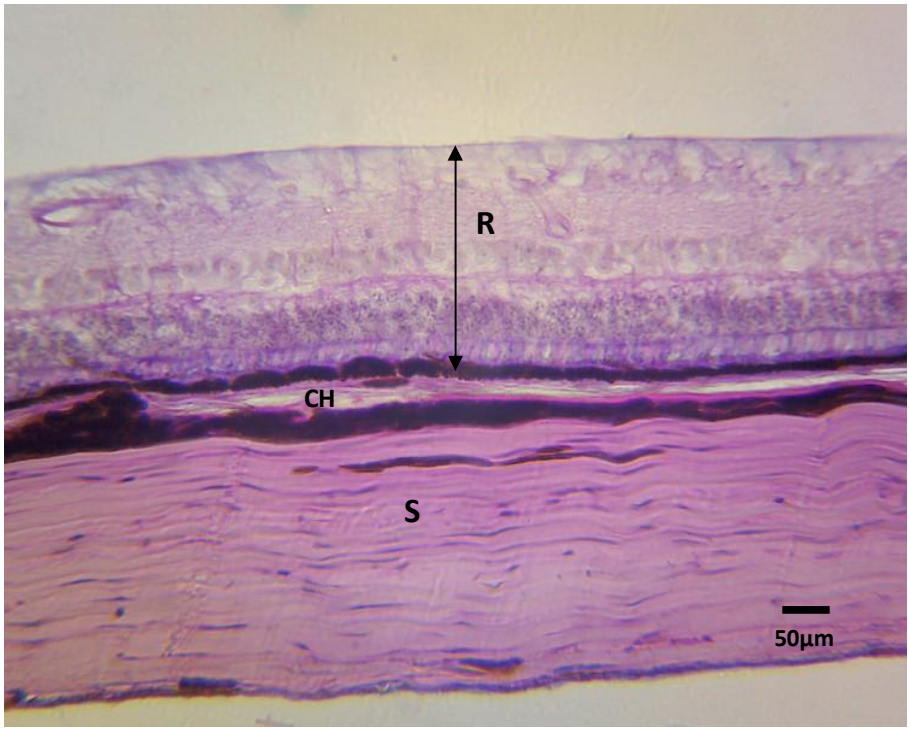

FIG. 118 Section of the eye showing the PAS positive sclera **S;** Choroid **CH;** Retina **R.** AB/PAS. pH 2.5

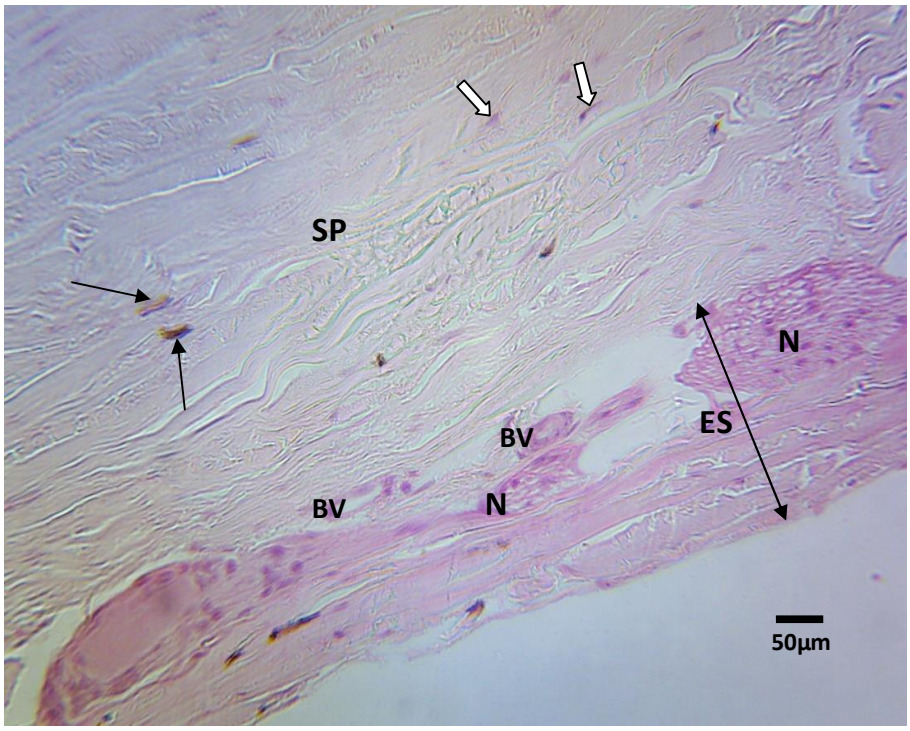

FIG.119 Section of the adult sclera showing nerves **N**, blood vessels **BV** within the episclera **ES;** sclera proper **SP**; Fibrocytes (white arrows); Melanocytes (black arrows).H&E.

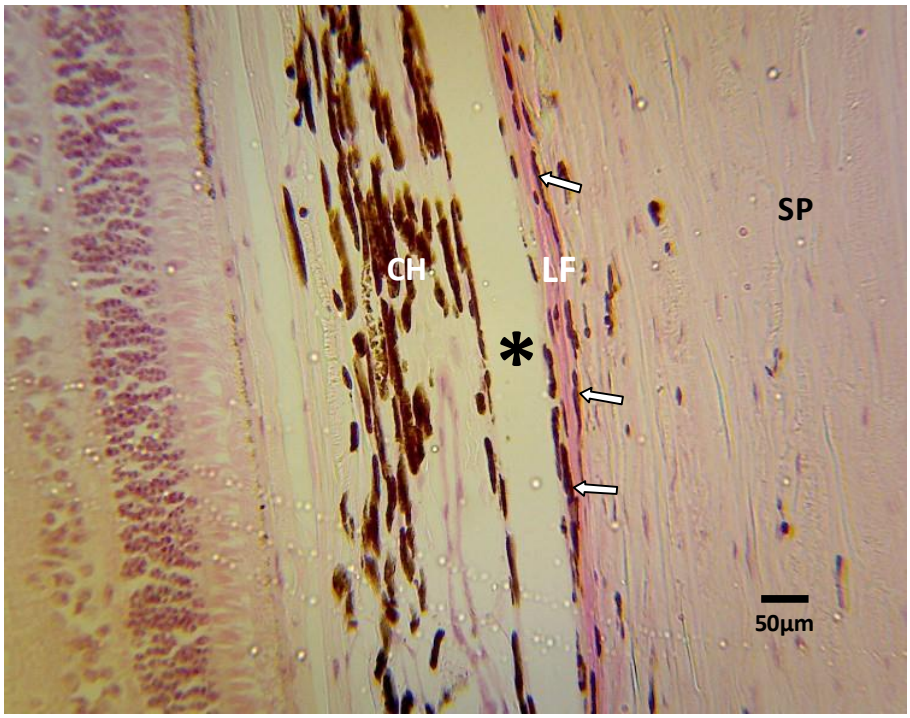

FIG. 120 Section of the adult eye showing the Sclera proper **SP**; Lamina fusca **LF**; Melanocytes (arrows); Choroid **CH**. Note that the space (asterix) is artifactual.H&E

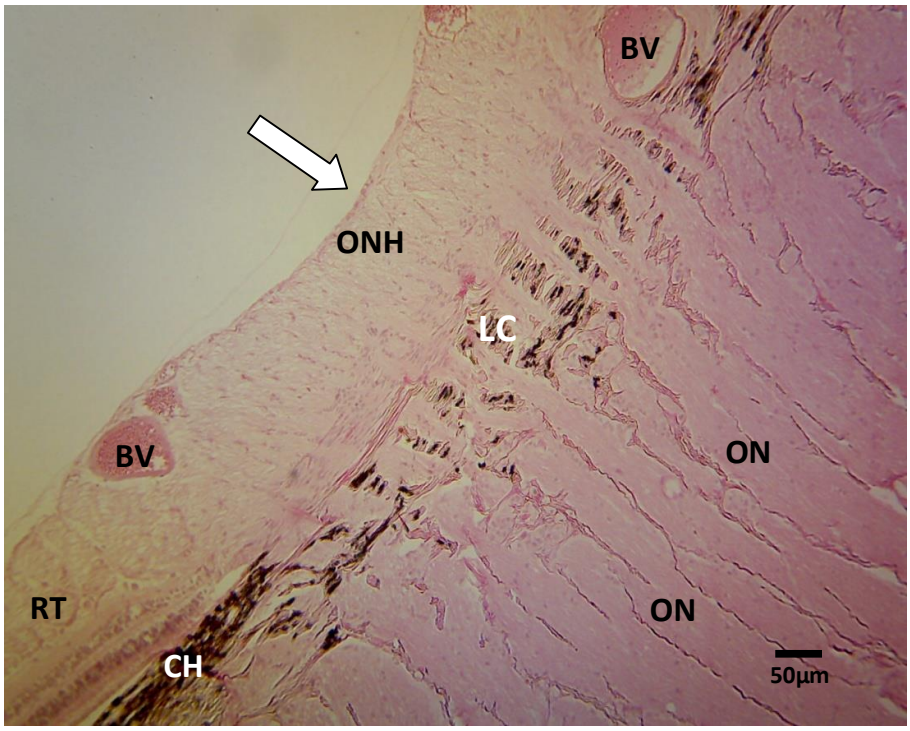

FIG. 121 Section of the adult optic nerve head (optic papilla) showing the Lamina cribrosa **LC**; Optic nerve head **ONH**; Choroid **CH**; Blood vessel **BV**; Optic nerve bundles **ON**; Retina **RT**; Physiologic cup (arrow). H&E.

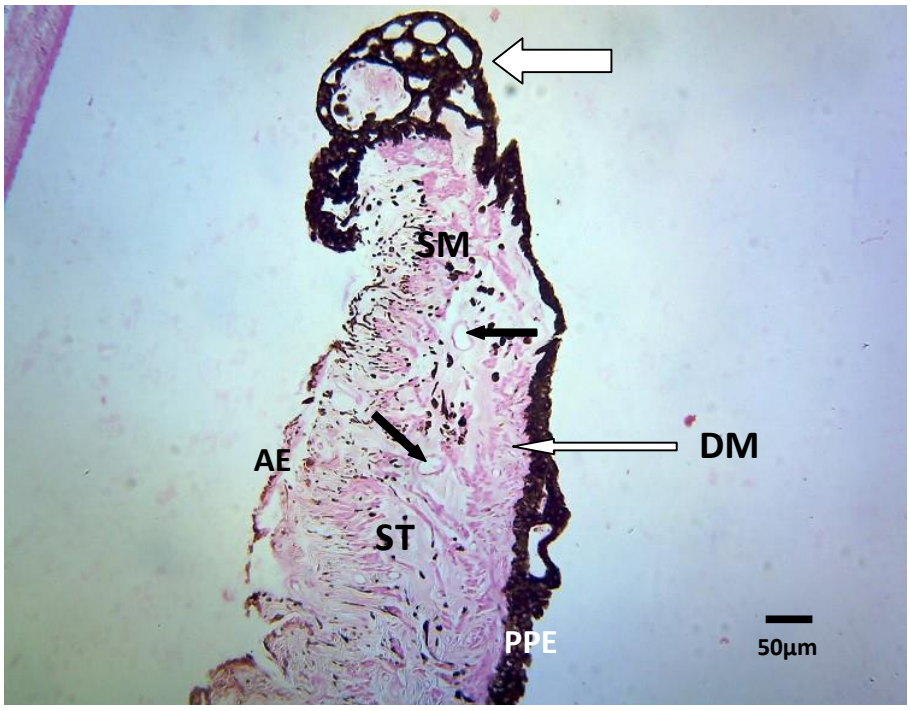

FIG. 122 Section of adult iris showing the anterior epithelium **AE**; Stroma **ST**; Pigmented posterior epithelium **PPE**; Sphincter Pupillae muscle **SM**; Dilator pupillae muscle **DM**; Granula iridica (big arrow); Blood vessels (black arrows). Note the pigment cells scattered within the stroma. H&E

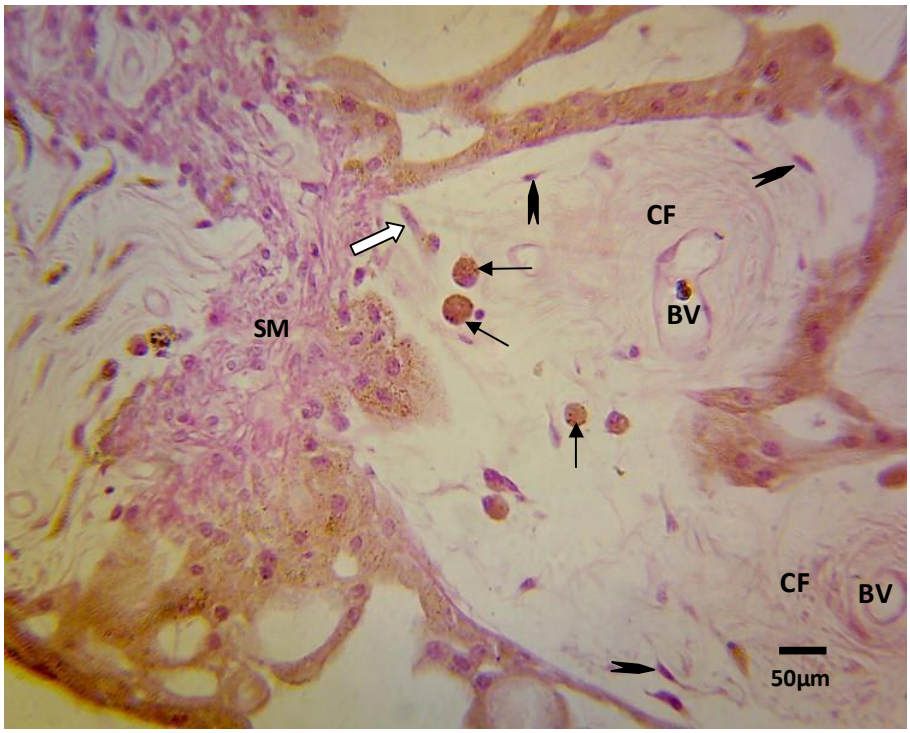

FIG.123 Section of the adult granula iridica showing blood vessels **BV**; Connective tissue fibres **CF**; Fibrocytes (black arrow heads); Sphincter pupillae muscle **SM**; Smooth muscle cell (white arrow); pigment cells (black arrows).H&E

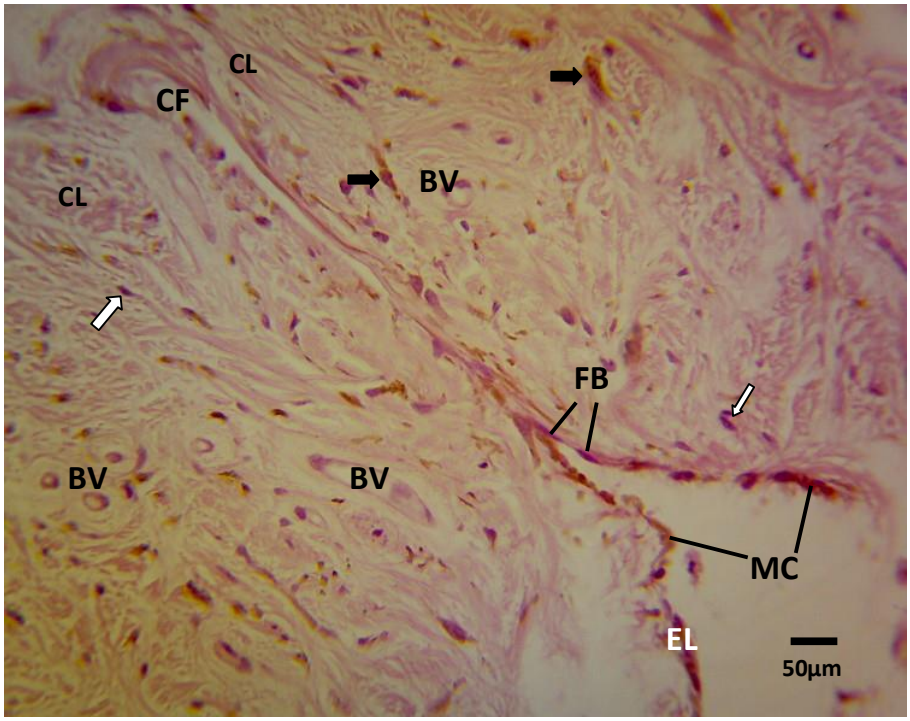

Fig. 124 Section of the Adult iris showing the anterior epithelial layer **EL** and stroma; Melanocytes **MC**; Fibroblasts **FB**; Blood vessels **BV**; Crypts of Fuchs **CF**; Collagen fibres **CL**; Chromatophores (black arrows); Fibrocytes (white arrows).H&E.

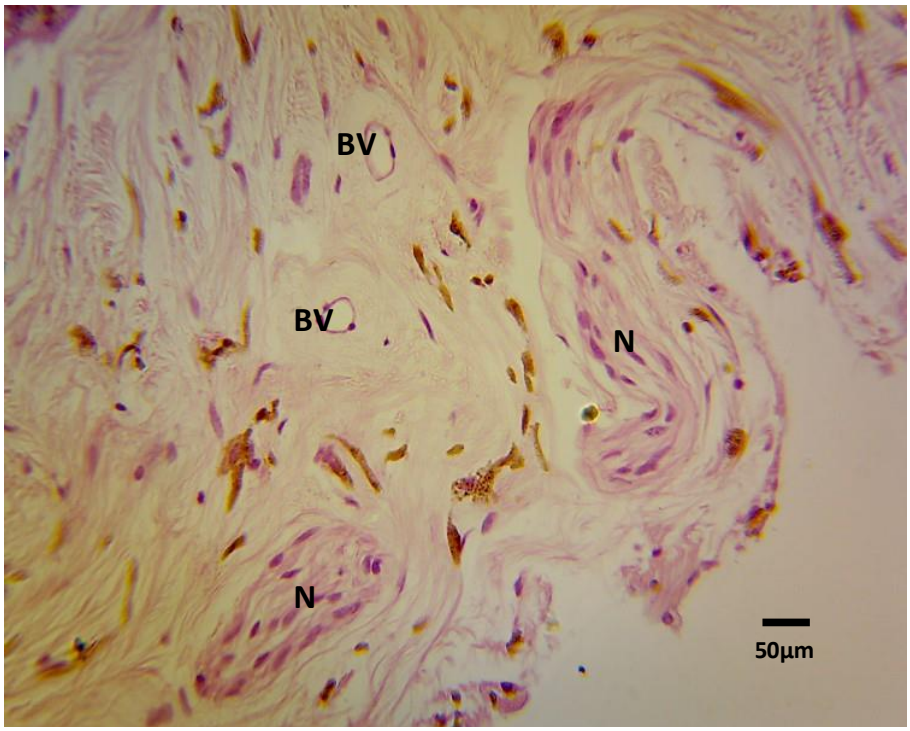

FIG. 125 Section of the adult iris showing Nerves **N**; Note the concentric collagen fibres around the vessels **BV**.H&E.

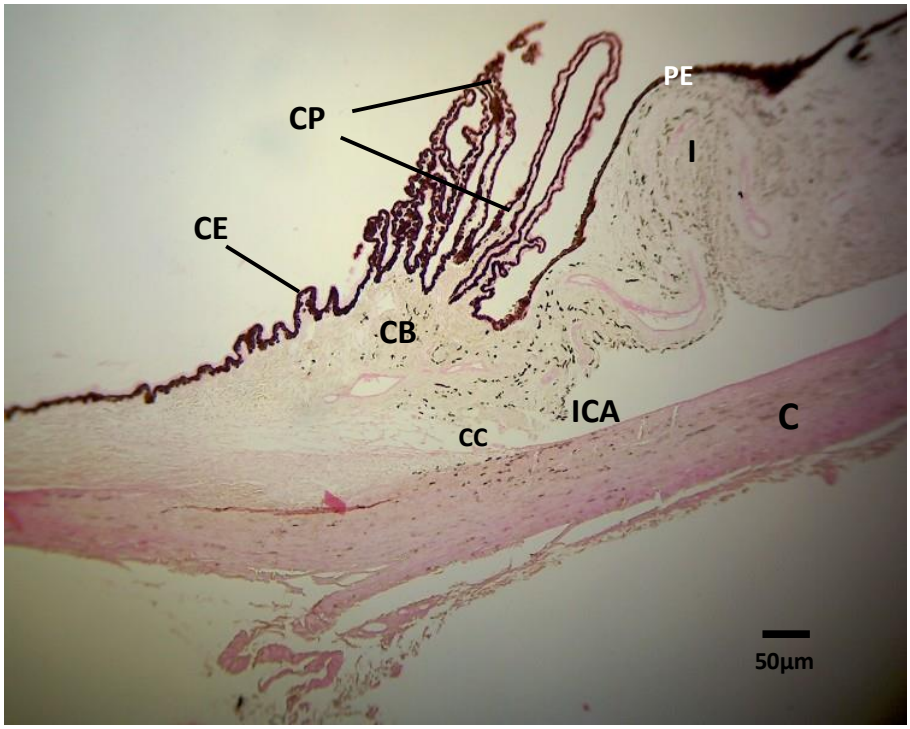

FIG. 126 Section of the adult anterior eye showing the Iris **I**; pigmented posterior epithelium of iris **PE**; Iridocorneal angle **ICA**; Ciliary cleft **CC**; Cornea **C**; Ciliary body **CB**; Ciliary processes **CP**; Ciliary body epithelium **CE.**H&E**.**

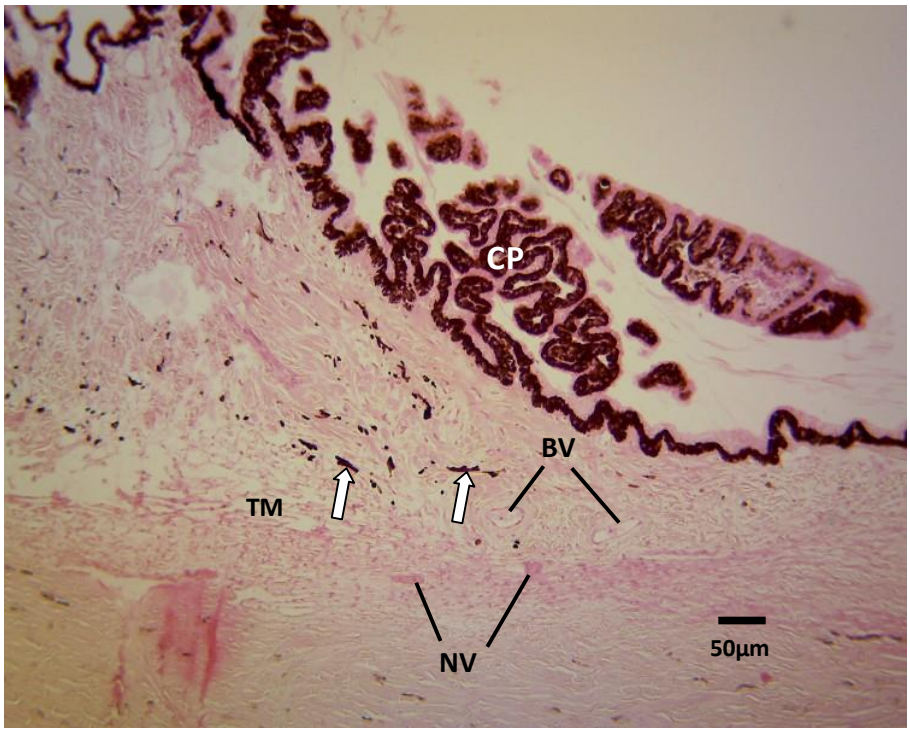

FIG. 127 Section of the adult Ciliary body showing trabecular meshwork **TM**; Ciliary Processes **CP;** Blood vessels **BV**; Nerves **NV.**H&E

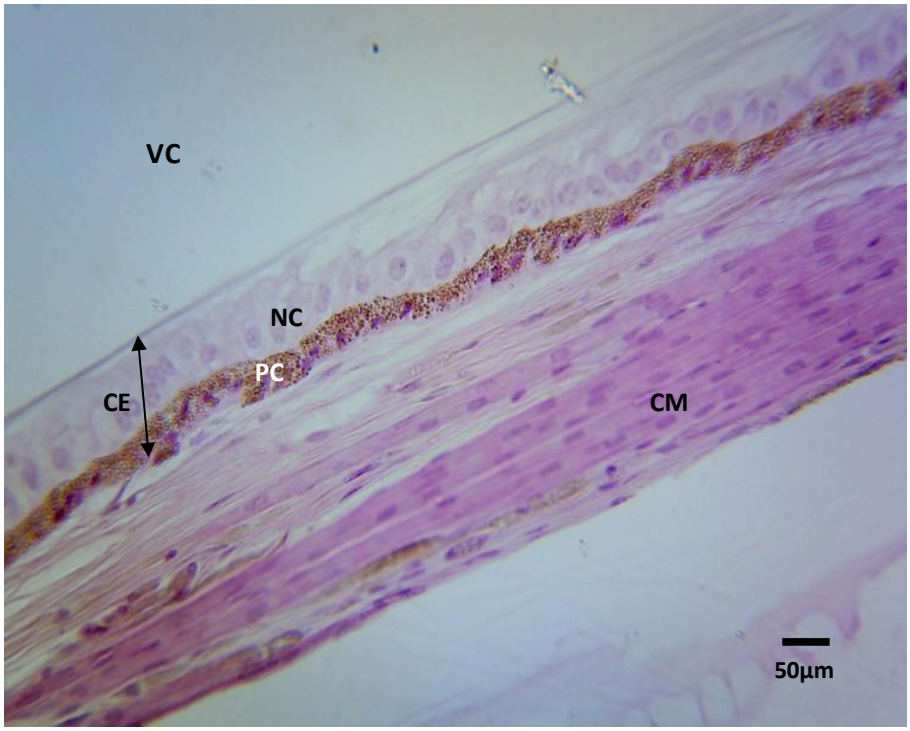

FIG. 128 Section of the adult ciliary body showing the outer pigmented cell layer **PC,** the inner non-pigmented cell layer **NC** of the bilayered columnar ciliary epithelium **CE;** Ciliary musculature **CM** (smooth muscles); Vitreous chamber **VC.**H&E.

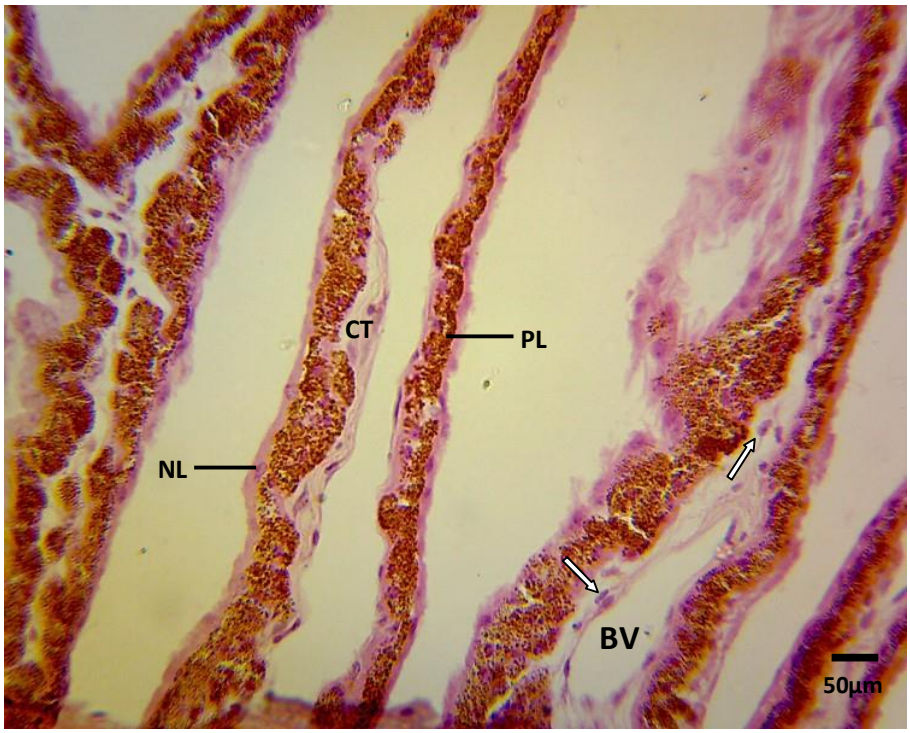

FIG. 129 Section of adult ciliary processes showing Blood vessels **BV;** Connective tissue stroma **CT;** pigmented outer cuboidal cell layer **PL;** Non-pigmented inner cuboidal cell layer **NL;** Fibroblasts (arrows). H&E.

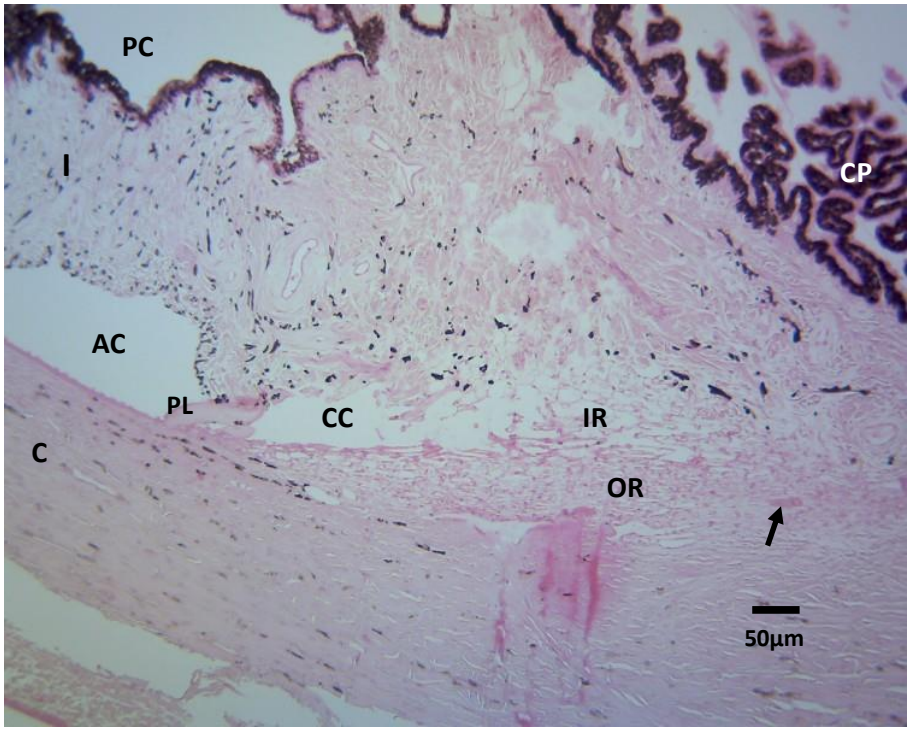

FIG. 130 Section of the adult anterior eye showing Cornea **C**; Anterior chamber **AC;** Iris **I**; Posterior chamber **PC;** Pectinate ligament **PL;** Ciliary cleft **CC;** Inner region of Ciliary cleft **IR;** Outer region of Ciliary cleft **OR;** Nerve (arrow)**;** Ciliary processes **CP**.H&E

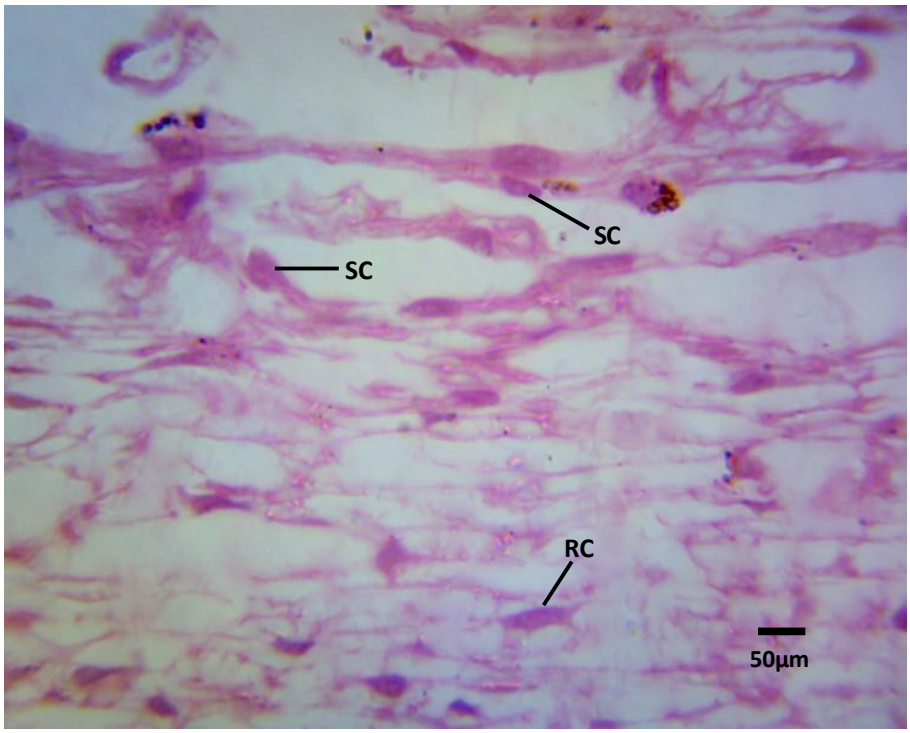

FIG. 131 Section of adult ciliary body trabecular meshwork showing Reticular cell **RC;** Squamous cells **SC,** lining the spaces. H&E.

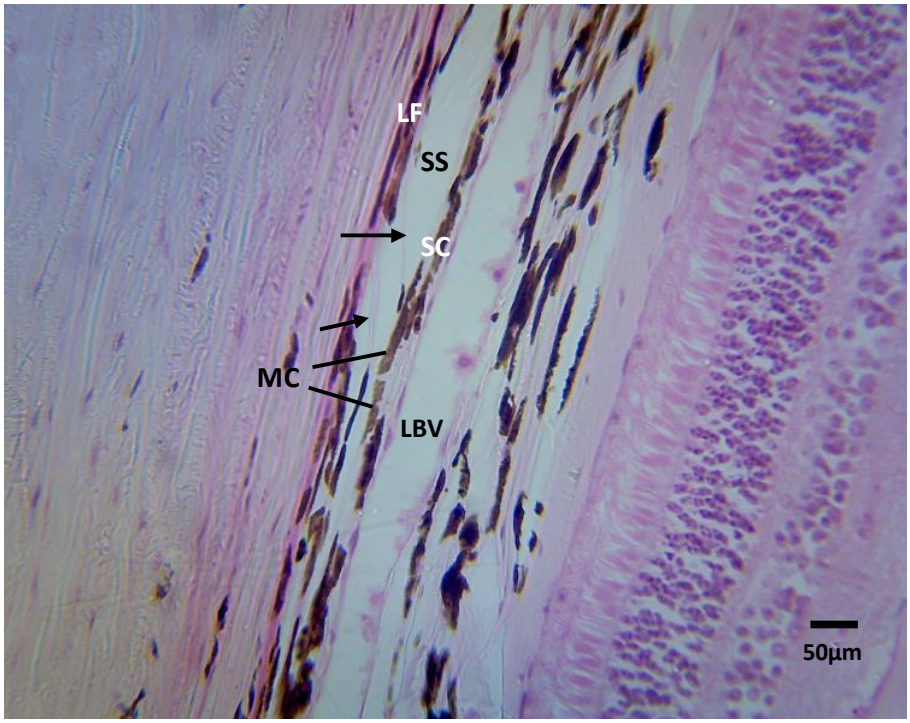

FIG.132 Section of the adult eye showing Lamina fusca **LF;** Suprachoroidal space **SS**; Suprachoroidea **SC;** Collagenous lamellae (arrows); Melanocytes **MC;** Choroidal large blood vessel **LBV.** H&E

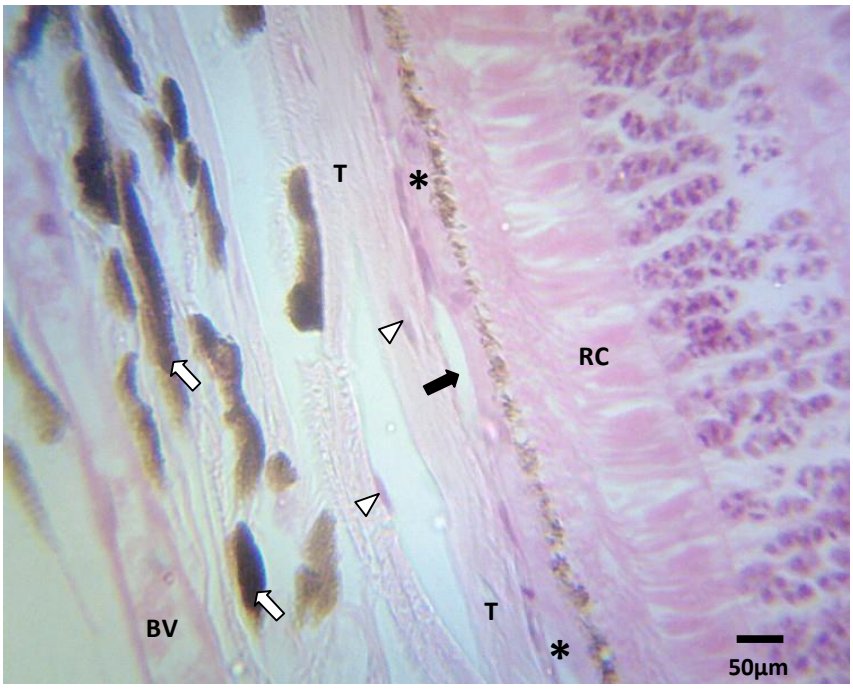

FIG.133 Section of the adult choroid (transition region) showing blood vessel **BV;** Melanocytes (white arrows); Choriocapilaris (black arrow); Tapetum fibrosum **T;** Fibrocytes (arrow heads); retinal pigment epithelium (asterix); retinal rod and cone layer **RC**. Note the scanty pigment deposition in the retinal pigment epithelium. H&E.

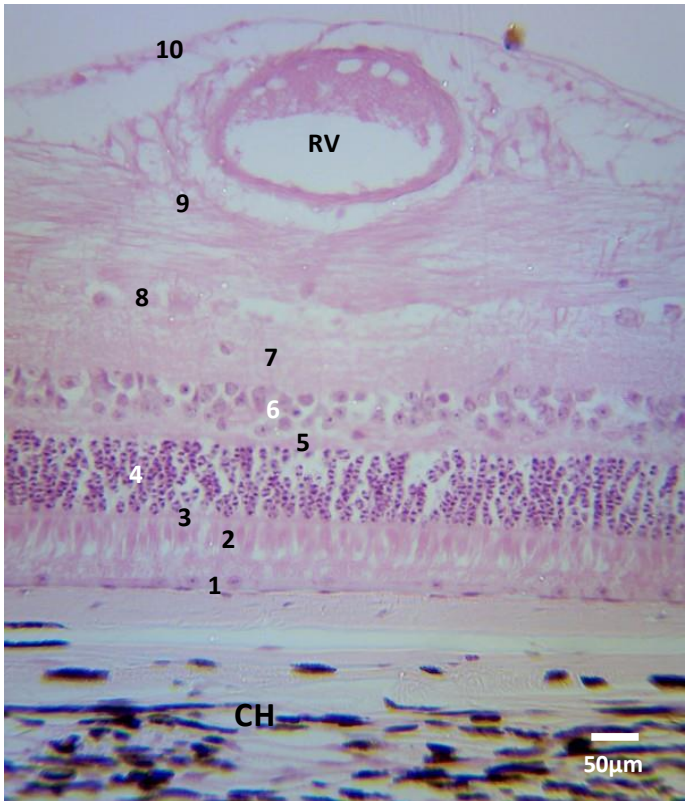

FIG.134 Section of the adult retina; **1**, Retinal pigment epithelium**; 2**, rod and cone layer; **3**, external limiting membrane; **4**, Outer nuclear layer; **5**, outer plexiform layer; **6**,inner nuclear layer; **7**, inner plexiform layer; **8**, ganglion cell layer; **9**, nerve fibre layer; **10**, inner limiting membrane; retinal blood vessel **RV;** Choroid **CH.**H&E.

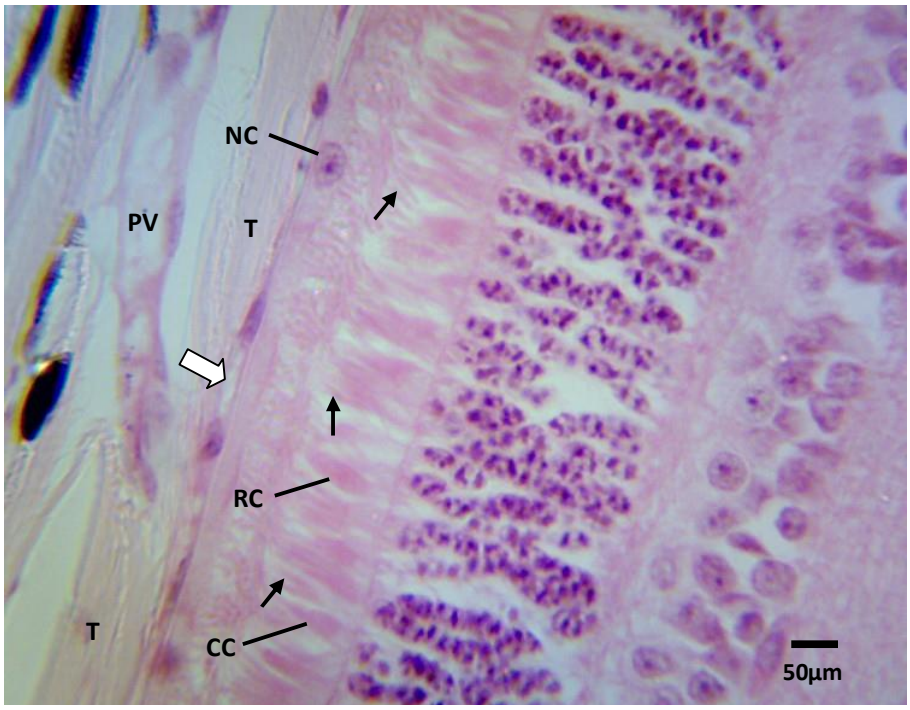

FIG. 135 Section of the adult retina (tapetal region); Nucleus of a retinal pigment epithelial cell **NC;** apical processes of the pigment epithelium (black arrows); Rod cell **RC;** Cone cell **CC;** Tapetum fibrosum **T;** perpendicular choroidal vessel **PV;** Choriocapilaris (white arrow). Note the absence of pigments in the retinal pigment cells. H&E.

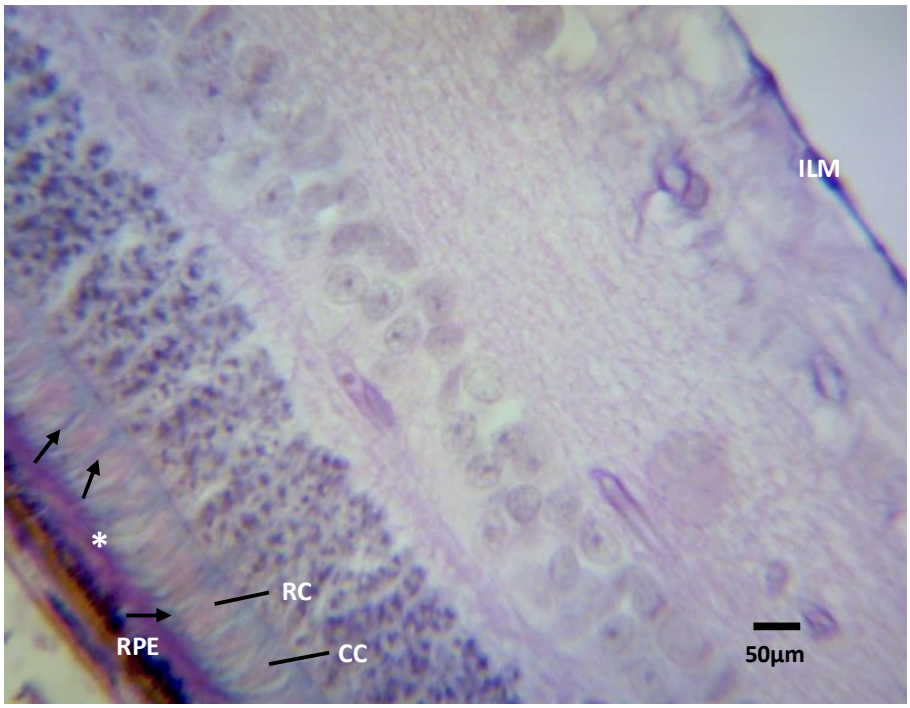

FIG. 136 Section of the adult retina (non-tapetal region).AB positive apical processes of the pigment epithelium (black arrows); Rod cell **RC;** Cone cell **CC;** Retinal pigment epithelium **RPE**; outer segments of rods and cones surrounded by retinal pigment apical processes (asterix); Inner limiting memberane **ILM.** Note the presence of pigments in the retinal pigment cells. AB-PAS. pH 2.5

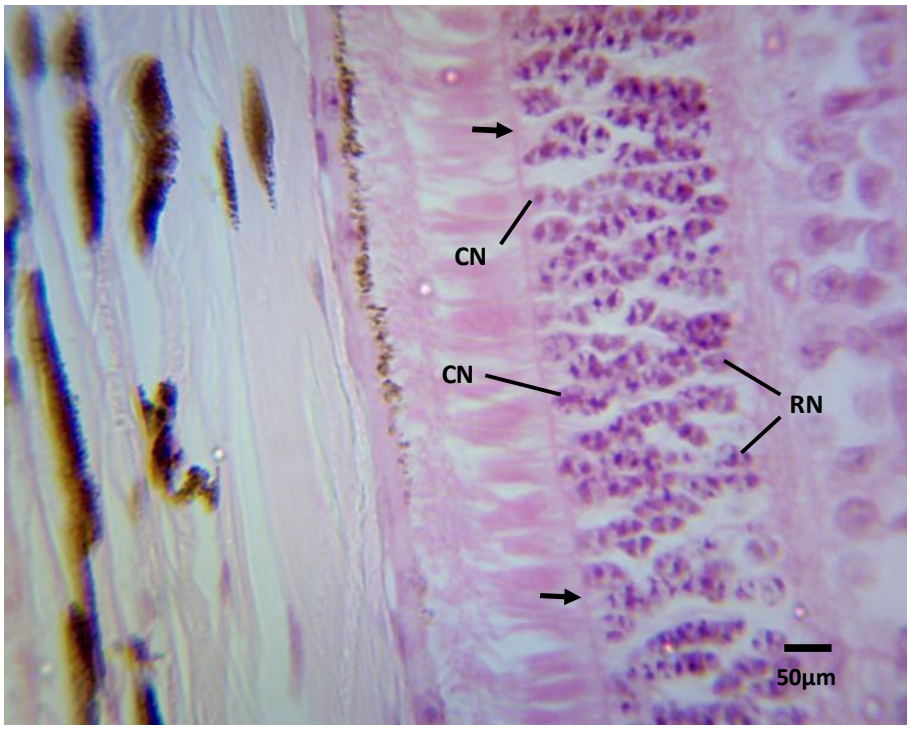

FIG.137 Section of the adult retina showing Cone cell nuclei **CN;** Rod cell nuclei **RN;** Outer limiting membrane (arrows).H&E.

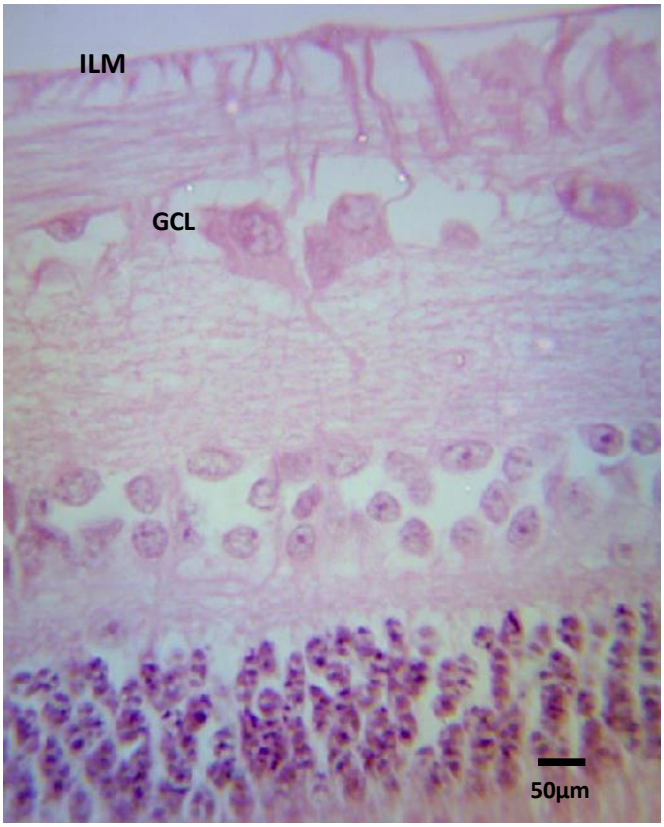

FIG.138 Section of the adult retina showing Inner limiting membrane **ILM;** Ganglion cell layer **GCL.**H&E.

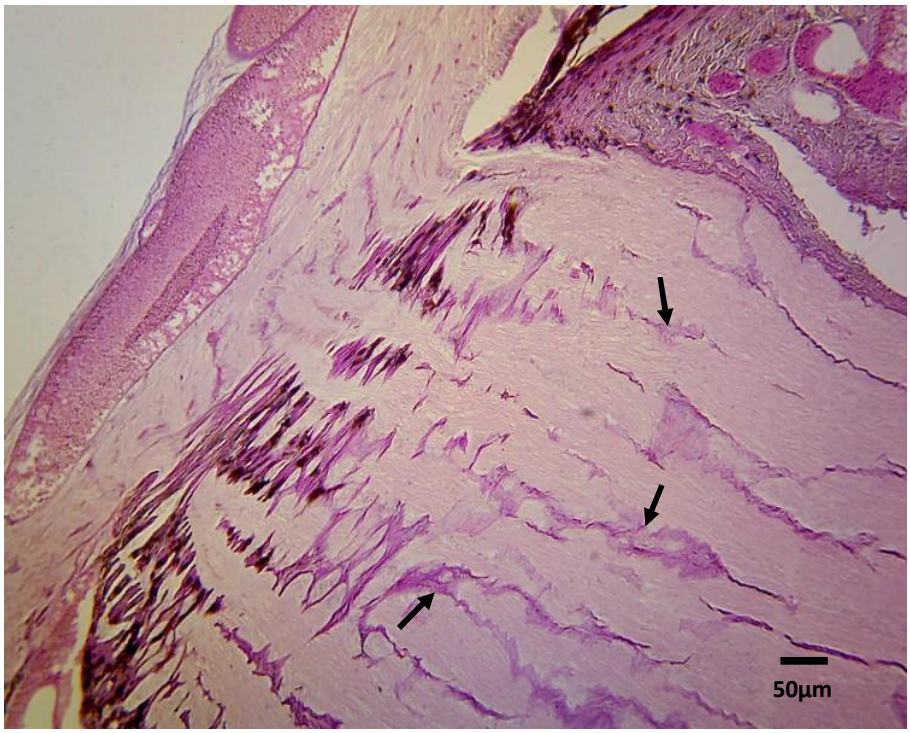

FIG. 139 Section of the adult Optic nerve head showing the PAS positive connective tissue septae (arrows) dividing the optic nerve into bundles. X 100 AB-PAS. pH 2.5

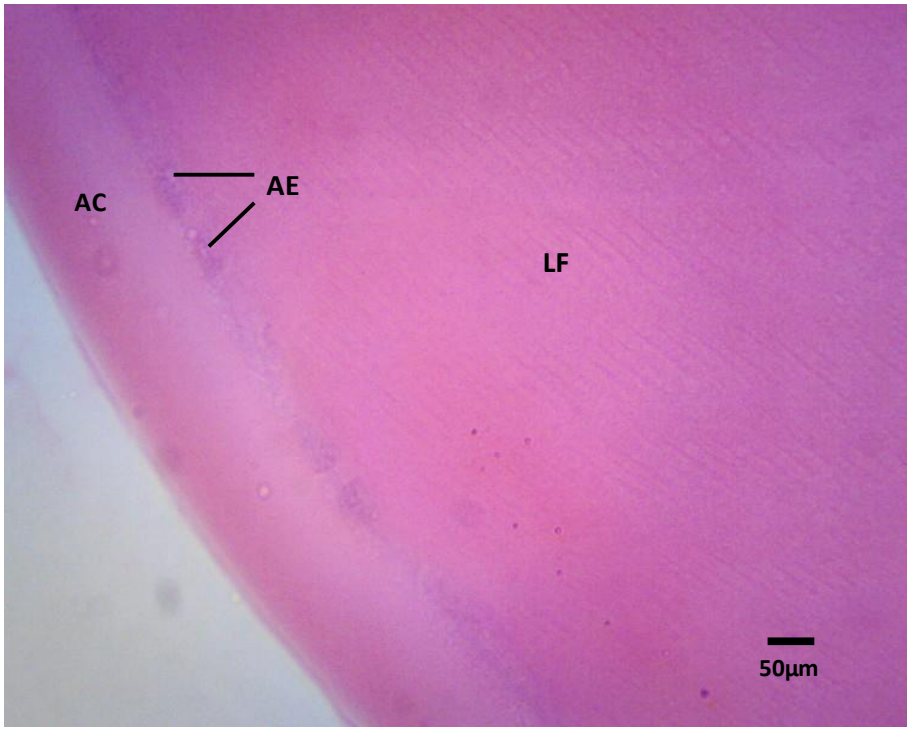

FIG. 140 Section of the adult lens showing the anterior capsule **AC;** anterior epithelium **AE;** Lens fibres **LF.** H&E.

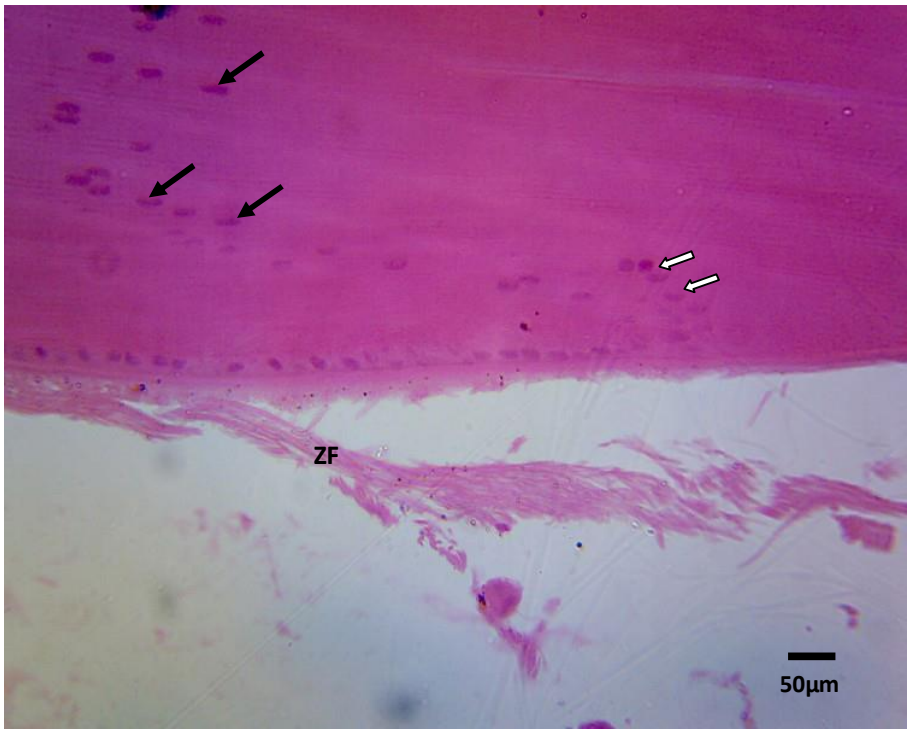

FIG.141 Section of the adult lens (equatorial region) showing Lens bow (white arrows)**;** Lens nuclei (black arrows); Zonular fibres **ZF.**H&E.

TABLE 1. Comparative histometric values of the corneal thickness at different stages (Mean ± SE)

PARAMETER	2nd MONTH	3rd MONTH	4th MONTH	NEONATE	ADULT
CCT	68.50±7.14[a]	90.47±8.85[a]	81.71±14.11[a]	91.61±15.81[a]	242.29±23.95[b]
PCT	71.20±6.93[a]	68.35±4.73[a]	88.87±15.26[a]	90.99±9.08[a]	179.34±8.37[b]

KEY: CCT - Central corneal thickness; **PCT –** Peripheral corneal thickness.

* Different superscripts in a row indicate significant difference between the stages ($p<0.05$).

CHAPTER FIVE

DISCUSSION

Most of the specimens used in this study were of abattoir origin and age estimates were based on crownï toï rump lengths (CRL) of fetuses. Age estimation by dentition was also employed for the adults. The slight variations with the ages of the specimens do not invalidate the developmental chronology described since the specimens were cheap, allowing for the confirmation of observations in multiple specimens. Also the CRL was measured by the same individual following the same procedure. Age estimation using crown to rump length and dentition have been employed in studies (Waziri *et al*., 2012; Nwaogu and Anya, 2009; Igbokwe, 2006).

There have been no previous studies, from available literature, on the developmental morphology of the eyeball of the Red Sokoto goat spanning the prenatal, neonatal and adult stages. Studies on the eyeball were generally limited to only adult eyeballs in goats (Olopade *et al*., 2005), cattle (Khaled, 2003) and histogenesis in camel (Osman *et al.*, 2013). The present study showed that the eyeball of the Red Sokoto goat as in other mammals is composed of three concentric layers arranged concentrically; the outer fibrous layer, middle vascular layer and inner nervous layer.

The histogenesis and morphology of these layers are similar to earlier reports in other mammals (Samuelson, 2007; Samuelson, 1999; Cook, 1999).

Generally, the developmental changes observed, progressed posterior-anteriorly for the inner nervous and the middle vascular layers, and anterior ï posteriorly for the outer fibrous layer.

5.1 OUTER FIBROUS LAYER

5.1.1 Cornea

The corneal layers were similar to those of other mammals (Zieske, 2004). During their development, the epithelium and endothelium appeared before their basement membranes could be demonstrated suggesting that they could have contributed to the basement membrane formation.

The corneal primordium in fetuses aged about 45 days had an epithelium composed of a layer of cuboidal cells centrally which acquired a superficial layer of spindle shaped cells towards the periphery. The epithelium added another layer at about 59 days. The epithelium was continuous and similar to the developing epidermis of the eyelid bud. This is supported by the findings that both the corneal epithelium and the epidermis are derived from the surface ectoderm (Sinowatz, 2010; Cook, 1999). The stroma at this stage was composed of loosely arranged mesenchyme that originated from the neural crest cells (Reme *et al*., 1983).

Development of the limbus as at day 75 was obvious with blood vessels and few melanocytes appearing in the stroma in the limbal region along with fibrocytes and fibroblasts which had started depositing collagen fibres.

The vesicular nuclei of the basal layer of cells of the corneal epithelium as at day 105 indicates that the cells were active and probably synthesizing and secreting the Bowmanôs layer which was demonstrated at this stage in the specimen studied. This finding is supported by Sinowatz (2010) and Gordon *et al*., (1994) who demonstrated that the corneal epithelial cells from embryos are capable of synthesizing type V collagen, which is one of the constituents of the Bowmanôs layer. The Bowmanôs layer appears at about day 135 in man (Cook, 1999).

In the neonate, the epithelium was 3-4 cell layers deep. The basal layer of the epithelium had tall columnar cells with vesicular nuclei which indicated that they were active, probably secreting more of the basement membrane. The cell layer superficial to the basal cells became increasingly heterochromatic and flat towards the outer layer where they are desquamated. The stratified squamous epithelial type is necessary for the cornea at this stage since it is exposed to abrasion thus necessitating high cell turn over. Peripherally, at the limbus some cells of the basal layer of the epithelium acquired pigment granules. The appearance of the pigment granules at this stage could be as a result of light incidence on the globe and the need to restrict the passage of light into the eyeball through the cornea alone. In the adult, the corneal epithelium was composed of 5 ï 8 layers of cells. The increase in the number of cellular layers is for protection since the adult eye is exposed to irritation and possible abrasion during blinking as the blinking reflex has fully developed at this stage. The mitotic basal cells observed in this layer at this stage served to replace the desquamated cells from the superficial layer of cells (Freshney *et al*., 2007). The lymphocytes observed in the epithelium at this stage are for immunological defense since the cornea is exposed to pathogens. The cornea was thicker centrally than peripherally; this finding is supported by earlier findings in most other domestic animals (Samuelson, 1999). This feature probably gives the cornea the optimum curvature to enhance its light gathering and refraction properties. The basal cells at the limbus were pigmented thereby restricting the passage of light rays into the eyeball through the cornea only.

The stroma of the cornea as at day 45 was made up of mesenchymal cells and PAS positive fibroblasts with an extracellular matrix that was slightly AB positive. The PAS positive cells indicate the presence of mucosubstances in the cytoplasm of the fibroblasts while the slightly AB positive extracellular matrix shows the presence of sulphated connective tissue mucin which may include chondroitin-4-sulphate (CS) and keratan sulphate (KS) (Cook, 1997;

Junqueira and Carneiro 2005). Mesenchymal cells at the region of the limbus were differentiating into pigment cells evidenced by the deposition of pigment granules deposited in their cytoplasm. These pigment cells differentiated further and got more numerous as the eye developed further.

The corneal endothelium was observed earlier in the third month of gestation in this study. The endothelium formation is initiated early during gestation in other mammals; at about day 34 in monkeys and day 39 in man (Cook, 1999). As at day 59, the stroma was more compact because more mesenchymal cells had differentiated into fibroblasts which were laying down collagen. This fibroblast differentiation was more obvious in the anterior and posterior regions of the developing cornea probably because they had more access to nutrition as the cornea is devoid of blood vessels and depended on diffusion through the anterior epithelium and posterior endothelium.

The posterior region of the corneal stroma was slightly PAS positive at this stage while at the neonatal stage, the stroma reacted positively to both PAS and AB techniques indicating the presence of both sulphated connective tissue mucin and carbohydrate. The adult corneal stroma was predominantly AB positive anteriorly and PAS positive posteriorly. This could be due to the higher concentration of the sulphated connective tissue mucin (keratan sulphate and chondroitin-4-sulphate) in the anterior region and a higher concentration of carbohydrates in the posterior region. This disagrees with previous findings in bovine corneal stroma where the KS/CS ratio is higher posteriorly than anteriorly (Bettelheim and Plessy, 1975; Castro *et al.* 1988). The concentration of keratan sulphate and chondroitin-4-sulphate in the anterior region could have protective functions from pathogens. Since they have high water affinity, they could also contribute to the hydration of the corneal epithelium along with the secretions of eye glands to avoid dryness of the corneal epithelium. The concentration of carbohydrate in the posterior region could be as a result of its diffusion

through the endothelium (Samuelson, 1999) from the aqueous humor into the cornea. The stroma in all the stages studied was devoid of lymphoid cells or other defensive cells. This disagrees with the findings of Maggs (2008). However, it is supported by the findings of Knop and Knop (2005). The presence of these lymphoid cells would have reduced the transparency of the cornea thus, leading to reduced visual acuity.

The endothelium as at day 45 was composed of low cuboidal cells up to the adult stage where it was made up of fewer low cuboidal to flattened cells suggesting that the individual cells were spreading out to compensate for the increase in the corneal surface area as growth and development increased the size of the cornea as well as death/loss of endothelial cells. This is because the endothelial cells do not divide. These findings are corroborated by those of Van Horn *et al*. (1977), Gwin *et al*. (1983) and Bourne (2003).

In this study, the Descemetôs membrane was first demonstrated at about day 75 while the Bowmanôs membrane was demonstrated at about day 105. However, the Descemetôs membrane got thicker with age and was thickest at the adult stage. This suggests that it was constantly being produced. The loss of endothelial cells, which are important for maintaining the transparency and nutrition of the cornea, and the increase in the thickness of the Descemetôs membrane with advancing age, could be one of the reasons for reduced visual acuity in old individuals.

The central and peripheral corneal thicknesses of the adults (242.29 ± 23.95; 179.34 ± 8.37) were significantly higher ($p < 0.05$) than those of the fetuses of 2nd (68.50 ± 7.14; 71.20 ± 6.93), 3rd (90.47 ± 8.85; 68.35 ± 4.73) and 4th (81.71 ± 14.11; 88.87 ± 15.26) months of gestation and also that of the neonate (91.61 ± 15.81; 90.99 ± 9.08). For the adult stage, the higher central corneal thickness finding is in consonance with that of most other domestic animals (Samuelson, 1999).

5.1.2 Sclera

The scleral primordium as at day 45 was only represented by the PAS positive outer region of mesenchymal condensation surrounding the globe. This condensation was continuous with the corneal primordium at the limbus which at this stage had started developing. This has been reported in the developing human eyeball which showed developmental process in the limbus by the sixth week (Sellheyer and Spitnaz, 1988; Snell and Lemp, 1989).

Development of the sclera proceeded with the appearance of blood vessels and nerves in the outer region of the scleral primordium by day 59. This represents the primordia of the episclera which became quite recognizable by day 75. The scanty collagen fibres seen in the region of the future scleral stroma were being laid down by the fibroblasts in that region initiating the development of the sclera stroma. The caprine scleral layers therefore differentiates from outside to inside. This differs with human sclera which has been reported to differentiate from inside to outside (Duke ï Elder and Cook, 1966; Weal, 1982; Sellheyer and Spitnaz, 1988). The scleral layers were complete by day 105 with the appearance of the innermost layer of the sclera, the lamina fusca.

The number of melanocytes seen in the sclera and other regions of the eye increased with age especially after birth suggesting that the incidence of light on the cells may have stimulated increased production of pigments or the differentiation of the melanocytes from mesenchymal cells.

5.1.3 Lamina Cribrosa

Differentiation of the lamina cribrosa was obvious by day 75 as few melanocytes and collagen depositing fibroblasts were observed in the region destined to become the lamina cribrosa. This initiation of the differentiation starts at about day 90 in man (Cook *et al*., 1991).

At the neonatal stage, it was fully differentiated and was composed of astrocytes, fibroblasts and collagenous fibres. The fibroblasts lay down the collagenous fibres that provide structural support and the astrocytes are essential for the maintenance of the axons of neurons of the optic nerve that passes through the lamina cribrosa.

5.2 MIDDLE VASCULAR COAT

5.2.1 Iris

A clump of mesenchymal cells at the rim of the optic cup represented the iris as at day 45. The iridopupillary membrane and tunica vasculosa lentis were also seen at this stage. The iridopupillary membrane has been reported to be present in the human fetal iris by the 11^{th} to 12^{th} gestational week (Wai e*t al*., 2002). Pigmentation of the iridal stroma had started by this stage as shown by the presence of melanocytes within the mesenchymal cells of the iridal primordium. Developing iridal smooth muscle and blood vessels were seen in the iridal stroma as at day 59. These muscle cells contained pigment granules and were in close association with the posterior pigmented epithelium of the iris and also contained pigment granules suggesting that the muscle myocytes could have differentiated from the posterior pigment epithelium. This finding is supported by previous findings by Yamashita and Sohal, (1986). This radially oriented developing muscle is the dilator pupillary muscle which is radially oriented in the adult. This dilator muscle orientation and early appearance is similar to the findings in the rabbit where the dilator pupillary muscle appears within the first trimester (Tamura and Smelsa, 1973). The iridal endothelial layer, which was present at this stage, met the corneal endothelium at the junction of the iris and the cornea forming the closed iridocorneal angle. The vessels of the iridopupillary membrane were continuous with the annular sinusoid surrounding the optic cup. These vessels supply the anterior surface of the developing lens and the anterior segment of the developing eye.

By day 75, the stroma of the iris became more vascularized with many blood vessels seen just posterior to the iridal endothelium (anterior epithelium of the iris). The constrictor pupillary muscle was present as at day 75 along with early formative stage of the granula iridica seen at the tip of the iris. The formation of the pectinate ligament was also obvious by day 75.

By day 105, the pupillary muscles had fully differentiated with the constrictor pupillary muscle appearing more developed than the dilator pupillary muscle. This concurs with early findings in species with horizontally elongated pupils (Prince *et al*., 1960). The dilator muscle appeared before the constrictor muscle; however it appears that its development was slowed down as the constrictor muscle was better developed than it at a later stage.

At the neonatal stage, the iridopupillary membrane had regressed. This membrane has been reported to regress within the first two postnatal weeks in dogs and around parturition in some domestic mammals (Aguirre *et al*., 1972; Cook, 1999). The regression of this membrane at this stage is to rid the eye of structures that will reduce vision because the goat, being a precocious animal, relies mostly on its vision for nutrition as well as detection and evasion of predators soon after birth. The melanocytes seen in the iridal stroma of neonates had few pigments in their cytoplasm unlike in the adult suggesting that exposure to light may have stimulatory effects on pigment production in ocular melanocytes. Strands of smooth muscle cells were observed running from the dilator muscle to the anterior layers of the posterior pigment epithelium peripherally. The strands probably serve as anchors and support for the underdeveloped dilator pupillary muscle.

In the adult, the granula iridica was made up of cysts lined by pigmented epithelium. Smooth muscle cells were seen at the basal region of the granula iridica. These features had been observed by Samuelson (1999). The granula iridica helps to control the amount of light that enters the eye through the pupil. The presence of muscle cells at its base suggests that it could

have some contractile properties and may augment the contraction of the constrictor pupillary muscle since they are in close proximity. The crypts of Fuschs seen in the iris of this species have been reported in other animals. It could be a feature that helps in draining the aqueous humour since they extend deep into the iridal stroma which contains numerous capillaries that could help drain off excess aqueous humour. Furthermore, these crypts only appeared in the adult when the fully matured ciliary body would have started producing large volumes of aqueous humour necessitating increased drainage rate to maintain the correct intraocular pressure. The posterior layers of the pigmented epithelium were composed of pigmented transitional epithelium which is an important feature as the pupillary muscles lend distensibility properties to the iris.

5.2.2 Ciliary Body

The initial development of the ciliary body appeared as vascular enlargements in the region of the ciliary body as at day 45. By day 59, the ciliary body had fully differentaited and was made up of ciliary folds and the developing iridocorneal angle. This is similar with reports on the development of the ciliary body in sheep (Saadatlou *et al*., 2013). The ciliary body and the ciliary folds are covered by bilayered epithelium made up of an inner layer of non pigmented columnar cells and outer layer of pigmented columnar cells. The iridocorneal angle at this stage was still closed. However, the trabecular meshwork had already started forming at this stage with the condensation of the corneoscleral and iridociliary regions. This pattern of development has been documented in rats by Reme *et al*. (1983a, 1983b). The vessels seen at this stage between the two regions of the developing trabecular meshwork were the anlage of the angular aqueous plexus. Development of the trabecular meshwork continues postnatally in animals born with their eyes still closed like the dog and cat (Williams, 1993; Samuelson and Gellat, 1984).

The initiation of the development of the ciliary body musculature and ciliary processes was evident by day 75. This was indicated by the presence of smooth muscle cells in the posterior region of the ciliary body as well as the branching of the ciliary folds into ciliary processes. The development of the ciliary body musculature therefore, starts at the posterior ciliary region while that of the ciliary processes proceeds anterior ï posteriorly because the folds just at the base of the iris started branching before the more caudal folds. The cores of these processes were vascularized. The ventral iridocorneal angle was also fully differentiated and opened at this stage suggesting that the ciliary processes could have started the production of aqueous humour and the pressure built up by the accumulation of the aqueous humour within the anterior chamber may have contributed to the opening of the iridocorneal angle at this stage to facilitate the drainage of the aqueous humour. The angular aqueous plexus was also well differentiated at this stage. This is probably because it is mainly involved in the drainage of the aqueous humour. These developmental changes suggest that the production and drainage of the aqueous humour starts late in the 2nd gestational month in this species.

The formation of the ciliary processes of the ciliary body was completed by day 105 evidenced by the branching of the caudal Ciliary folds of the ciliary body into ciliary processes. The inner non pigmented layer of the bilayered epithelium, covering the pars plana, was of the pseudostratified type and gradually changed to the simple columnar type towards the processes and finally to the simple cuboidal type over the processes with larger blood vessels in the core of the ciliary processes. The increased vascularization was to support the increased metabolic activities of the cells of the ciliary processes as the aqeous humour production increased. The iridocorneal angle was open at this stage. This could be as a result of the need to evacuate the aqueous humour produced by the fully differentiated ciliary body and ciliary processes. The meshworks of the ICA were lined by trabecular cells (fusiform shaped cells). The ciliary body musculature was fully differentiated and was mostly

made up of only meridionally oriented smooth muscle fibres. The pectinate ligament was observed at this stage anchoring the base of the iris to the inner surface of the peripheral cornea. It probably functions to strengthen the base of the iris as the opening of the iridocorneal angle could have weakened the iridal base.

At the neonatal stage, most of the features observed in the earlier stage matured further. However, the iridocorneal angle extended further into the ciliary body forming the ciliary cleft which would increase the efficiency of the outflow of the aqueous humour. The iridocorneal angle was also obviously differentiated into two regions; the uveal trabecular meshwork and the corneoscelral trabecular meshwork. This is in agreement with earlier reports on the iridocorneal angle in other mammals (Samuelson, 1999). The trabecular meshwork had vessels associated with it through which the aqueous humour is drained. The trabecular meshwork functions to sieve out particles moving through the iridocorneal angle while the trabecular cells lining the space also ingest particles giving the iridocorneal angle the ability to remove debris thereby serving a defensive function (Smith *et al*., 1986; Samuelson *et al*., 1985; Garcia, 1986; Johnson *et al*., 1990; Samuelson *et al*., 1984; Grierson and Lee, 1973; Sherwood and Richardson, 1981).

In the adult, numerous blood vessels melanocytes and nerves were observed in the ciliary body. This agrees with earlier findings in the sheep (Saadatlou *et al*., 2013). The increase in the number and size of blood vessels and melanocytes with development and maturation is for the supply of nutrients to the increasingly metabolically active cells and for regulation and control of light falling on the light sensitive parts of the eye respectively. The nerves serve to transmit impulses to and from the ciliary body for its accommodative activities.

Plasma cells were observed within the trabecular meshwork indicating that the trabecular meshwork could also have immunodefensive functions. The ciliary processes were covered

by a bilayered cuboidal epithelium. This agrees with the findings of Khaled, (2003) in the bovine eye and disagrees with that of Samuelson, (1999). This variation could be as a result of the seasonal variations in the activities of these cells in the production of aqueous humour since increased evaporation during the dry season can lead to faster depletion of the aqueous humour necessitating an increase in its production by these cells to balance the intraocular pressure. It could also be species dependent. Fibroblasts were also seen in the stroma of the ciliary processes suggesting that collagen deposition was still ongoing at this stage.

The ciliary body musculature was mainly made up of meridionally oriented fibres running from the ciliary body towards the ora serrata and few circularly and radially oriented myocytes, unlike in primates where the ciliary body musculature is more developed (Henderson, 1926; Tripathi, 1974) suggesting that the accommodative capability of this specie is very weak.

Fibrocytes of the anterior iridal surface lined the anterior surface of the pectinate ligament at its junction with the iris while cuboidal endothelial cells of the cornea lined it at its junction with the posterior surface of the cornea. Posteriorly, the pectinate ligament was lined by the squamous trabecular cells of the trabecular meshwork.

5.2.3 Choroid

As at day 45, the choroidal layers were not differentiated. However, the choriocapillaris was represented at this stage by the annular sinusoid. This agrees with the findings by Sellheyer, (1990) who documented that the choriocapillaris starts to differentiate during the 4^{th} and 5^{th} weeks. Periocular mesenchymal cells lined this vessel. The blood vessels seen at this stage in the future choroidal region are the choroidal blood vessels. Differentiation of the choroid starts with the choroidal blood vessels which is indicative of the structural and functional importance of vasculature to this eye coat (Cook, 1999).

By day 59, mesenchymal cells were seen differenting into fibroblasts in the posterior choroidal region, while the choroid was only represented anteriorly by the iridocorneal condensations indicating that choroidal differentiation and development starts from the posterior choroidal region and proceeds to the anterior region. There was also proliferation of blood vessels and pigments cells within the region of the future choroidal stroma at this stage. The fibroblasts in the tapetal region were bigger with more euchromatic nuclei than those in the non-tapetal region indicating that they were active cells, probably synthesizing materials for the formation of the tapetum fibrosum.

The large vessel layer of the choroid had appeared by day 75, represented by a region containing large blood vessels within the developing choroid. These vessels were connected to the choriocapillaris by smaller vessels traversing the choroid at perpendicular angles. The choroid as at this stage was made up of loose connective tissue containing fibroblasts and spindle shaped melanocytes oriented parallel to the retina. The orientation of the melanocytes serves to block out light from getting to the retina except through the pupil. Fibroblasts and fibrocytes were seen stacking up in the region dorsal to the optic nerve. The retinal pigment epithelial cells over this region were devoid of pigments. This is the initiation of the formation of tapetum fibrosum. The retinal pigment epithelium over this region is unpigmented; this feature allows light rays that escaped the photoreceptors to pass through the epithelium to the tapetum which in turn reflects it back to the photoreceptors thereby providing them with a second chance of stimulation.

By day 105, the choroid had fully differentiated as all the layers were present. The tapetum fibrosum was fully differentiated at this stage containing vesicular fibroblasts and occasional melanocytes. This agrees with earlier reports that the tapetum is fully developed before birth in ungulates (Samuelson, 1999) unlike in the dogs and cats where ocular development

continues postnatally. The vesicular fibroblasts suggest active protein synthesis; probably synthesizing connective tissue fibres of the tapetum fibrosum.

In the neonate, the choroid matured further with an increase in the number and size of melanocytes and blood vessels.

In the adult, the suprachoroidal space appeared between the outermost layers of the choroid (suprachoroidea) and the innermost layer of the sclera (lamina fusca). The two layers were attached to each other by collagenous lamellae traversing this space.

The outermost layer of the choroid contains elastic connective tissue (Samuelson, 1999) which suggests that it can expand and contract. This space may therefore be functioning as an expansion gap for the highly vascularized choroid. It could have appeared in the adult because the animal at this stage would be involved in activities that could raise the blood pressure which can engorge and expand the highly vascular loose connective tissue that makes up the choroidal stroma.

The dorsal portion of the choroid contained a layer of regularly arranged collagenous fibres, the tapetum lucidum. This structure is seen in most vertebrates and invertebrates except pigs, humans and squirrels (Shinozaki *et al*., 2010; Young and Braekevelt, 1993). It was oriented parallel to the retina. It functions to reflect light back to the photosensitive cells of the retina, providing another opportunity of stimulation of these cells, thus increasing retinal sensitivity in animals possessing it (Ollivier *et al*., 2004; Schwab *et al*., 2002). Its location and arrangement, behind the retina and parallel to it, positions it properly to do this function. The tapetum is useful to animals that are active in low light conditions (scotopic vision). It is responsible for the ñeye-shineò seen in the eyes of animals in dim light conditions. Only fibrocytes were seen interposed between the tapetal collagenous fibres indicating that they are

no longer actively laying down collagen fibres. The choroid in the adult also showed an increase in the size and number of melanocytes.

5.3 INNER NERVOUS COAT

The primordial retina was present as at day 45. It was made up to two layers; the outer retinal pigment epithelium and the inner neuroblastic layer. This level of retinal development is seen in most mammalian fetuses at this early stage (Cook *et al*., 1991) and also in fish (Gerd, 1975). The retinal pigment epithelial cells were cuboidal in the posterior region of the optic cup, and gradually changed to columnar cells from the equator to the rim of the optic cup. This suggests that the differentiation of the retinal pigment epithelium (RPE) proceeded from the posterior region to the rim of the cup since the primitive optic vesicle cells are columnar before becoming cuboidal and filled with melanin granules (Cook, 1999). The RPE over the region dorsal to the optic nerve head had fewer pigment granules in their cytoplasm. This was to allow light to get to the tapetum and be reflected from it as the tapetum fibrosum developed within the choroid in this region.

The inner neuroblastic layer was divided into an outer nuclear zone containing columnar cells in a pseudostratified arrangement and an inner anuclear zone. Mitotic cells were observed in the outer region of the nuclear layer. The mitoses seen commenced in the primitive single layered columnar cell layer of the optic cup and transformed it to the thickened pseudostratified columnar epithelium seen at this stage (Sinowatz, 2010). Mitotic cells seen at this stage in the outer region of the outer nuclear layer suggests that the retina is populated through this means before the differentiation into different retinal cell types. The outer nuclear zone showed signs of separation into two nuclear layers by the transient layers of Chievitz centrally (posteriorly), this division at this stage was not seen peripherally (anteriorly) suggesting that the retina has a horizontal differentiation gradient going from the

center towards periphery. This is similar to that reported in the dog and other mammals at this stage (Aguirre *et al*., 1972; Sinowatz, 2010). At this stage in the specimens studied, the developing optic nerve lacked connective tissue sheats and septae. The lamina cribrosa was also not seen. This suggests that the retinal ganglion cells whose axons make up the optic nerve had not differentiated at this stage. Axons from the ganglion cells have been reported to grow towards and into the optic stalk at a later stage of retinal differentiation (Sinowatz, 2010).

By day 59, the retina was clearly divided into two neuroblastic layers by the transient layer of Chievitz centrally while the peripheral retina still lagged behind in this aspect. The developing nerve fibre layer was observed at this stage along with nerve fibres of the optic nerve indicating that the ganglion cells had started differentiating by this stage. In man, the retinal ganglion cells are the first retinal cells to develop within the inner neuroblastic layer and its axons form the optic nerve (Spira and Hollenberg, 1973). Spherical cells were seen differentiating and migrating viterad within the inner neuroblastic layer. These cells could be the developing ganglion cells since they are the first to develop.

Differentiating spherical cells were seen in the outer nuclear layer. These could be the cell bodies of the Müller, amacrine and horizontal cells since they develop within the outer nuclear layer along with the rods and cones which mature last in the outermost region of the retina (Spira and Hollenberg, 1973). Large blood vessels were observed within the developing nerve fibre layer. They are for nutrition since the cells of the developing retina are very active at this stage.

The optic nerve, at this stage, had connective tissue sheats covering it and connective tissue septae separating the bundles. In man, optic nerve sheat formation commences in the 7th week but becomes defined in the 5th month (Cook, 1999). These sheats and septae serve to

protect the nerves and also as scaffold for the blood vessels supplying the optic nerve. Glial cells were seen. They serve to support the optic nerve cells. Fibrocytes were seen perpendicularly oriented to the nerve fibres at the cribiform. This indicates the initiation of the formation of the cribriform plate (lamina cribrosa) as it forms around the axons of the optic nerve presenting the sieve ï like structure (Cook, 1999). Towards the vitreous, the hyaloid vessel was surrounded by cells. These cells are glial cells displaced towards the center of the optic stalk by the axons of the optic nerve when they turned into the optic stalk. This tuft of cells is called the Bergmeisterôs Papilla (Kuwabara, 1975).

By day 75, the nerve fibre layer was very distinct with numerous blood vessels within it. The ganglion cell layer was evident throughout the retina. This layer appears at about the 7^{th} week in man (Cook, 1999). However, more cells were still differentiating and migrating to the developing ganglion cell layer from the inner neuroblastic layer. The ganglion cell layer contained cells at different stages of differentiation and maturation. Hence, differentiation and migration from the inner neuroblastic layer continued up to this stage even after the ganglion cell layer has been established. Centrally, the inner nuclear layer had separated from the outer neuroblastic layer. This separation had not taken place peripherally as the differentiating cells were still migrating inwards. A plexiform layer was seen between the inner nuclear layer and the ganglion cell layer. This plexiform layer contains fibres of the ganglion cells and fibres of the bipolar cells that constitute the inner nuclear layer along with the Müller cells, horizontal cells and the amacrine cells. Thus the connection between these two layers was established at this stage. The developing inner nuclear layer was also separated from the outer neuroblastic layer by another plexiform layer. These Plexiform layers, separating the ganglion cell layer from the inner nuclear layer and the inner nuclear layer from the outer neuroblastic layer are the inner plexiform and outer plexiform layers, respectively. Hence, the two plexiform layers appeared at this stage. A single layer of cells

was seen differentiating at the outer margin of the outer neuroblastic layer. This layer represents the outer nuclear layer containing the cell bodies of the rods and cones. The primitive optic cup columnar cells were still seen in all the layers formed probably providing a pool from which specific retinal cells differentiate.

By day 105, the retina had all the 10 retinal layers present. The retina is fully developed in precocial animals including ungulates before birth.The region of interdigitation between the retinal pigment epithelium and the tips of the rods and cones was AB positive. This could be due to the presence of a viscous ground substance of glucosaminoglycans that surrounds the outer segments of the rods and cones (Spitznas and Hogan, 1970; Fine and Yanoff, 1979; Weale, 1963). This ground substance, because of its viscosity, could function as a barrier against penetration of pathogens as well as a lubricant between the processes of the retinal pigment epithelium and the outer segments of the rods and cones since they are interdigitated. Numerous spherical cells were arranged into 8-9 rows in the outer nuclear layer. Cells in the outermost row were larger, more euchromatic and better differentiated than the other cells in this layer that were smaller and more heterochromatic. The larger cells situated in the outer margin are the cell bodies of the cones while the smaller cells are the cell bodies of the rod cells. The cell bodies of the cone cells are bigger than those of the rod cells probably because of the number of protein pigments it synthesizes and stores. It has been reported to contain three pigments unlike the rod cell that contains just one pigment (Junqueira and Carneiro, 2005). It has been reported that cone photoreceptors differentiate before the rod photoreceptors (Levin *et al*., 1997). This explains the lag in differentiation between the cone and rod cell bodies.

The inner nuclear layer contains the cell bodies of bipolar cells, horizontal cells, Müller cells and amacrine cells arranged in 5 ï 6 rows. These cells function to support the other retinal

cells (Müller cells) and integrate, modulate and transmit impulses from the photoreceptors to the ganglion cells (horizontal, amacrine and bipolar cells) (Ofri, 2008b).

The inner plexiform layer was thicker than the outer plexiform layer. This is because it contains the fibres of all cell types in the inner nuclear layer and that of the ganglion cells as well as their synapses which are complex and integrated while the outer plexiform layer contains the fibres of the photoreceptors, bipolar cells and horizontal cells and their synapses which are simpler (Samuelson, 1999). Neuronal supportive cells were observed in the ganglion cells layer. They function to support the ganglion cells. The internal limiting membrane that was present was formed by the endfeet of the Müller cells. The nerve fibre layer which was mainly composed of axons of the ganglion cells extends between the ganglion cell layer and the internal limiting membrane. Blood vessels were seen in the entire retinal layer except the outer nuclear layer, photoreceptor layer and the retinal pigment epithelium. Large blood vessels were present in the nerve fibre layer and ganglion cell layer and gradually thinned down to capillaries at the inner nuclear layer. This vascular pattern conforms to the holangiotic pattern of vascularization that has been documented in other domestic ruminants and most other mammals (Ofri, 2008b).

The physiologic cup (a depression within the optic nerve head) appeared at this stage. This depression at the optic nerve head is probably due to increased intraocular pressure pushing against the optic nerve head and the cribriform plate which is not as strong as the other regions because of the openings in that region. Neuroglial cells were seen within the nerve bundles of the optic nerve. They serve as supportive cells to the nerves cells of the optic nerve.

In the neonate, the retinal layers seen in the fetus of about day 105 were all present and were undergoing maturation. The eosinophilic cuboidal retinal pigment epithelial cells had

euchromatic nuclei that were basally located. These are in agreement with Ts'o and Friedman, (1967) who documented that retinal pigment epithelial cells are generally mononucleate in most species except in rat and rabbit. These cellular features were seen only in the tapetal region as the cells in the non tapetal regions were all densely packed with pigment granules that masked their features while the cells in the tapetal regions were devoid of the granules. Over the transitional region (region between tapetal and non tapetal regions) the retinal pigment cells had less pigment granules in them and also presented alternating groups of pigmented and non pigmented cells. This gave it a sieveï like appearance.

The photoreceptor layer contained the rods and cones. The visual cell layer was separated from the outer nuclear layer by a thin PAS positive membrane. This membrane is seen in other mammals (Ofri, 2008b). This membrane holds the layer of rods and cones together (Samuelson, 1999) and could also function as a shield to protect the photoreceptor cell bodies from the metabolic wastes and heat from the highly metabolic layer of rods and cones. The outer nuclear layer contained 7- 8 rows of heterochromatic nuclei centrally but tapered down to 2 ï 3 rows peripherally. This reduction in number of nuclear layer was because of the reduction of the number of the photoreceptors towards the periphery as the sensory retina ends at the ora serrata (Junction of the sensory and non sensory retina).

The inner nuclear layer was composed of euchromatic nuclei arranged in 5 ï 7 rows centrally but tapered down to 1 ï 2 rows peripherally at the ora serrata. The ganglion cell layer was a single row of retinal ganglion cells and neuroglial cells. However, within the tapetal region, there was an area that contained large retinal ganglion cells arranged in 3 ï 4 rows. These ganglion cells were large with intense eosinophilic cytoplasm. This region is the area centralis or visual streak. This is an area of acute vision and usually contains numerous photoreceptors (mainly cones) therefore needs more ganglion cells for the transmission of visual impulses to the brain. The visual streak has been documented in many vertebrate

species (Silveira *et al.*, 1989; Wong, 1989; Henderson, 1985; Wakakuwa *et al.,* 1985). The ganglion cell layer can be made up of 6 ï 9 rows of cells in primates and various non mammalian animals (Samuelson, 1999).

The adult retina had similar anatomy with that of the neonate apart from the relative increase in size and other few differences.

The outer nuclear layer contained nuclei of rods and cones arranged in 6 ï 7 rows. The outer nuclear layer presented a decrease in the number of rows of cells from the fetal stage to the adult; Day 105 (8 ï 9 rows), Neonate (7 ï 8 rows) and adult (6 ï 7 rows). It could be that there was a pool of undifferentiated cells within the layer and was being gradually drawn from and depleted as differentiation/maturation progressed. It also suggests that non viable cells were removed to create space for the viable ones in the very competitive microenvironment of the retina; this presupposes that one of the retinal cell types has phagocytic capabilities. This cell type is probably the Müller cell since is does the supportive function to the other cell types.

The nerve fibre layer was composed of unmyelinated axons of the ganglion cells. The unmyelination of the axons could be to increase the retinal transparency. The optic nerve and optic nerve head were similar to that of the neonate.

5.4 LENS

The developing lens had already separated from the surface epithelium as at day 45. This separation occurs at about day 33 during gestation in man and day 25 in the dog (Cook *et al.*, 1991; Cook, 1999). At this stage, a vague lens capsule was present; the posterior lens surface was devoid of epithelium while the anterior lens epithelium was a mixed type of epithelium. The anterior lens epithelium was composed of cuboidal cells and columnar cells with some of

the cuboidal cells sitting on other cuboidal cells while the columnar cells traversed the thickness of the epithelium. Towards the equator, the epithelium was thicker, packed with cells and presented a pseudostratified arrangement. The epithelium extended beyond the equator slightly where it was still pseudostratified but had fewer cells with euchromatic nuclei. These findings disagree with Ofri (2008a) and Cook (1999) who documented that the anterior epithelium was cuboidal at this stage. The change in the epithelia cell type at this stage could be attributed to the fact that the cells were active at this stage synthesizing materials for their basement membrane (lens capsule) thus needed more space for storage. At the equator and beyond, the lens cells presented a pseudostratified arrangement because they were elongating in a diagonal direction to form the lens fibres. Lens fibres are formed only at the equator after the initial enlongation and obliteration of the lens cavity by the posterior lens epithelium (Cook, 1999). The euchromatic nature of the nuclei seen in cells at the equator is due to their active state.

The lens bow was present at this stage. It was formed by the nuclei of the elongated posterior epithelium of the optic vesicles. The tunica vasculosa lentis was present at this stage around the developing lens. It was formed by vessels of the hyaloid system (posteriorly and laterally) and vessels of the iridopupillary membrane (anteriorly). These vessels serve nutritional purpose for the otherwise avascular developing lens with very active cells undergoing rapid differentiation.

By day 59, the anterior lens epithelium had simple columnar cells centrally and pseudostratified columnar at and near the equator. This could be due to a reduced stage of activity for the cells lining the central portion since the lens capsule was better defined at this stage. However the still very active equatorial cells still retained their morphology and arrangement. The lens bow and the tunica vasculosa lentis were still present.

By day 105, the lens capsule was thicker anteriorly than posteriorly indicating that the lens capsule was continuously being laid down by the lens epithelium. This agrees with earlier reports in other mammals (Ofri, 2008a). The posterior lens capsule was thin because that surface is devoid of epithelial cells as the posterior lens epithelial cells had gone into the formation of the primary lens fibres. The central anterior epithelial cells, at this stage, were composed of cuboidal cells which became simple columnar cells at and near the equator where they elongate into secondary lens fibres. The vessels of the tunica vasculosa lentis were smaller and sparsely distributed at this stage indicating the initiation of its regression. The definitive form of the ciliary body also appeared at this period suggesting that there is a relationship between the appearance of the ciliary body and the regression of the tunica vasculosa lentis. One of the major functions of the ciliary body is the production of the aqueous humour which bathes the anterior surface of the lens and nourishes it. The ciliary body probably secretes a substance in the aqueous humour that inhibits and initiates the regression of the tunica vasculosa lentis since its nutritional function has been taken over by aqueous humour production. More so, the vessels would occlude the light path into the eye if they do not regress. In the dog and man, the pupillary membrane and tunica vasculosa lentis regresses on the 14th day post partum and in the last trimester respectively (Aguirre *et al*., 1972; OôRahilly, 1983).

In the neonate, the anterior epithelium was composed of cuboidal cells with euchromatic nuclei centrally and columnar cells with slightly heterochromatic nuclei equatorially. The lens bow was still present. It is formed by the nuclei of the cells at the equator when they elongate to form the lens fibres; their nuclei retain their central positions in the cytoplasm as the cells grow longer thus the different cell lengths present a curved nuclear arrangement referred to as the lens bow. The anterior vessels of the tunica vasculosa lentis had disappeared by this stage leaving few small blood vessels at the equator and on the posterior surface of the

lens. The anterior vessels may have regressed before the posterior vessels because the vitreous, which provides nourishment for the lens posteriorly, is very viscous which may lead to slower movement of molecules through it to the lens. This could necessitate the need for a longer duration of nutrient transport to the posterior lens surface via the hyaloid vessels than the anterior surface. The zonular fibres attaching the lens to the ciliary bodies were seen at this stage.

In the adult, the lens capsule was thicker anteriorly than posteriorly. The anterior lens epithelium was composed of squamous to cuboidal cells centrally and low columnar cells at the equator where they elongate and form secondary lens fibres. This finding agrees with that of Samuelson (1999). The lens capsule getting thicker at this stage implies that its lay down by the lens epithelial cells is a continuous process. The squamous shape of the epithelial cells from the initial columnar shape could be because the cells were wearing out with age from the continuous production of the lens capsule since there is no evidence of renewal for the cells. The lens bow was still seen at this stage especially close to the equator. Most of the lens fibres (lens cells) are devoid of nuclei as they lose their nuclei during maturation getting filled with proteins called crystallins (Sinowatz, 2010).

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