Kafkas Univ Vet Fak Derg
19 (2): 337-342, 2013
DOI: 10.9775/kvfd.2012.7846

Journal Home-Page: http://vetdergi.kafkas.edu.tr
Online Submission: http://vetdergikafkas.org

RESEARCH ARTICLE

The Study of Histogenesis of Sheep Fetus Iris

Mohammad Ali Ebrahimi SAADATLOU [1] Hamed TAVOUSI [2] Rana KEYHANMANESH [3]

[1] Department of Basic Sciences, Faculty of Veterinary Medicine, Tabriz Branch, Islamic Azad University, Tabriz - IRAN
[2] Graduated of Veterinary Medicine, Islamic Azad University of Tabriz, Tabriz - IRAN
[3] Department of Physiology, Faculty of Medicine, Tabriz University of Medical Sciences, Tabriz - IRAN

Makale Kodu (Article Code): KVFD-2012-7846

Summary

In this study, for determination of histogenesis of iris in sheep, 32 samples of sheep fetuses at different and certain ages were used. After sample preparation and fixation, their age were determined and histological and anatomical studies were done. In histological studies, the H&E staining and two other specific staining (verhoeff and Masson's Trichrome) were used. Obtained results demonstrated that a two-layered cup formed in first month but iris separation from the anterior and ceca part of retina occurred in second month. The separation of iris from ciliary body was completed in third month. From the beginning of third month, the iris formed changes and its circular shape changed to oval form so that its internal angle was larger than its external angle and this condition was retained until the end of pregnancy. General shape of pupil conformed to general shape of iris. It was histologically revealed that iris was formed from the anterior part of the optic cup just at the connection place of outer and inner layers in second month and thereafter a part of superficial ectoderm and choroid was added to it. The first signs of elastic fibers in iris as well as smooth muscles appearance were significant in second month. The posterior cells are pigmented from the beginning of the iris cell formation. Anterior epithelial tissue of the iris was simple squamous and two layer cuboidal cells formed its posterior epithelium which were stabilized in third month. The number of collagen and elastic fibers and smooth muscles and their size increase with age.

Keywords: *Iris, Sheep fetus, Histogenesis*

Koyun Fetus Irisinin Histogenesisi Üzerine Bir Çalışma

Özet

Bu çalışmada, koyunlarda iris histogenezinin belirlenmesi için farklı ve belirli yaşlarda 32 adet koyun fetus örneği kullanıldı. Numune hazırlama ve tesbit sonrası, yaş durumları belirlenerek histolojik ve anatomik çalışmalar gerçekleştirildi. Histolojik çalışmalarda, H & E boyama ve diğer iki spesifik boyama (Verhoeff ve Masson's Trichrome) kullanıldı. Elde edilen sonuçlar, iki katmanlı çanak formasyonunun ilk ayda oluşmasına rağmen retinanın anterior ve ceca bölümlerinden iris ayrılmasının ikinci ayda meydana geldiğini gösterdi. İrisin siliyer cisimden ayrılması üçüncü ayda tamamlandı. Üçüncü ayın başından itibaren, iris değişiklikleri oluşmuş ve onun iç açısının dış açısından daha büyük olabilmesi için sirküler şekli oval forma değiştiği ve bu durumun gebeliğin sonuna kadar muhafaza edildiği gözlemlendi. Pupilin genel şekli iris genel şekli ile uyumlu bulundu. Histolojik olarak ortaya çıkan bulguda, iris ikinci ayda dış ve iç katmanların bağlantı yerindeki optik çanağın anterior bölümünü oluşturduğu ve bundan sonra yüzeysel ektodermin bir kısmının ve koroidin buna eklendiği belirlendi. Iris elastik liflerinin yanı sıra düz kasların görünümünün ilk belirtileri ikinci ayda anlamlı bulundu. Posterior hücreler iris hücre formasyonu başlangıcından itibaren pigmentli halde gözlemlendi. Irisin anterior epitel dokusu, üçüncü ayda stabilize olan onun posterior epitelini oluşturan basit skuamöz ve iki katmanlı kübik hücreler şeklinde olduğu belirlendi. Kollajen ve elastik lifler ve düz kasların sayısı ve onların büyüklüğünün yaş ile arttığı sonucuna varıldı.

Anahtar sözcükler: *Iris, Koyun fetusu, Histogenez*

INTRODUCTION

Eye is one of important and sensory organs of the body and has many roles in relationship between animal and environment. Because of the importance and sensitiveness of eye, some researchers have worked about this organ in many years [1-7].

The eyeball has three thin tunics that, being in close apposition, form a laminated sheet that surrounds the partly liquid, partly gelatinous center [8]. The three tunics are an external fibrous tunic that gives form to and protects the eye-ball, it is the only complete tunic, a middle vascular

İletişim (Correspondence)
+0411 3364664
anatomist_2001@yahoo.com

tunic that consists largely of blood vessels and smooth muscles and is concerned with the nutrition of the eyeball and the regulation of the shape of the lens and size of the pupil, and an internal tissue and is the layer most directly concerned with vision [9].

The vascular tunic consists of three zones: choroid, ciliary body and iris. The third and smallest part of the vascular tunic is the iris, which is suspended between the cornea and lens. It is a flat ring of tissue attached at its periphery to the sclera and to the ciliary body the opening in the center is the pupil [9]. Iris is susceptible to various injuries because it is anterior part of eye. The normal structures of eye in sheep fetus have not been showed clearly yet so before studying about any abnormal cases, it seems helpful to evaluate the normal conditions of this organ. Therefore, this study proposed to demonstrate the histogenesis of iris in sheep fetus.

MATERIAL and METHODS

The present study was conducted on 32 samples of sheep fetus which were gathered from slaughterhouse in Tabriz city. The age of gathered fetuses were determined by the following formula [10];

X = 2.1(17+y), y = Fetus length, X = fetus age

Understudied groups included 30, 40, 50, 60, 80, 110, 135, and 150-day old fetuses (n=4 for each group). The fetuses with length less than 7 cm were fixed completely in 10% formalin and the eyes of others (fetuses with length more than 7 cm) were placed in 10% formalin for 48 h. For completing the fixation for internal parts of eye, 10% formalin was injected to internal part of the eye. Also, the dimension measurements were done by ruler and caliper. Tissue samples were prepared by usual histological method. Histological sections were stained with three staining methods; H&E (for general examination of tissue), Verhoeff (for examination of elastic fibers), Masson's tricrome (for examination of collagen fibers and muscle cells) [11]. Finally the samples were studied under light microscope.

RESULTS

The Results of the First Month Group or 30-Day Old Fetuses

Histological findings suggested that two-layer optic cup was form in this stage and its two inner and outer layers were separated from each other by intra retinal space. Outer layer of optic cup was clear with its small granules and formed retinal pigmented layer.

However, the anterior fifth of the inner layer and the pars ceca retinae of optic cup which was the separated place of iris and other tissues were seen circular and there was no sign of iris. The retinal granular layer was obvious clearly in Verhoeff staining and there was no sign of elastic fibers. In Masson's tricrome staining, the fine collagen fibers and mesenchyme cells were observable clearly and most of them were not differentiated *(Fig. 1)*.

Results of 40-Day Old Fetuses

In the early of second month, there was no clear sign of iris. The anterior fifth of the inner layer, the pars ceca retinae, remained one-layer thick. The pars ceca retina was not divided to iris and ciliary body yet. At this stage, retinal pigmented layer producing the iris pigmented layer was seen and the anterior part of choroid originated plenty of iris blood vessels. In addition, the influence of superficial ectoderm in forming of iris muscles was obvious. In Verhoeff staining, little signs of elastic fibers in undifferentiated part of iris was observable. These fibers were concentrated mostly in superficial ectoderm. In Masson's tricrome staining, the pigments continued from retina and collagen fibers accompanied with muscular cells differentiated from superficial ectoderm were obvious. Separation of iris from superior parts of eye has not been done yet and this demonstrated that the differentiation of this part was done in the middle of the second month.

The Results of 50-Day Old Fetuses

In the middle of second month, the separation of iris and ciliary body was very little and has not been separated completely. Iris had squamous epithelium in anterior part and unclear two-row epithelium in posterior part. The posterior layer cells were completely pigmented and their granules were clear. There was loose connective tissue among epitheliums consisted of fibrocytes, fibroblasts, blood

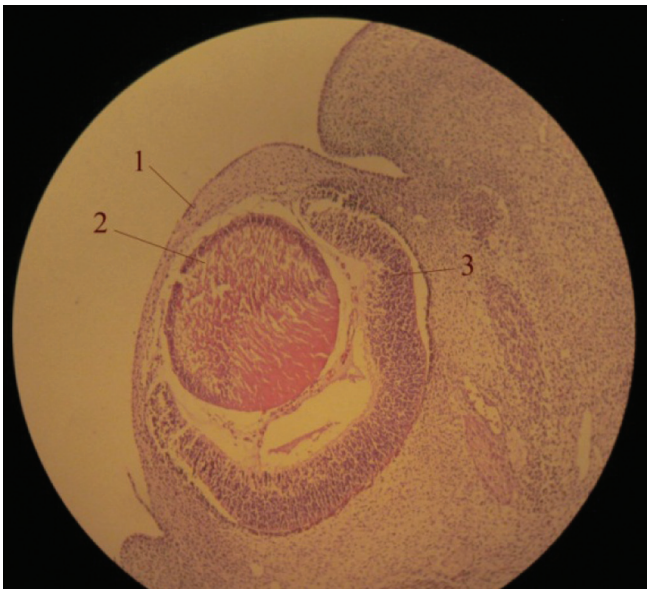

Fig 1. Histological findings in the first month group or 30-day old fetuses (H&E, ×100); **1**- Cornea, **2**- Lens, **3**- Optic cup

Şekil 1. Birinci ay veya 30-günlük fetus gruplarında histolojik bulgular (H&E, × 100); **1**- Kornea, **2**- Lens, **3**- Optik çanak

vessels and muscular cells. In Verhoeff staining, elastic cells were very weak and limited to vascular wall. The pigments of epithelium cells were obvious. In Masson's tricrome, blue-colored fine collagen fibers in connective tissue and flat muscle cells were significant under the epithelium *(Fig. 2)*.

The Results of 60-Day Old Fetuses

Cornea was oval, iris was dark and pupil was relatively circular. The average length and transverse of external diameter of iris and were 0.46±0.081 cm and 0.30±0.051 cm respectively and the average length and transverse diameter of pupil were 0.44±0.071 cm and 0.4±0.31 cm respectively *(Table 1)*.

In the late of this month, the iris existed but less separated. Iris was seen in the form of a very fine pigmented layer in anterior part of the eye. Iris was separated from other tissues slowly and grew like a small mass between the lens and cornea. Epithelium cells of anterior part were as simple squamous but posterior part of epithelium cells was stratified and much pigmented. The border among posterior epithelial cells was not clear due to compression. Fibrocytes were seen in middle position and fine muscular cells were visible. In Verhoeff staining, elastic fibers were very limited and posterior layer pigments were visible strongly. In Masson's tricrome staining, the fine collagen fibers in loose connective tissue among epithelium tissues were seen. Also smooth muscular cells in iris were significant. The general result showed that the iris was separated from ciliary body slowly in the late of second month *(Fig. 3)*.

The Results of 80-Day Old Fetuses (Third Month)

In this period, iris color was dark brown. The iris followed cornea shape and its internal angle was larger than external angle. The average length of external diameter and transverse of iris were 1.0±0.51 cm and 0.7±0.51 cm respectively and the average of length and transverse diameter of pupil were 0.7±0.032 cm and 0.3±0.24 cm respectively *(Table 1)*.

In this month, the iris was fine and separated from choroid. The anterior part of iris is smoother and covered with squamous epithelium. There was a loose connective tissue under the epithelium. There were fibroblasts, fibrocyts and smooth muscles under the epithelium. Blood

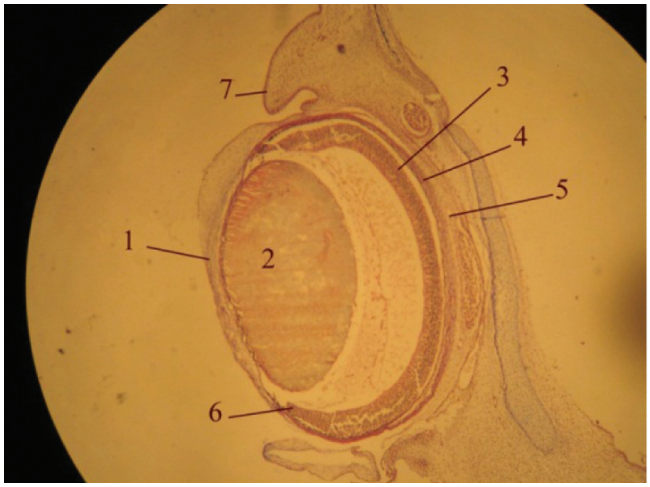

Fig 2. Histological findings in the middle of second month or 50-day old fetuses (Masson's Trichrome, ×40); **1-** Cornea, **2-** Lens, **3-** Retina, **4-** Choroid, **5-** Sclera, **6-** Root of iris and ciliary body, **7-** Eyelid

Şekil 2. İkinci ayın ortasında veya 50 günlük fetuslarda histolojik bulgular (Masson Trichrome, × 40); **1-** Kornea, **2-** Lens, **3-** Retina, **4-** Koroid, **5-** Sklera, **6-** İrisin kökü ve siliyer cisim, **7-** Gözkapağı

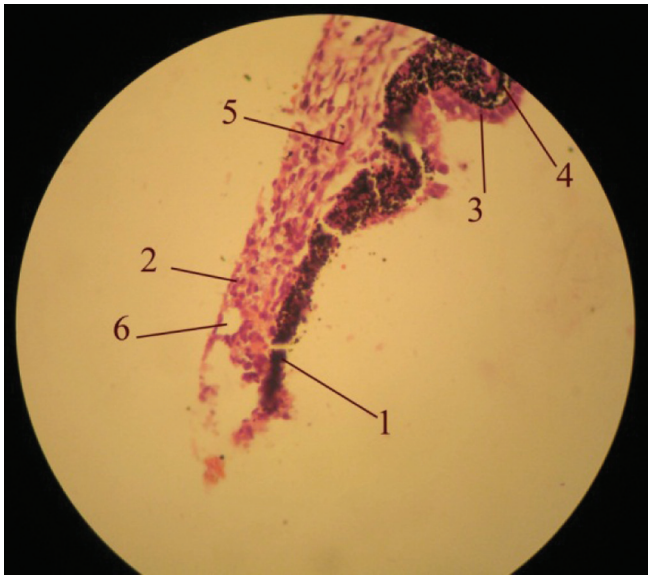

Fig 3. Histological findings in 60-day old fetuses (H&E, ×100); **1-** Posterior pigment epithelium of iris, **2-** Anterior epithelium of iris, **3-** Superficial cells of ciliary body, **4-** Deep cells of ciliary body, **5-** Smooth muscles, **6-** Blood vessels

Şekil 3. Otuz günlük fetuslarda histolojik bulgular (H & E, × 100); **1-** İrisin posterior pigment epiteli **2-** İrisin anterior epiteli, **3-** Siliyer cisim yüzeysel hücreleri, **4-** Siliyer cismin derin hücreleri, **5-** Düz kaslar, **6-** Kan damarları

Table 1. *Average length and transverse of external diameter of iris and average length and transverse of diameter of pupil in sheep fetuses at different ages*
Tablo 1. *Farklı yaşlardaki koyun fetuslarında iris dış çapının ortalama uzunluk ve eni ile pupil dış çapının ortalama uzunluk ve eni*

Fetus Age	Iris		Pupil	
	Average Length	Average Transverse of External Diameter	Average Length	Average Transverse of Diameter
Second month	0.46±0.081 cm	0.30±0.051 cm	0.44±0.071 cm	0.4±0.31 cm
Third month	1.0±0.51 cm	0.7±0.51 cm	0.7±0.032 cm	0.3±0.24 cm
Fourth month	1.3±0.51 cm	0.8±0.51cm	0.7±0.32 cm	0.3±0.77 cm
Fifth month	1.6±0.41 cm	0.9±0.72 cm	0.8±0.22 cm	0.4±0.35 cm

vessels were more than the previous months. The superior epithelium was relatively smooth and formed of stratified cuboid cells. Internal cells had more pigments. Masson's tricrome staining showed fine blue collagen fibers in connective tissue. Also, there were smooth red muscles under epithelium and in the center of iris *(Fig. 4)*.

The Results of 110-Day Old Fetuses (Fourth Month)

The 110-day old fetuses eyelids were semi open and iris was dark brown. The iris had oval shape and followed the shape of cornea; internal angle of iris was larger than its external angle. The pupil dimension in internal angel was larger than the external one too. The average length and transverse of external diameter of iris was 1.3±0.51 cm and 0.8±0.51cm respectively and the average length and transverse diameter of pupil were 0.7±0.32 cm and 0.3±0.77 cm respectively *(Table 1)*. Also, the third eyelid was clear in internal angle of the eye. The separation of ciliary body and iris from the choroid was clear completely in fourth month and they separate from each other in the limbus region. The iris was thin and fine and moved between cornea and lens. Iris had an anterior irregular simple squamous epithelium. The loose connective tissue consisted of fibrocytes, fibroblasts and blood vessels were seen under this tissue. Melanocyte cells were low in connective tissue. Epithelium cells of posterior layer were relatively irregular and consisted of one or two rows of cuboid cells with plenty of melanocytes. There were a lot of melanocytes with not observable cytoplasm. In the center of iris, there were significant muscular cells placed in a row behind each other. In Verhoeff staining, there was no important sign of elastic fibers in iris connective tissue and there were elastic fibers only in vessels wall. In Masson's tricrome staining, the dispersed blue collagen fibers in loose connective tissue were seen especially under the anterior epithelium. In addition, the existence of many muscular cells in red ribbon form at the center of iris was confirmed. The muscular cells adjacent to choroid were greater *(Fig. 5)*.

The Results of 135-Day and 150-Day Old Fetuses (Fifth Month)

In fifth month, the eyelids were open and the third eyelid was seen in internal angle. Iris was dark brown and rested between cornea and lens. Iris resembled cornea and its internal angle was larger than external one. Pupil was oval and its internal angle was larger than external one. Two small nodes were seen in lower edge of pupil in the

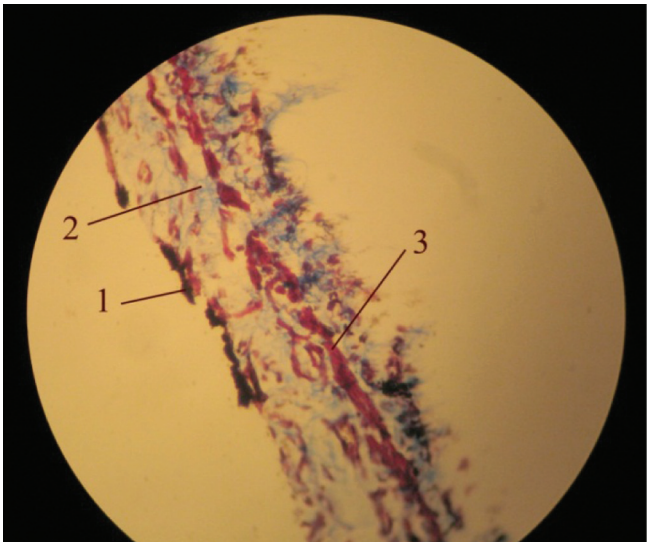

Fig 5. Histological findings in the fourth month (Masson's Trichrome, ×400); **1**- Posterior pigment epithelium of iris, **2**- Collagen fibers, **3**- Smooth muscle cells

Şekil 5. Dördüncü ayda histolojik bulgular (Masson Trichrome, × 400); **1**- İrisin posterior pigment epiteli, **2**- Kolajen lifler, **3**- Düz kas hücreleri

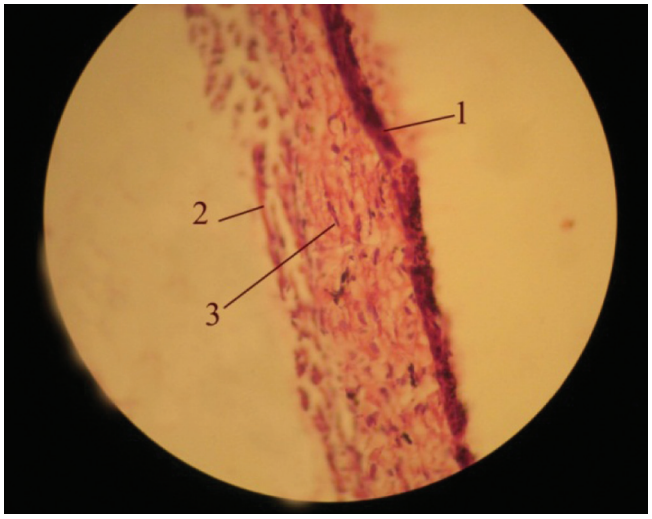

Fig 4. Histological findings in the third month (H&E, ×400); **1**- Posterior pigment epithelium of iris, **2**- Anterior epithelium of iris, **3**- Connective tissue with fibrocyte

Şekil 4. Üçüncü ayda histolojik bulgular (H & E, × 100); **1**- İrisin posterior pigment epiteli, **2**- İrisin anterior epiteli, **3**- Fibrositli bağ doku

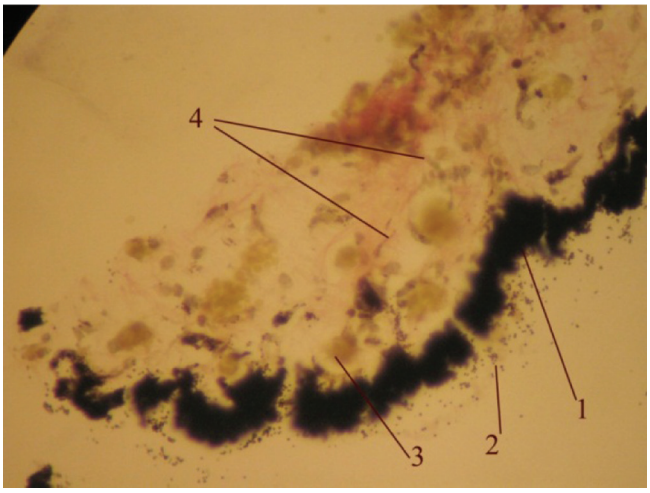

Fig 6. Histological findings in the fifth month (verhoeff, ×400); **1**- Posterior pigment epithelium of iris, **2**- Pigments, **3**- Blood vessels, **4**- Elastic fibers

Şekil 6. Beşinci ayda histolojik bulgular (Verhoeff, × 400); **1**- İrisin posterior pigment epiteli, **2**- Pigmentler, **3**- Kan damarları, **4**- Elastik lifler

late of fifth month. The average length and transverse of external diameter of iris were 1.6±0.41 cm and 0.9±0.72 cm respectively and the average of length and transverse diameter of pupil were 0.8±0.22 cm and 0.4±0.35 cm respectively *(Table 1)*. A part of choroid had inclined to the eyeball adjacent to limbus and divided to two parts; anterior part (iris) and superior part (ciliary body). Iris was seen finer than cornea and had two different epitheliums. Anterior epithelium of iris was irregular and covered with simple squamous or cuboid cells. There was a loose connective tissue with plenty of blood vessels or accumulated red blood cells under this tissue. Fibrocytes, fibroblasts and melanocytes were very low in connective tissue. In connective tissue, plenty of vessel sections were significant because iris was member of vascular layer of eye. The smooth muscle cells and fine myoepithelial cells for iris contractions were seen in connective tissue. Most of these muscular cells exist under the iris epithelium. The posterior epithelium of iris was relatively smooth and covered with two rows of irregular cuboidal cells. These cells were completely pigmented and had more intracellular black granules. The muscular and myoepithelial cells under the epithelium became greater at the adjacent to free edge of iris. In Verhoeff staining, there were not any signs of elastic fibers but the pigmented cells and plenty of blood vessels in internal layer of iris and smooth muscular cells in connective tissue were significant. In Masson's tricrome staining, the collagen fibers of connective tissue and red smooth muscles with myoepithelial cells were observable. The muscular cells were concentrated under the epitheliums and blood vessels gathered in the center of iris *(Fig. 6)*.

DISCUSSION

Anatomical examinations weren't possible in one-month old or 30-day old samples due to smallness of samples so only histological examination was conducted. As it was mentioned in results section, in this month optic cup was formed relatively and was clear as circle mass under microscope. These optic cups grew bilaterally from the head of fetus. But there was not any sign of optic cup and mesenchymal differentiation to choroid, sclera, iris and ciliary body in sheep fetus in this stage.

In human fetus the developing eye appears in the 22-days embryo as a pair of shallow grooves on the sides of the forebrain [12]. Also it has been reported that in human fetus with closure of the neural tube these grooves form outpocketings of the forebrain, the optic vesicles. Shortly thereafter the optic vesicle begins to invaginate and forms the double-walled optic cup. The inner and outer layers of this cup are initially separated by a lumen, the intra retinal space [12]. In 16-18 days old dogs the optic vesicles are formed while it is formed on 5-30 days old cattle [13]. Based on this report the optic cup is formed on 19-day old in dogs and on 30-day old in cattle [14]. The reports suggest that in most animals the optic cup is formed relatively in first month of pregnancy.

In the early of the second month's samples of sheep fetus it was clear that optic cup were deepen slowly and were formed double walled completely. The outer layer of the optic cup is known as the pigmented layer of the retina. Retina was the most clear and thick eye layer. This issue suggested that retina follows this rule that nervous part of the body is differentiated earlier than the other parts. Also, outer layer of optic cup which forms external part of retina has small pigmented granules and seen as a dark pigmented layer. In human fetus the optic vesicle moves to internal part and forms a two-layer cup on its 42-day old [15]. At first there is a space between these two layers but the space disappears after a short time and the two layers of cup appose each other [16]. In human fetus in this stage the outer layer of the optic cup which is characterized by small pigment granules, is known as the pigment layer of the retina. This issue added that development of the inner (neural) layer of the optic cup is more complicated [12]. The posterior four-fifth, the pars optica retina, differentiates into light-receptive elements. The anterior fifth of the inner layer, the pars ceca retinae, remains one cell layer thick. It later divides into ,the pars iridica retinae, which forms the inner layer of the iris, and the pars ciliaris retinae, which participates in formation of the ciliary body [12].

As it was mentioned the outer layer of optic cup in one-month old fetus was clear with its small pigmented granules and the pigmented layer is called retina [17]. This pigmented layer will form the pigmented layer of iris and ciliary body on fifth of anterior part of optic cup [13]. In this stage (first month) the bending part of optic cup's anterior layer which will divide to ciliary body and iris is seen circular yet [18]. Also, there is not any clear sign of iris and ciliary body in the early of the second month. In other words, one fifth of anterior part of optic cup; ceca part of retina has not been separated to iris and ciliary body. Moreover, in this period, the participation of choroid anterior part in forming of iris and ciliary body is seen and produces plenty of blood vessels in the structure of these parts. Meanwhile, in this stage the influence of superficial ectoderm on forming of iris and ciliary body's muscles is observable. In human fetus the region between the optic cup and the overlying surface epithelium is filled with loose mesenchyme [12,16]. The sphincter and dilator pupillae muscles form in this tissue. These muscles develop from the underlying ectoderm of the optic cup. In the adult the iris is formed by the pigment-containing external layer with the unpigmented internal layer of the optic cup, and a layer of richly vascularized connective tissue that contains the pupillary muscles [19,20].

In the middle of the second month, the separations of iris from ciliary body is very low but in the late of this month (near 60-day old) iris and ciliary body are separate from each other so that they have common root but they

are separated in anterior part. In general in the late of second month iris is separated from ciliary body slowly.

Complete separation and relative differentiation is done in third month and final specifications of these two parts are apparent in this month. In human fetus, iris and ciliary body is formed from one fifth of internal part of retina's ceca part [12].

Superior part of iris consists of granular epithelium cells which extend from anterior edges of optic cup to lens [18]. These conditions conformed to sheep samples. Furthermore, connective tissues of choroids, sclera, iris and some other parts of eye are formed from neural crest and small participation of mesoderm [21,22]. Also the iris muscles which are produced from pigmented epithelium cells, are placed in the edge of optic cup earlier [23,24].

In general, it is concluded that in third month (90-day old) the eyelids are opened. The iris is like a dark mass. The iris and pupil shape are horizontal oval and their sizes become larger by the time. These results confirm with the results about other animals and human [9].

Furthermore, histological examinations revealed that elastic and collagen fibers are clear slowly during fetus maturation. Based on the findings of this research, number and size of smooth muscle increases with evolution of fetus which confirms with the fetus growth rules [11]. It is worth to note that blood vessels and cell pigmentation increase significantly with fetus growth.

REFERENCES

1. Braekevelt CR: The retinal epithelial fine structure in the domestic cat. *Anat Histol Embryol*, 9, 58-94, 1990.

2. Churchill AJ, Booth A: Growth and differentiation sheep lens epithelial cells *in vitro* on matrix. *Br J Ophthalmol*, 80, 669-673, 1996.

3. Gould DB, Smith RS, John SW: Anterior segment development relevant to glocoma. *Int J Dev Biol*, 48, 1015-1029, 2004.

4. Link BA, Nishi R: Development of the avian iris and ciliary body: Mechanisms of cellular differentiation during the smooth -to-striated muscle transition. *Dev Biol*, 203, 163-176, 1998.

5. Oliver FJ, Samuelson DE, Brooks PA, Lewis ME, Kallberg AM: Comparative morphology of the tapetum lucidum (among selected species). *Vet ophthalm*, 7, 11-23, 2004.

6. Perione SM, Sistodaneo L, Filogamo G: Embryogenesis of the avian iris sphincter muscle: *In vivo* and *in vitro* studies. *Int J Dev Neurosci*, 8 (1): 17-31, 1990.

7. Thut CJ, Rountee RB, Hwa M, Kingsley DM: A large-scale in situ screen provides molecular evidence for the induction of eye anterior segment structures by the developing lens. *Dev Biol*, 231, 63-76, 2001.

8. Eleanor A, Blakely M, Kathleen A, Bjornstad P, Chang I, Morgan P: Growth and differentiation of human lens epithelial cells *in vitro* on matrix. *Embryo J*, 193 (6): 85-96, 2001.

9. Dyce KM, Sae DM, Wensing CYG: Text Book of Veterinary Anatomy. pp. 323-336, Saunders Company, 2002.

10. Hitchcock PF, Macdonald RE, Vande RE, Wilson RE: Growth and differentiation of goat lens cells *in vitro*. *Neurobiol*, 29, 399-413, 1996.

11. Imaizumi M, Kuwabara T: Development of the rat iris. *Invest Ophthalmol*, 10, 733- 744, 1971.

12. Sadler TW: Langmans`s Medical Embryology. 11th ed., pp. 394-404, Williams&Wilkins, 2012.

13. McGeady TA, Quinn PJ, FitzPatrick PJ, Ryan PJ: Veterinary Embryology. First ed., pp. 295-305, Blackwell Publishing, 2006.

14. Kassa A, Aogama M, Sugita S: The morphology of the iridocorneal angle of buffaloes *(Bos bubalis)*: A light and scaning electron microscopic study, Okajimas folia. *Anat Jpn*, 78 (4): 145-152, 2001.

15. Franco AJ, Masot PJ, Aguado MC, Gomez L, Redondo E, June N: Morphometric and immunohistochemical study of the eye development. *J Anatomy*, 204 (6): 501-510, 2004.

16. Cvekl A, Tamm ER: Anterior eye development and ocular mesenchymei new insights from mouse models and human diseases. *Bio Assays*, 26, 374-386, 2004.

17. Chow RA, Lang RA: Early eye development in vertebrates. *Ann Rev Cell Dev Biol*, 17, 255-296, 2001.

18. Silberman DN, Padan RA: Iris development in vertebrates, genetic and molecular considerations. *Brain Res*, 4 (1192): 17-28, 2008.

19. Sun G, Asami M, Otha H, Kosaka J, Kosaka M: Retinal stem/ progenitor properties of iris pigment epithelial cells. *Dev Biol*, 289, 243-252, 2006.

20. Thumann G: Development and cellular functions of the iris pigment epithelium. *Sur Ophthalmol*, 45, 345-354, 2001.

21. Haruta M, Kosaka M, Kanegae Y, Saito I, Inoue T, Kageyama R, Nishida A, Honda Y, Takahashi M: Induction of photoreceptor - specific phenotypes in adult mammalian iris tissue. *Nat Neurosci*, 4, 1163-1164, 2001.

22. Akagi T, Akita J, Haruta M, Suzuki T, Honda Y, Inoue T, Yoshiura S, Kageyama R, Yatsu T, Yamada M, Takahashi M: Iris-derived cells from adult rodents and primates adopt photoreceptor- specific phenotypes. *Invest Ophthalmol Visual Sci*, 46, 3411-3419, 2005.

23. Jensen AM: Potential roles for BMP and Pax genes in the development of iris smooth muscle. *Dev Dyn*, 232, 385-392, 2005.

24. Akagi T, Mandai M, Ooto S, Hirami Y, Osakada F, Kageyama R, Yoshimora N, Takahashi M: Otx2 homeoboyx gene induces photoreceptor - specific phenotypes in cells derived from adult iris and ciliary tissue. *Invest Ophthalmol Visual Sci*, 45, 4570-4575, 2004.