DEVELOPMENTAL STUDY OF THE OLFACTORY BULB IN RABBITS *Oryctolagus cuniculus*

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ABSTRACT

This study was conducted to investigate the development of olfactory bulb (OB) in indigenous rabbit. A total of 40 healthy rabbits were divided into two groups according to their ages, the first group involved the prenatal stages, while the second group involved the post natal ages. All specimens for this study processed by the routine paraffin method and stained with H and E stain. The light microscopy examination revealed that, At 20 day old fetuses the olfactory bulb consisted of two layers. The marginal zone formed of axons of the olfactory sensory neurons and the ensheathing progenitors cells surrounded amass of cells. At 28 day of gestation and one day postnatal, the olfactory bulb consisted of three layers, olfactory nerve layer, mitral cell layer, and granule cell layer and at (7-14) day old pups glomeruli appeared as spherical structure surrounded by periglomerular cells and the glomerular layer was clearly distinguished. The mitral cell layer appeared as cellular zone. 21 days old pups. The six layers of olfactory bulb was clearly appeared (olfactory nerve layer, glomerular layer, external plexiform layer, mitral cell layer, internal plexiform layer, and granule cell layer). At 30 day age pups the histological structure of the olfactory bulb was completely developed.

Key words: Development, olfactory bulb, rabbit pups.

INTRODUCTION

The olfactory bulb is laminal structure located at the most rostral region of brain protected by cribriform plat. It is part of limbic system, embryologically originated from the prosnencephalon. It consists of two types of neurons involve the mitral and tufted cells which considered as the principal neurons beside the granule cells and short axons cells which considered as intrinsic neurons. The olfactory bulb involved in the following function: Detection of odors, Discrimination between odors, Filtering out many back ground odors and Permitting higher brain areas involved in modify the odor detection and discrimination. The olfactory bulb is a path way to transduction of odor to the brain via the tract of olfactory bulb (Butter and Hodos, 2005 ; Benignus and

Prah, 1982 ; Afifi and Bergman, 1986). The sense of smell in animals is depended on the size and function of olfactory bulb, which is developed with age (Stephan, 1983 ; Buschhuter *et al.,* 2008 ; Kavio and Jameela, 2011). The processing of different odor and cues occur through olfactory bulb to hypothalamus, hippocampus and other part of brain. The olfaction controlling animals behavior in feeding, mating, maternal relation (Hardy *et al.,* 2005 ; Lin *et al.,* 2005 ; 2006 ; Sanchez-Andrad and Kendrick, 2009).

MATERIALS AND METHODS

The study involved ten (20, 28) day old, the age of fetuses detected by measured of crown-ramp length by digital caliber according to (Mc Laughlin and Chiasson, 1999). The group of postnatal ages was divided into six subgroups, each group involved five pups, according to their ages as following, one day old (P1), one week (P7) two weeks (P14), three weeks (P21), four weeks (P30) and eight weeks (P60). The animals of post natal group were sacrificed by over dose intra muscular injection of Ketamine 15 mg BW^{-1}, and 5 mg BW^{-1} Xylazine. The lower jaw, skin, muscles and the skull bones were cut and removed. The olfactory bulb fixed by using 15% formalin solution and 2 grams of ammonium bromide for at least 48 hours. All specimens processed upgrading with ethanol alcohol for paraffin section examination, then sectioned serially in frontal plane at 7 µm. The prepared sections were stained with Hematoxylin and Eosin according to Luna (1968).

RESULTS AND DISCUSSION

The current study revealed that the six layers of olfactory bulb showed several changes in their histological structure according to different ages included the following: Olfactory nerve layer (ONL): Fetus at 20 day of gestation period: This layer consisted of the axons which originated from the olfactory sensory neurons, these axons were grew and migrated with ensheathing progenitor cells through the mesenchymal tissue (Fig. 1). The axons penetrated and inter the developing olfactory bulb and forming marginal zone which later developed into the definitive olfactory nerve layer of olfactory bulb, Fig. 1. These findings agree with the results of Graziadie *et al*., (1980) and Doneett (1989) in mice and with Treloar *et al*., (1999). This result suggest that, the growth and migration of the axons of olfactory sensory neurons toward the developing olfactory bulb were under the effects of many growth factors, these factors include the factors release from the ensheathing glial cells, mesenchymal tissue and the factors release from olfactory bulb (Runyan and Phelps, 2009 ; Trcloar *et al*., 2010). Fetus at 28 day of gestation–One day old pup: The

olfactory nerve layer appeared as regular mass of axons and glial cells which surrounded the cellular population of developing olfactory bulb which consisted mainly of an mature mitral and tufted cells (Fig. 2). At 7 days old pup: This layer appeared more regular and their axons were clearly identified and presented many of glial cells (Fig. 3). The present result compatible with the results of Qin-guo *et al*., (2008). The olfactory nerve layer play a role in transduction the impulses which detected by the olfactory sensory neurons to the olfactory bulb (Benignus and Prah, 1982 ; Afifi and Bergman, 1986). Glomerular Layer (GL). Fetus at 20 day of gestation – one day old pup day: the glomerular layer was not distinguished and there were no individual glomeruli (Fig. 1). This observation was similar with result of Greer *et al*., (1982) in rat pups during sucklines period, and with result of Schneider *et al*., (2009) in the fetuse and neonate of tammar wallaby. At this age the axons of the olfactory sensory neurons made up the synapses with dendrites of mitral and tufted cells without forming the typical individual glomeruli (Treloar *et al*., 1999). At 7 day old pup: The individual glomerulus appeared as small spherical densely stained structure and wasn't completely surrounded by the periglomerular and glial cells (Fig. 3). At 14 day pup: The individual glomerulus was clearly distinguished, and appeared completely surrounded by round periglomerular cells of dark nucleus, and glial cells (Fig. 3). These results were corresponding with the results of Price and Powell (1970); Jeune and Jourdan (1991) and with result of Meisami and Sendero (1993); Willey (2004). At 21-30 days pup: The glomerular layer was very clearly distinguished and individual glomeruli were closely surrounded by the periglomerular and glial cells (Fig. 5 "a and b"). The present result shows that at P 30 days the glomerular layer appeared similar to that of adult. These results parallel result of Greer (1982); McLLean and Shipley (1987); Kathleen and Christina (2002). The olfaction information can be converged the olfactory impulse and conducted it to reach the mitral – tufted cells and periglomerular cells. The axons of the olfactory sensory neurons synapses with the dendrites of mitral and tufted cells and the periglomerular cells and short axons cells forming the individual glomeruli (Kosaka and Kosaka, 2005). The glomeruli with the olfactory nerve axons and the mitral–tufted dendrites forming functional unit, which distributed to form the odor map. Each individual glomeruli respond to specific odor molecule detected by specific olfactory sensory neurons, these specific glomeruli of olfactory bulb helping the brain to understand the odor stimulance, these opinion agree with results of Mori *et al*., (2006) and Lin *et al*., (2006). External plexiform layer and

the internal plexiform layers (EPL) (IPL). Fetus at 20 day of gestation: The external and internal plexiform layers were absent at this age (Fig. 1). Fetus at 28 day of gestation-one day old pup: The development of two layers could not be distinguished because the mitral cells was diffused (Fig. 2). These observation supported with the results of Green *et al*., (1982) in rat, Schneider *et al*., (2009) in tamor Wallaby and Yokosuka *et al*., (2011) in rat and Crow.

At 7 day old pup: The layers began to be noticed (Fig. 3). At 14 day old pup: The two layers were clearly distinguished where the mitral cell layer separated between them. The external plexiform layer consisted of few granule cells which appeared small rounded with dark stained nucleus and fusiform mitral cells of different sizes and pale stained nucleus (Fig. 4). At 21 day old pup: The two layers were taken the form of adult pattern (Fig. 5 a). These results confirmed to the finding of Creer *et al*., (1982) in rat and Qin-guo *et al*., (2008) in dog, and Young and Less (1999). Mitral cell layer (MCL), Fetus at 20 day of gestation: The development of mitral and tufted cells was not organized into layers, and appeared as mass of non-differentiated immature cells (Fig. 1). This observation confirmed the results of Hinds (1968); Hinds and Ruffett (1973); Baye (1983); Bergmann *et al*., (1993) and Winpenny *et al*., (2011). The development and maintenance of the mitral cells and tufted cells affected by the arrival of olfactory nerve axons and it's contact with developing olfactory bulb (Couperleo and Brunjes, 2003). Fetus 28 day of gestation-one day old pup: The mitral and tufted cells appeared diffused forming cellular band consisted of many cell layers. The mitral and tufted cells (large cells) in developing olfactory bulb originated and differentiated earlier before the interneuron (small cells) (Fig. 2). At 7 day old pup: The mitral and tufted cells formed an identitive band between the external and internal plexiform layers (Fig. 3). At 14 day old pup: the mitral cells layer consisted mainly of large pyramidal cells contained large and round pale nucleus, and few oval or fusiform tufted cells which were smaller than mitral cell. Small round with dark nucleus granule cells were noticed in the margin of the layer (Fig. 6). These finding agree with Greer *et al*., (1982), Schneider *et al*., (2009) and Yokoswka *et al*., (2011). At 21 day old pup: The mitral cells body arranged into 1-2 cells layers. The granule cells aggregated at the margin of the mitral layer and intermingling with its cells. The tufted cells were clearly present. (Rosselli- Austin and Altman, 1979 ; Royet *et al*., 1998). At 30 day old pup: The Mitral cells were similar to that of adult and no important changes were noticed. This result agree with results of Greer *et al*., (1982) and Qin – guo *et al*., (2008). The relationship between mitral-tufted cells

and the periglomerular cells is dendrodendritic synapses formed between this opinion is explained by Kasaka and Kosaka (2005). The secondary lateral dendrites of mitral and tufted cells form dendrodendritic synapses with granule cells in the external plexiform layer this agree with result which mentioned by Price and Pwell (1970). The axons of the mitral and tufted cells extended through the lateral olfactory tract to reach different regions in the brain this mentioned by many authors like the Afifi and Borgmeans (1986) ; Seveg (1999); Mast and Griff (2005) ; Nagayama *et al*., (2010). The Mitral and tufted cells different in their sizes, but both have similar function (Christie *et al.,* 2001). The mitral and tufted cells when stimulated by axon of olfactory sensory neurons which release glutamate that act as neurotransmitters causes stimulation to the periglomerular and granule cells, Ayluin *et al*., (2005) and Eyre *et al*., (2009). Granule cell layer (GCL) Fetus at 20 day of gestation: the granule cell layer was absent (Fig. 42). This finding agree with the result of Baye (1983) and Bergmann *et al*., (1993) and Winpenny (2011), they revealed that the interneuron are three cells (periglomerular cells, short axons cells and granule cells), these proregenerated at late prenatal and postnatal from the sub-ventricular zone of proencephalon (Wang *et al*., 2005). The interneuron continuous in neurogenesis even in adult life of animal, the same results observed by Baye (1983) ; Mayoshi *et al*., (2009). Fetus at 28 day of gestation–one day old pup: This layer was occupied by granule cells which scattered and diffused within layer spaces (Fig. 2). At 7 day old pup: the granule cells began to cluster with each other (Fig. 3) Its width was 517 µm. At 14-21 day old pups: The granule cells clusters to form nest of cells arranged centrifugally (Fig. 4 and 5 a). At 30-60 day old pups. There were no important histological changes (Fig. 8). The development sequence of the granule cells layer was proved the finding of Greer *et al*., (1982) and Kathleen and Christina (2002) in rat and Qin-guo *et al*., (2008) in dog, and Schneider *et al*., (2009) in Tammar Wallaby. The clustering of the granule cells was very important for cells, performance and response to the same impulses and participates in inhabitation and odor discrimination. The results go with the results of Reyha *et al*., (1991); Gheusi *et al*., (2000). The present result suggests that, the mechanism of neurogenesis of new granule cells due to the effects of new odor stimulants and its migration to the olfactory bulb by mechanism similar to that of lymphocyte by the lymphatic system when new irritant or antigen. This opinion supported by result of Dong *et al*., 2007 ; Heinbockel *et al*., 2007 ; Laaris *et al*., 2007). The results of this study showed that the width of olfactory bulb layers and the diameters of glomeruli of

glomerular layer were increased with age (Rossell-Asustin and Altman, 1979; Geune and Gordan, 1991 ; Mesami and Sendera, 1993 ; Qin-gou, 2009 ; Kilkash *et al*., 2010). The structural organization of olfactory bulb and the mature lamination reach the adult pattern at P 30 (Graziadei and Graziadei, 1980 ; McLen and Shipley, 1987 ; Mesami and Sendera, 1993 ; Gordan, 1991 ; Youn and Lee, 1994 and Kathleen and Christina, 2002).

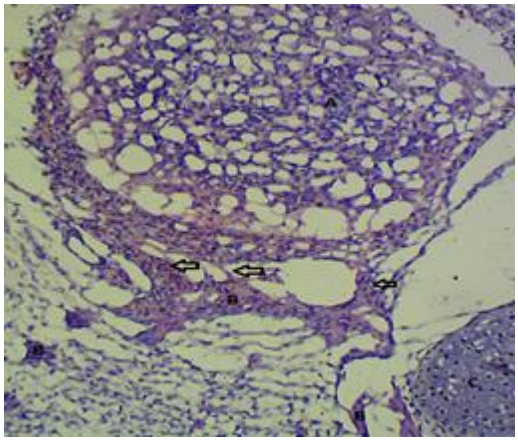

Figure 1. Histological section of OB. (E. 20) shows: (A) Neuroblast. (B) Nerve fibers. (C) Hyaline cartilage. (Arrows show penetrations of nerve fiber to olfactory N L. (H and E x 100)

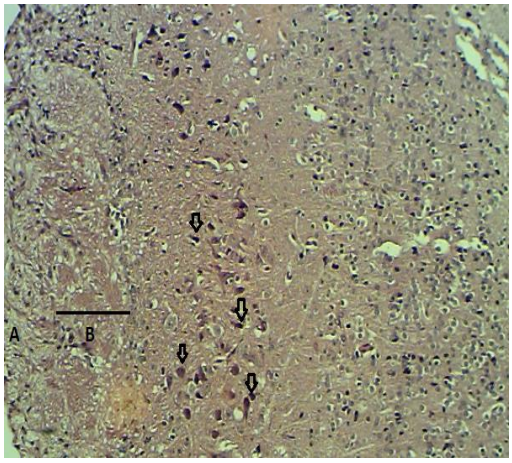

Figure 2. Histological section of Olfactory bulb (E28-P1) day shows: (A) olfactory nerve layer. (B) Non differentiated G L. Arrows show diffused cells. (H and E x 40)

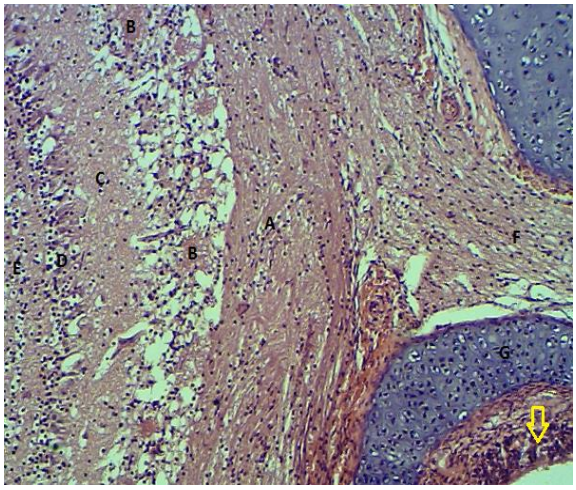

Figure 3. Histological section of OB. (P7) shows: (A) ONL (B) Glomeruli of GL. (C) EPL. (D) Band of mitral cells. (E) IPL. (F) Projected nerve from olfactory epithelium toward olfactory bulb. (G) Cribriform plate. Arrow shows olfactory epithelium (H and E x 100)

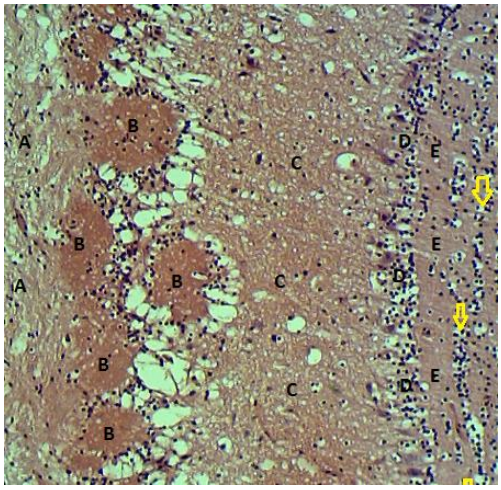

Figure 4. Histological section of Olfactory bulb (P14) days shows: (A) ONL. (B) Glomeruli of GL. (C) EPL. (D) Mitral cells layer (E) IPL. (F) GCL. aggregation of granule cells (Arrows head) (H and E x 200)

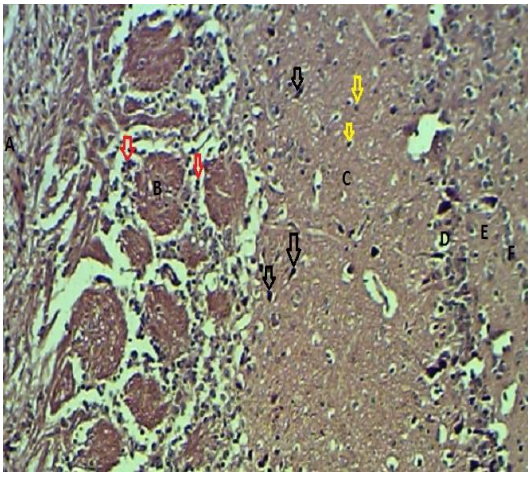

Figure 5 a. Histological section of OB (21-30) day shows: (A) ONL. (B) Glomeruli of GL. (C) EPL (D) MCL (E) IPL (F) GCL(Black arrow shows tufted cells, granule cells (yellow arrow), periglomerular (red arrow) (H and E x 200)

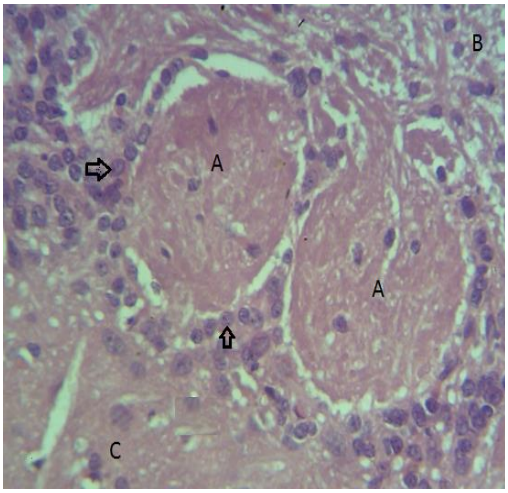

Figure 5 b. Histological section of GL (30-60) day shows: (A) Individual glomerulus. (B) ONL (C) EPL. (Arrows show periglomerular cells. (H and E x 400)

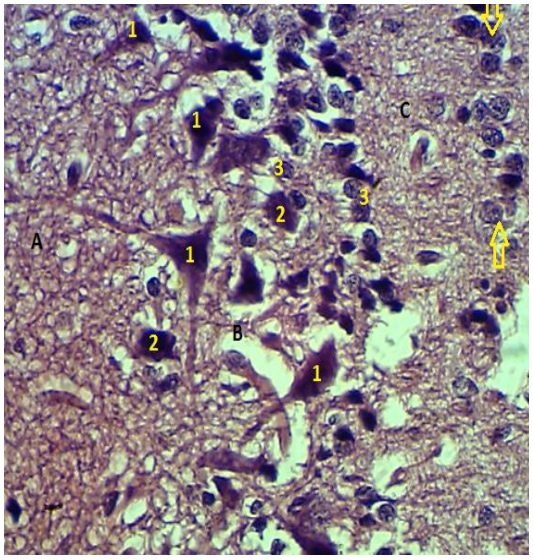

Figure 6. Histological section of mitral cells layer (14 day): (A) EPL (B) MCL. (C) IPL. (1) Mitral cells. (2) Tufted cells. (3) Granule cells. GCL (Arrows showed). (H and E x 400)

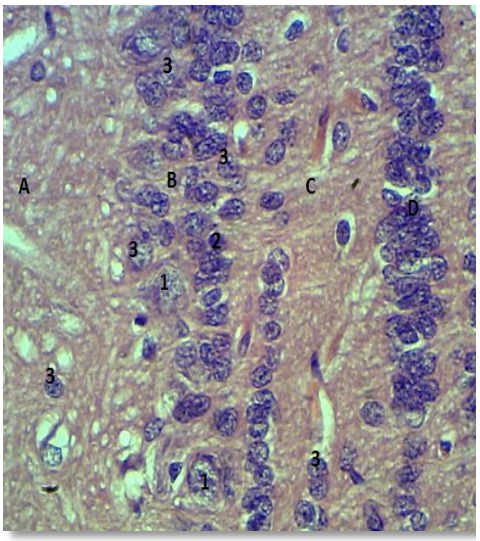

Figure 7. Histological section of MCL (30-60) day old pup shows: (A) EPL (B) MCL. (C) IPL. (1) Mitral cells. (2) Tufted cells. (3) Granule cells. (D) Granule cells layer. (H and E x 400)

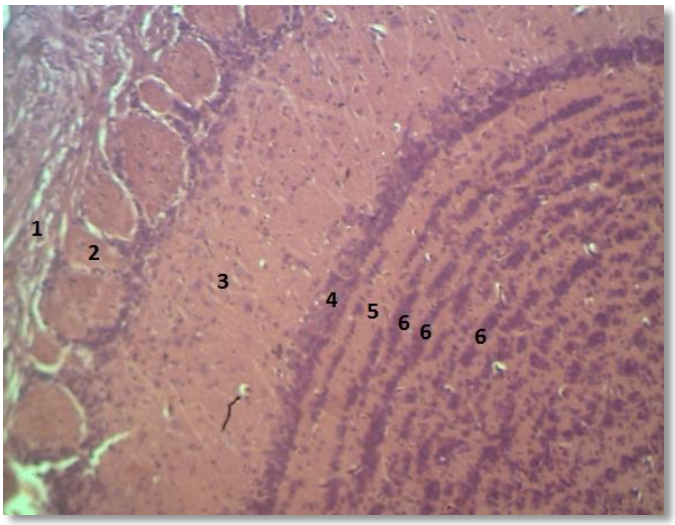

Figure 8. Histological section of OB (60) day shows:1- ONL. 2- GL. 3- EPL. 4- MCL. 5- IPL. 6- GCL. (H and E x 400)

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دراسة تطورية للبصلة الشمية في الارانب
Oryctolagus Cuniculus

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المستخلص

أجريت هذه الدراسة لبحث تطورالبصلة الشمية للارنب، فقد تم استخدام 40 ارنباً سليماً قسمت الى مجموعتين اعتمادا على العمر، ضمت المجموعة الاولى اعمار ماقبل الولادة وضمت المجموعة الثانية اعمار ما بعد الولادة. جميع العينات (البصلة الشمية) تم تمريرها بطريقة البرافين العادية واستخدمت صبغة الهيماتوكسلين ايوسين للتصبيغ. اظهر الفحص بالمجهر الضوئي ان البصلة الشمية في جنين عمره 20 يوماً تتكون من طبقتين، الطبقة الخارجية وتتألف من محاور الخلايا الشمية الحسية للظهارة الشمية المبطنة للتجويف الانفي وتحيط بهذه المحاور الخلايا المولدة للخلايا الغمدية، اما الطبقة الداخلية فتتكون من كتلة من خلايا غير متمايزة، أما في الجنين بعمر 28 يوماً وعمر يوم واحد بعد الولادة فتتكون البصلة الشمية من ثلاث طبقات، الطبقة الخارجية تمثل طبقة العصب الشمي، والطبقة الثانية تتكون من الخلايا المترالية التي تكون منتشرة التوزيع، والطبقة العميقة او الداخلية فتمثل الخلايا الحبيبية. تبدأ الكبيبات بالظهور عند عمر7- 14 يوماً بعد الولادة بالظهور على شكل تراكيب كروية داكنة الصبغة ومحاطة بشكل غير كامل بالخلايا الدبقية لتكون طبقة رابعة (الطبقة الكبيبية) وبهذا العمر ايضا تبدأ طبقة الخلايا المترالية بالظهور على شكل حزام خلوي مما يؤدي الى بداية ظهور الطبقات التشابكية الداخلية والخارجية، وعند عمر21- 30 يوماً تلاحظ الطبقات الست النموذجية المكونة للبصلة الشمية كاملة. استنتجت الدراسة ان البصلة الشمية للارنب تتغير نسجيا بتقدم العمرويكتمل نموها بعمر شهر من الولادة.

الكلمات المفتاحية: التطور، البصلة الشمية، جراء الأرنب.