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Histology

Prenatal Development of Rabbit Cerebellum

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ABSTRACT

The present study's purpose was to give an overview of the rabbit cerebellum's prenatal developmental stages. Forty embryos of prenatal ages (E10, E15, E17, E20, E25, E25 and E30) were obtained from the rabbit. The cerebellum primordium appeared on day 10 (E10). At E25 the cerebellar primordium formed from the neuroepithelium, mantle layer and marginal layer. The peripheral layer of neuroepithelium transformed to external granular layer (EGL). This EGL is termed as second germinal layer. The EGL molecular development was a distinct structure in the developing cerebellum that afterward disappeared. On day 30 prenatal embryos of rabbits, the cerebellum was not fully differentiated. The cerebellar cortex consisted of external granular and internal granular layers. An extended process that takes place during prenatal and postnatal life gives the mature cerebellum its ultimate structure. In the current study, the rabbit cerebellum's prenatal development and age-related histogenesis were connected.

Key words: Histology, Cerebellum, Prenatal development, Rabbits, Histogenesis.

INTRODUCTION

Rabbits are regarded a standard laboratory animal (excellent experimental models) for embryological research. Due to its high similarity to human anatomy and histology, adequate size, simplicity of handling and care, and ease of housing and feeding, the transgenic rabbit is used as an animal model for a number of human diseases and antibody generation (BÕsze and Houdebine, 2006; Brewer, 2008; De la Portilla et al., 2011; Soliman et al., 2015).

Rabbits exhibit placental development, like humans and have a short reproductive cycle (Fischer et al., 2012; Julik et al., 2012). Rabbits have a high economic value and are used in a wide range of commercial purposes such as meat and hair, in the production of coats and regal dresses (Al-Mahmodi, 2016). The meat of the rabbit is easily digested and provides a good source of protein for infants, sick, aged and healthy people (Zotte, 2002).

The cerebellum is considered the largest motor organ responsible for balance, coordinates the muscular tone and equilibrium of the body (Mariën and Borgatti, 2018; Schmahmann, 2019 and Hatten, 2020).

The cerebellum's development provides an excellent model for researching neurogenesis because the cerebellum has a simple and largely standardized cytoarchitecture (Sillitoe and Joyner, 2007).

The cerebellar primordium is described as a crescent- shaped cluster of cells

that appear on E15 in rabbit (Lossi et al., 1995; De Almeida da Anunciaçao et al., 2021), on E15-16 in mice (Chen et al., 2017), on E 15 in rats (Dziegielewska et al., 2001; Alicelebic et al., 2004), on E 14-16 in Pygmy mice (Favaron et al., 2012), on E 22-25 in cat (Knospe, 2002), on E 60 in bovine(Ferreira et al., 2018), on E 38 in equine (Franciolli et al., 2011; Rigoglio et al., 2017), on E 90 in Alpaca (Montelli et al., 2019) and on E 32 in human (Menu, 2009; O'Rahilly and Müller, 2010; Ten Donkelaar et al., 2014; Shiraishi et al., 2015). The nervous system is one of the earliest systems to develop and the last one to develop after birth (Nathia et al., 2016).

Therefore, the present study aimed to throw spotlights on the histological and histochemical features of the rabbit cerebellum during prenatal development by using light and transmission electron microscope.

Table 1. Biometric data and estimated age according to the crown-rump length (Evans & Sack, 1973) of rabbit embryos and fetuses.

Gestational stage	Number of animals	Weight (g)	Crown-rump (cm)	Gestational age (days)
Beginning of gestation	8	3-4	3.9-4.3	10
Mid-gestation	8	3.8-5	5.1	15
Mid-gestation	8	5.5	7.3	20
End of gestation	8	24-26	10	25
End of gestation	8	44	11.5-12.4	30

MATERIALS AND METHODS

2.1. Examined animals

The present study was carried out on forty females of balady rabbits. They were collected from different popular markets in Menoufia Governorate, Egypt, where all rabbits were kept and managed under the same conditions of feeding, water supply and temperature. The ovulation in the rabbits is induced; it happened only a few hours after mating (Sirotkin et al., 2010). They submitted to natural mating and embryonic day (E0) was the day following mating. Forty prenatally developed embryos (E10, E15, E16, E20, E25, and 30 days of embryonic day were attained from the coupled does. The experimental protocols were approved by the Institutional Animal Care and Use Committee (IACUC) with oversight of the faculty of Veterinary Medicine, University of Sadat City, and protocol no: VUSC-041-1-20.

2.2. Histological study

The embryos of (E10, E15, E17 and E20) were fixed in 10% formalin for light microscopic examination. The cerebella of embryos (E25 and E30) were fixed in 10% formalin for at least 48 hours. All specimens were pounded briefly in tap water, dehydrated in ascending grades of ethyl alcohol, cleared in methyl benzoate or xylene, and embedded in paraffin wax. Cross sections (5-7µm) in thickness of each stage were cut on a microtome (LEICA RM-2155, Germany) (Luna, 1968). The sections were stained with:

a) Harri′s hematoxylin and eosin (H&E).

b) Crossmon′s trichrome stain (CT).
c) Weigert′s elastic tissues stain (WE).
d) Gomori′s reticulin stain (GR).
e) Periodic acid Schiff technique (PAS).
f) Alcian blue at pH2.5 (AB).
g) Alcian blue at pH2.5 – periodic acid Schiff combination (AB+ PAS).

All the aforementioned stains and techniques were described by Bancroft and Gamble (2008). The Olympus CHS microscope (Olympus Optical CO, LTD, Tokyo, Japan; Leica DM 2500, Leica Germany) was used to examine each stained section.

2.3. *Photomicrography*

A digital camera (Olympus DP71 camera) mounted on an Olympus IX71 microscope was used to capture digital pictures. Software included DP Manager, DP Controller, and an image analysis application. The only adjustments made to the captured images were in Adobe Photoshop Elements (Adobe Systems, Tokyo, Japan) for contrast and brightness.

2.4. For Transmission Electron Microscope Study

Small specimens (1 mm^3) from different parts of the cerebellum at prenatal ages (E25 and E30) were taken and fixed in 2.5% buffered glutaraldehyde in 0.1 M phosphate buffer solution pH 7.4 at 4^oC for 2 hours, washing three times with phosphate buffer solution (PBS) (10 min, each), Post fixed in 1% Osmic acid for (30 min), washing three times with PBS (10 min. each), then dehydrated with ascending series of ethyl alcohol (30, 50, 70, 90% and absolute alcohol) infiltrated with acetone, for 30 min. After dehydration samples were embedded in Araldite 502 resin. The plastic molds were cut in the Leica ultra-microtome, stained with 1% toluidine blue then photographed. After examination of semi- thin sections, ultra-thin sections were cut, stained with uranyl acetate. Then counter stained with lead citrate (Hayat, 2000). Ultrathin sections were examined & photographed by using JEOL-JEM-100 SX transmission electron microscope, Japan, electron microscope unit, Tanta University.

RESULTS

3.1. Biometric data and external morphological features

Morphological features of each age are described in figure 1 and table 1 contained biometric data (crown-rump length and weight) of each age.

3.2. Microscopic features of the cerebellum

At 10^{th} day of rabbit embryos, the primitive encephalic vesicles (prosencephalon, mesencephalon, and rhombencephalon) were observed. Rhombencephalon was formed from metencephalon (cerebellum and pons) and myelencephalon (medulla oblongata). The primordium of cerebellum originated from the dorsolateral portion of the metencephalon's alar lamina. It was formed from a mass of undifferentiated cells, dorsal to choroid plexus, project into the fourth ventricle (Fig. 2a). The primordium of cerebellum was formed of two distinct germinal parts: an intraventricular part, that appeared projected into the cavity of the developing fourth ventricle of the brain as swelling, and an extra intraventricular part (rhombic lip) that developed as posterolateral outgrowth from alar plates of the metencephalon as thickening. The intraventricular part was larger than rhombic lip (Fig. 2a, 2b, and 2c). Choroid plexus presented on the roof plate (Fig. 2a). Primordium of cerebellum showed weak positive reaction to PAS technique, so it contained few amounts of neutral mucopolysaccharide substance (Fig. 2d

and 2e). The elastic fibers appeared as thin fibers in connective tissue capsule (Fig. 2f).

At the 15th day of rabbit embryo age, the cerebellar primordium (the cerebellar plate) grows, increased in size and thickening and extended medially in the roof plate of the metencephalon, located dorsal to the fourth ventricle cavity (Fig. 3a). Primordia of pons and medulla oblongata had appeared at this stage (Fig. 3a). The primordium of cerebellum was differentiated into thin light less cellular zone and thick dense one. The less cellular zone showed fibroblasts and collagen fibers, with blood capillaries. The thick zone showed oval cells with large, oval nuclei, their cytoplasm was few and lightly basophilic. These cells were arranged as radiated cords. Some cells showed large spherical darkly stained nuclei (Fig .3b and 3c). Some of blood capillaries that contain nucleated red blood corpuscles had appeared (Fig. 3b and 3c). The choroid plexus appeared as irregular, thin folds which were lined with cuboidal to columnar cells, rested on loose connective tissue containing blood capillaries and fibro blast cells (Fig. 3b). The thickness of the fourth ventricle was increased (Fig. 3a). The cerebellar primordium showed weak positive reaction to PAS technique (Fig. 3d and 3e). By using AB pH 2.5 – PAS stain combination showed purple color (Fig. 3f and 3g). The collagen fibers appeared as thin fibers (Fig. 3h). The elastic fibers also, appeared as thin fibers (Fig. 3i and 3j). The reticular fibers appeared as fine fibers (Fig. 3k).

At the 17th day of rabbit embryo age, cerebellar primordium development was well in advance, and differentiation of cells was seen. The ventricular cavity lined with neuroepithelium layer, Purkinje cell migration wave layer that appeared as a dense layer of long, spindle-shaped cells, deep plexiform layer that appeared as fibrous layer, nuclear migration wave (cellular layer) and another fibrous layer that was extremely superficially dorsal and was called the superficial plexiform layer. (Fig. 4a and 4b). The cerebellar primordium showed positive reaction to PAS technique (Fig. 4c and 4d). The elastic fibers appeared as thin fibers (Fig. 4e). The reticular fibers appeared as fine fibers that showed positive reaction to Gomori′s reticulin stain (Fig. 4f).

At the 20th day of rabbit embryo age, cerebellum showed no folds and fissures. With increasing development of the cerebellum, it increased in size (Fig. 5a). The cells were arranged in different layers: neuroepithelium (ventricular zone), intermediate (mantle layer) and marginal layer. Neuroepithelium appeared as thin layer densely crowded, mitotically active and randomly arranged cells. The cells appeared large, with large and darkly stained nuclei and basophilic cytoplasm. The cytoplasmic processes were branched and darkly stained (Fig. 5b and 5c). The intermediate layer was thick lightly stained. Their cells were not crowded, triangular, polyhedral, and oval in shapes. The nuclei were large and lightly stained, and the cytoplasm was light with few cytoplasmic processes (Fig. 5b and 5c). The marginal zone was hypocellular with large spherical and lightly stained nuclei. The cytoplasmic processes were very few. It was formed of myelinated nerve fibers. (Fig. 5b and 5c). The cerebellar primordium showed moderate positive reaction to PAS technique (Fig. 5d and 5e) and weak reaction to alcian blue stain (Fig. 5f). By using AB pH 2.5 – PAS stain combination purple color appeared (positive reaction to PAS and negative reaction to alcian blue) (Fig. 5g and

5h). Collagen fibers were distributed as thin fibers in the cerebellar primordium (Fig. 5i and 5j). The elastic fibers were demonstrated in neuroepithelium (ventricular zone), intermediate (mantle layer) and marginal layer as thin fibers (Fig. 5k and 5l). The reticular fibers were distributed as fine fibers in neuroepithelium and primitive cerebellar cortex (Fig. 5m).

At the 25th day of rabbit embryo age, the cerebellum increased with more differentiation and appearance of fissures on its surface (Fig. 6a). Cerebellar cortex was arranged as the previous stage of development. Neuroepithelial cells give rise to external granular layer (EGL). EGL was known as the second embryonic layer that divided mitotically into different types of cells. This layer developed during fetal life as a result of cells migrating from the neuroepithelial layer via the intermediate and mantle layers. The EGL development was a distinct structure in the developing cerebellum that appeared in the late developmental stages of the embryo and persisted during first post-natal period, given rise to neurons of the internal granular layer (IGL), the basket and stellate cells. Some cells of mantle layer migrated to surface to form the cerebellar cortex (Golgi type II neurons and Purkinje cells), and the remaining cells form cerebellar nuclei. The marginal layer given rise to white matter (cerebellar medulla). The white matter and deep cerebellar nuclei were observed at E25 (Fig. 6b and 6c). The white matter showed nerve fibers, few glia and few blood capillaries. The cerebellum showed moderate positive reaction to PAS technique (Fig. 6d) and faint positive reaction to alcian blue stain (Fig. 6e and 6f). By using AB pH 2.5 – PAS stain combination showed purple color (Fig. 6g and 6h). The elastic fibers appeared distributed in the dura matter, intermediate (mantle layer) and between cells of marginal layer as few thin fibers (Fig. 6i and 6j). Collagen fibers were distributed as a few thin fibers in dura matter and between cells of marginal layer at this age (Fig. 6k and 6L). The reticular fibers appeared distributed in dura matter, neuroepithelium and cells of marginal layer (Fig. 6m and 6n).

Semithin sections showed three cortical layers neuroepithelial, intermediate (mantle layer) and marginal layer (Fig. 6o and 6p). The ultrastructural examination showed mitotically active cells in neuroepithelium (ventricular zone) with large euchromatic nucleus and apparent nucleolus. The cytoplasm contains Golgi bodies and numerous mitochondria (Fig. 6q and 6r). Marginal zone (Mz), containing myelinated nerve fibers (NF), filled with mitochondria (Fig. 6s).

At the 30th days of rabbit embryo ages, the cerebellum was not fully differentiated. The fissures were increased and more in depth (Fig. 7a) Cortex was comprised of external granular layer, molecular layer, and internal granular layer (Fig. 7b). External granular layer was polymorphic cells. Their nuclei were large elliptical in shape. This cell became swollen and spherical in shape due to migration (Fig. 7c and 7d). Molecular layer consisted of little, tiny granular cells with nuclei stained darkly and few cytoplasm with increased branched fibers. The fibers were increased and arranged in groups (Fig. 7c and 7d). The internal granular layer consisted of closely packed small granular cells with darkly stained, spherical nuclei and a few cytoplasm with few fibers (Fig. 7c and 7d). The white matter appeared fibrous with few oval lightly stained cells. (Fig. 7b, 7c, and 7d). The cerebellum showed moderate positive reaction to PAS

technique (Fig. 7e and 7f) and positive reaction to alcian blue stain (Fig. 7g and 7h). By using AB pH 2.5 – PAS stain combination showed purple color (Fig. 7i and jk). Collagen fibers appeared distributed in dura matter, and in external and internal granular layers (Fig. 7k). The elastic fibers appeared distributed in dura matter, in molecular, and internal granular layers (Fig. 7L). The reticular fibers were demonstrated in dura matter, external granular and internal granular layers (Fig. 7m).

Semithin sections showed three cortical layers the external granular, molecular, and internal granular layers (Fig. 7n and 7o). The ultrastructural examination showed the molecular layer of cerebellar cortex contained multiple myelinated nerve fibers filled with mitochondria (Fig. 7p). Ultrastructural examination of the cerebellar cortex revealed small sized Purkinje cells. Their nuclei were euchromatic and had pronounced nucleoli. They had rough endoplasmic reticulum strands, mitochondria, and the Golgi apparatus in their cytoplasm (Fig. 7q and 7r). An ultrastructural analysis of the granular layer showed that the spherical nuclei of the granule cells had peripheral heterochromatin clumps and mitochondria. There were neuroglial cells and myelinated nerve fibers (Fig. 7s).

At later postnatal stages, the EGL progressively disappeared, while there was a net increase in the thickness of the molecular layer and the IGL until the morphological features of the mature cerebellum are reached from P60 onward.

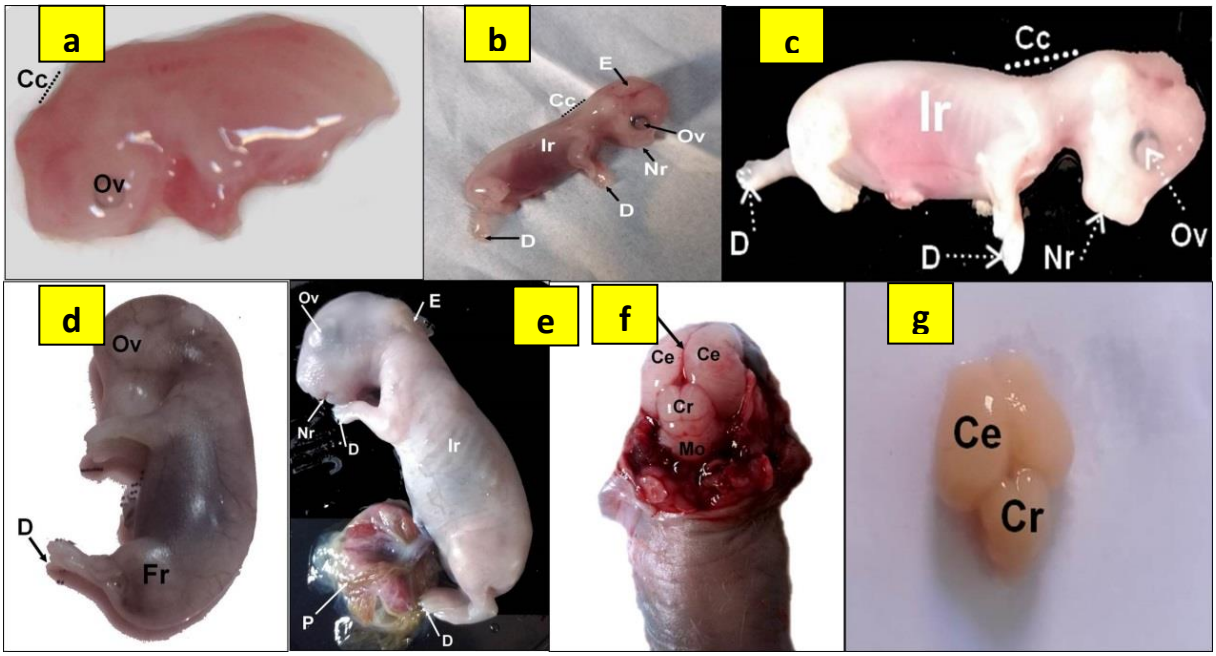


Figure 1. Developmental stages of cerebellum in balady rabbits during gestation. a) 10th day of gestation, b) 15th day of gestation, c) 16th day of gestation, d) 20th day of gestation, e) 25th day of gestation and f) 30th day of gestation. g) cerebellum at the day of parturition. E, Ear; Ir, Impression of ribs; D, Digit buds; Nr, Nasal region together with the mandible; Cc, Cervical curvature; Ov, Optical vesicle; fr, femoral region; P, placenta; Cr, Cerebellum; Ce, cerebral hemisphere; black arrow in number f, cerebral fissure.

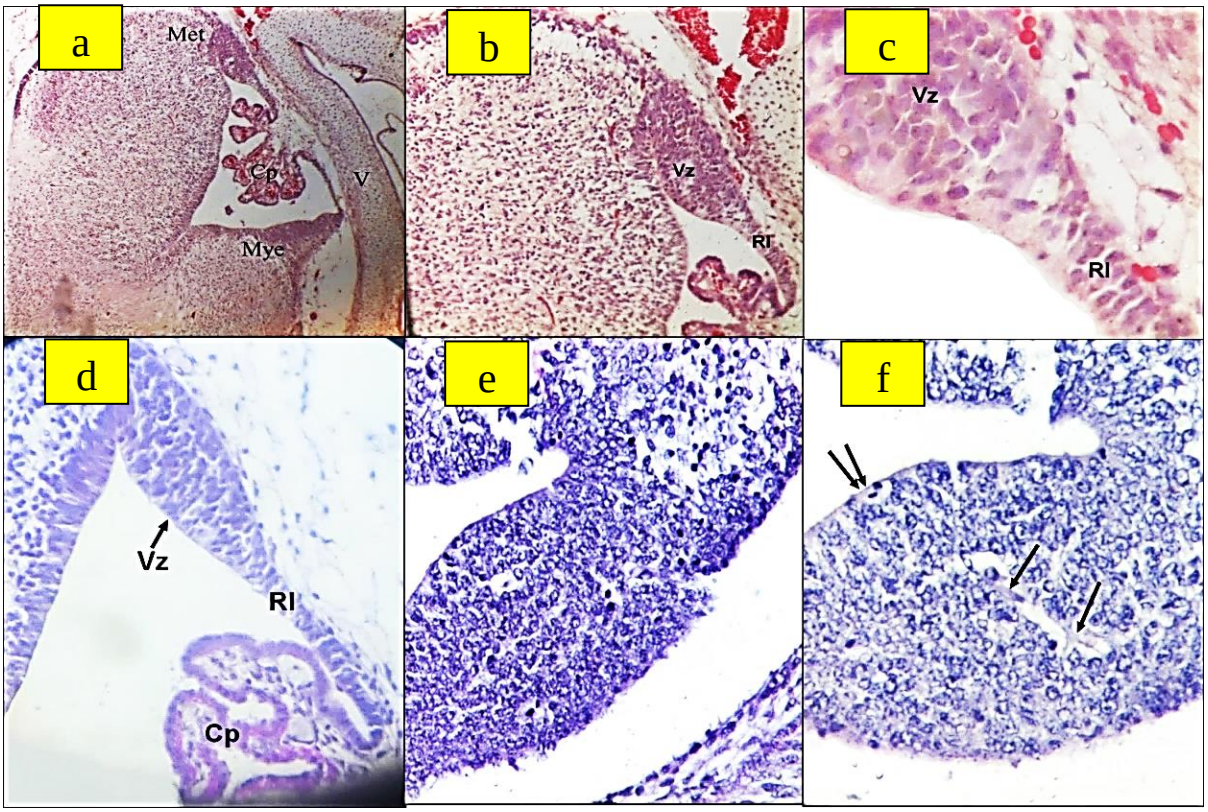


Figure 2. Photomicrograph of the rabbit cerebellum at embryonic age (E) 10. (a): showing metencephalon (Met), myelencephalon (Mye), vertebrae (V) and choroid plexus (Cp). H&E×40. (b): showing ventricular zone (Vz) and rhombic lip (Rl) of the cerebellum. H&E ×100. (c): Higher magnification of (a & b). H & E ×200. (d): showing weak positive neutral mucin reaction. (PAS stain X 200). (e): showing weak positive neutral mucin reaction. (PAS stain X 400). (f): showing distribution of elastic fibers (black arrow). (Weigert′s elastic tissues stain X 200).

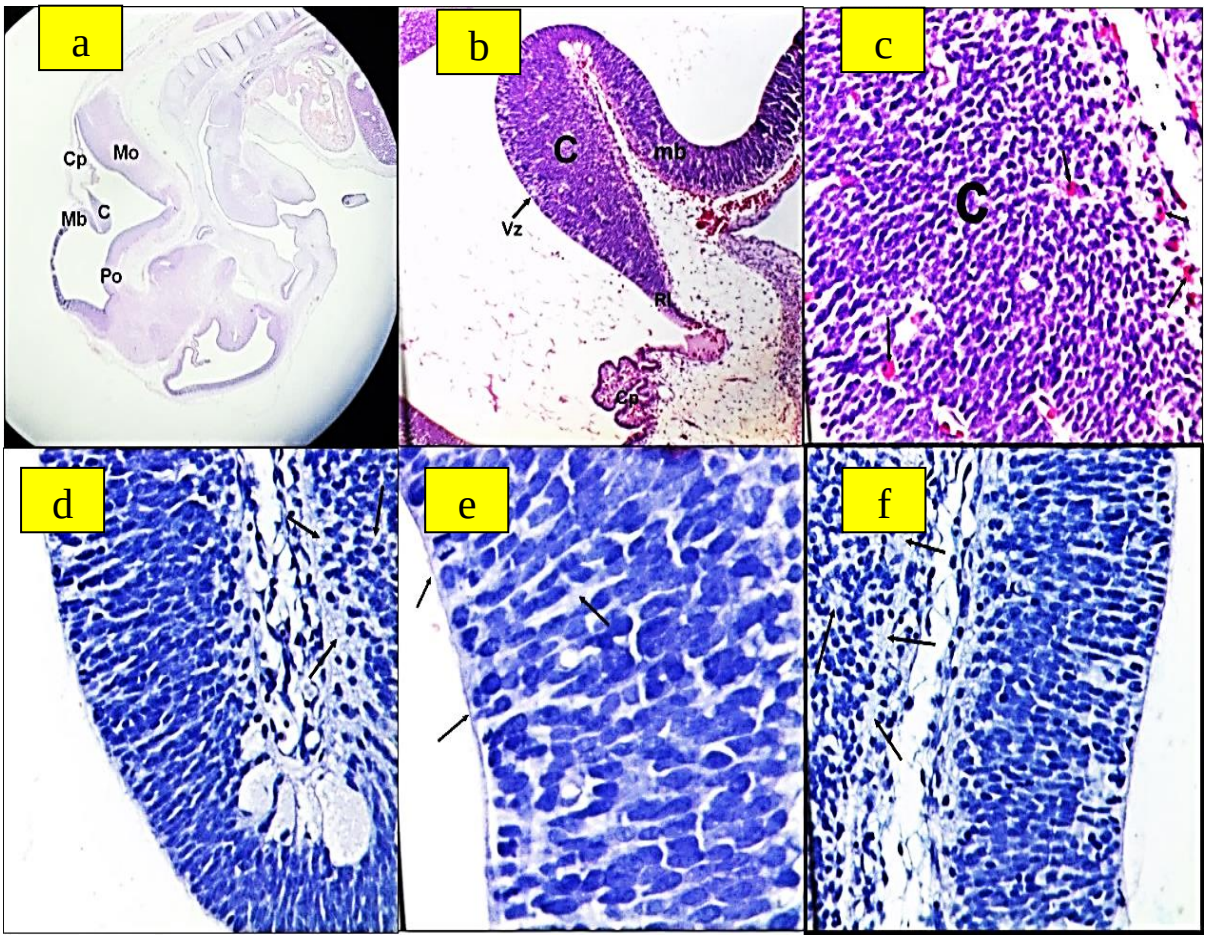
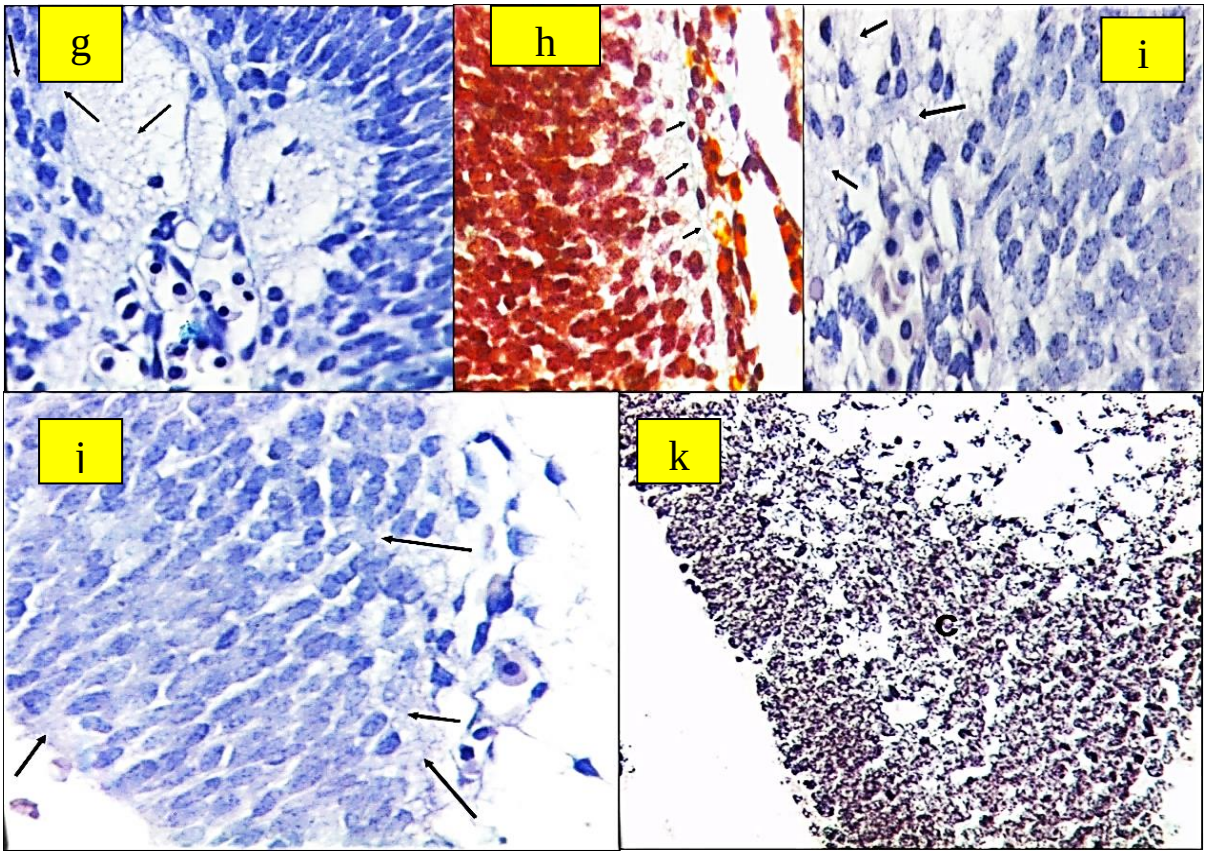


Figure 3. Photomicrograph of the cerebellum at E 15. (a): showing cerebellar primordium (C), choroid plexus (Cp), pons (Po), mid brain (Mb), and medulla oblongata (Mo). H&E stain stereomicroscope×5. (b): showing rhombic lip (Rl) and ventricular zone (Vz) of the cerebellum primordium (C), choroid plexus (Cp), mid brain (Mb). (H&E ×40). (c): Higher magnification of (a&b), cerebellar primordium (C) and capillaries (black arrows). (H&E × 200). (d): showing weak positive reaction to PAS (black arrows). (PAS stain X 200). (e): showing weak positive reaction to PAS (black arrows). (PAS stain X 400). (f): showing weak positive reaction to alcian blue – PAS combination method (AB pH 2.5 – PAS stain X 200).



(g): Higher magnification of (f)**.** (AB pH 2.5 – PAS stain X 400). (View h): showing positive reaction to Crossmon′s trichrome stain (black arrows). (CT X 400).(i&j): showing distribution of elastic fibers in cerebellum primordium (black arrow). (Weigert′s elastic tissues stain X 400). (k): showing distribution of reticular fibers in cerebellum primordium. (GR stain X 200)**.**

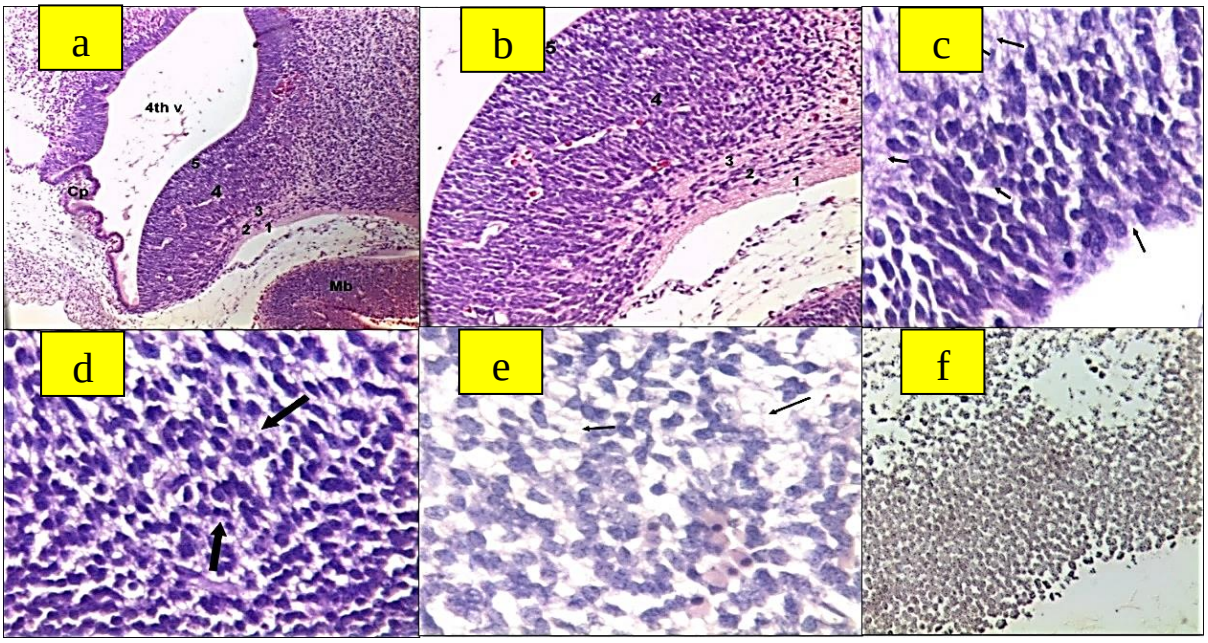


Figure 4. Photomicrograph of rabbit cerebellum at E (17). (a): showing 5, neuroepithelium; 4, Purkinje cell migration wave; 3, deep plexiform layer; 2, nuclear migration wave; 1, superficial plexiform layer; Cp, choroid plexus; 4th primitive 4th ventricle; midbrain (Mb). H&E X 40. (b): High magnification of (a). H&E X100. (c & d): showing positive reaction to PAS (black arrows). (PASstainX400). (e): showing distribution of elastic fibers in cerebellum primordium (black arrow). (Weigert′s elastic tissues stain X 400). (f): showing distribution of reticular fibers in cerebellum primordium. (GR stain X 400).

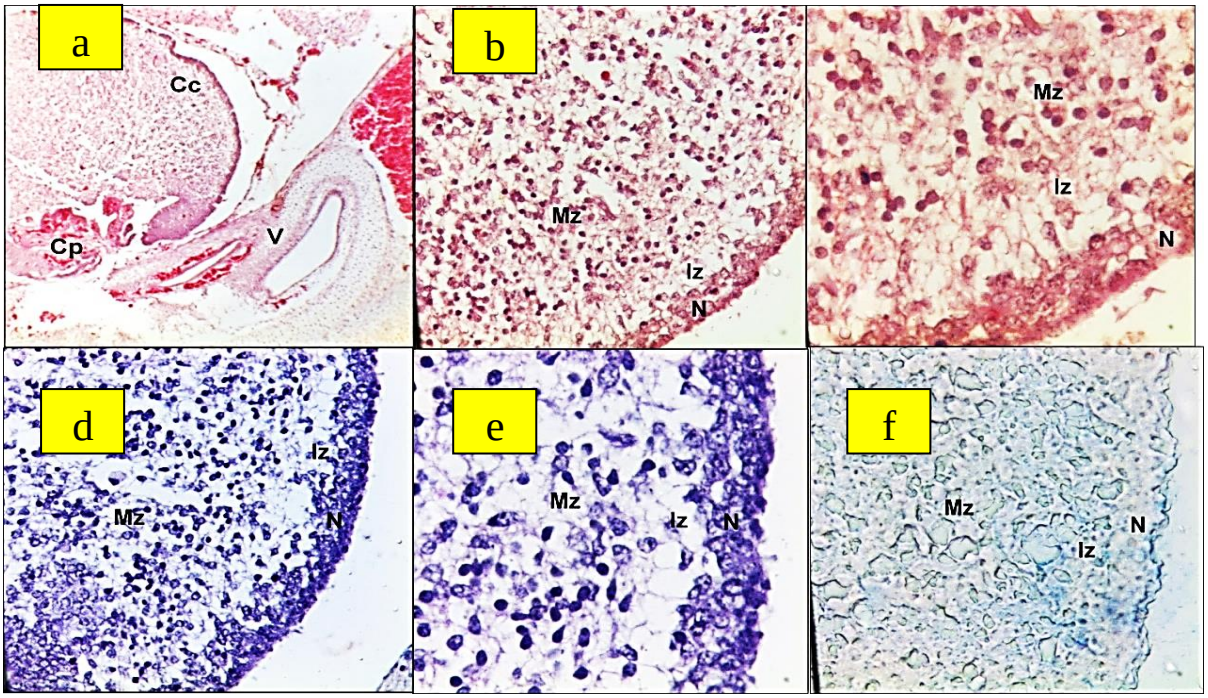
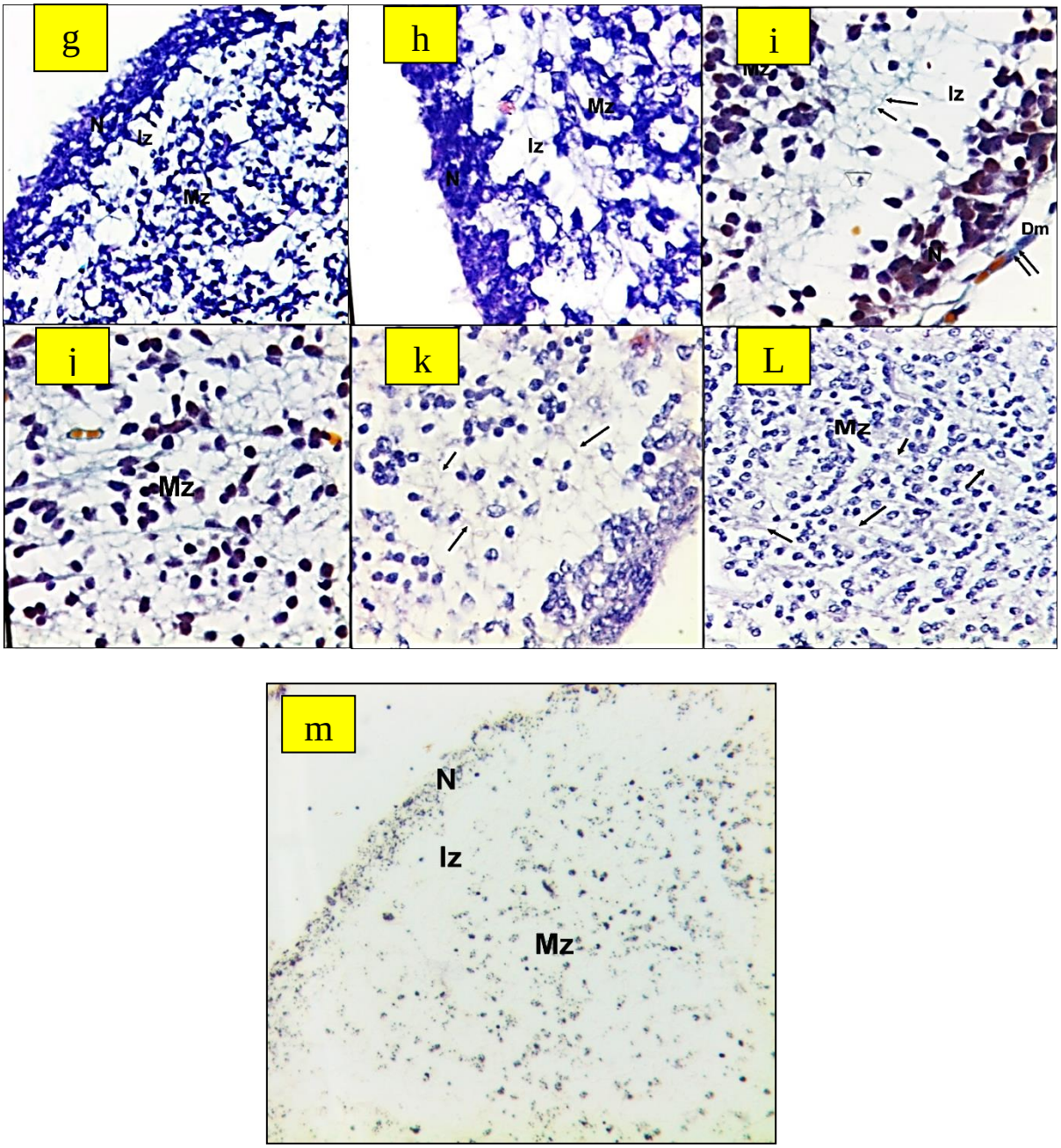


Figure 5. Photomicrograph of rabbit cerebellum at E 20. (a): showing choroid plexus (Cp), cerebellar cortex (cc) and vertebrae (V). (H&E X 40). (b): showing neuroepithelium (N), intermediate zone (Iz), marginal zone (Mz). (H&E X 200). (c): A higher magnification of (b). (H&E X 400). (d): showing positive reaction to PAS in neuroepithelium (N), intermediate zone (Iz), marginal zone (Mz). (PAS stain X 200). (e): A higher magnification of (d). (PAS stain X 400). (f): showing weak reaction to alcian blue stain. (AB pH 2.5 X 400).



(g): showing positive reaction to PAS and negative reaction to alcian blue. (AB pH 2.5 – PAS stains X 200). (h): A higher magnification of (g). (AB pH 2.5 – PAS stains X 400). (i): showing positive reaction to Crossmon′s trichrome stain (black arrow) in neuroepithelium (N), intermediate zone (Iz), marginal zone (Mz) and dura matter (Dm). (CT X 400). (j): showing distribution of collagen fibers in marginal zone (Mz). (CT X 400). (k): showing distribution of elastic fibers in cerebellum primordium (black arrow). (Weigert′s elastic tissues stain X 400). (L): showing distribution of elastic fibers in cerebellum primordium (black arrow). (Weigert′s elastic tissues stain X 200). (m): showing positive reaction to Gomori′s reticulin stain (black arrow). (GR stain X 200).

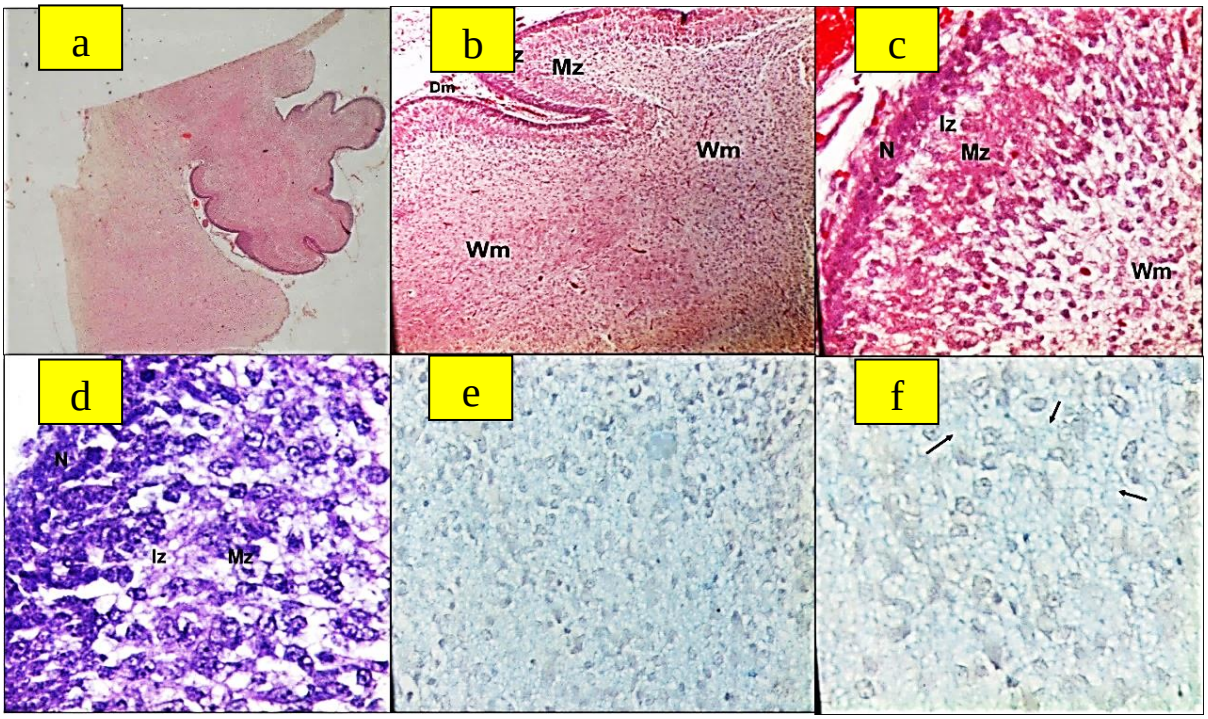
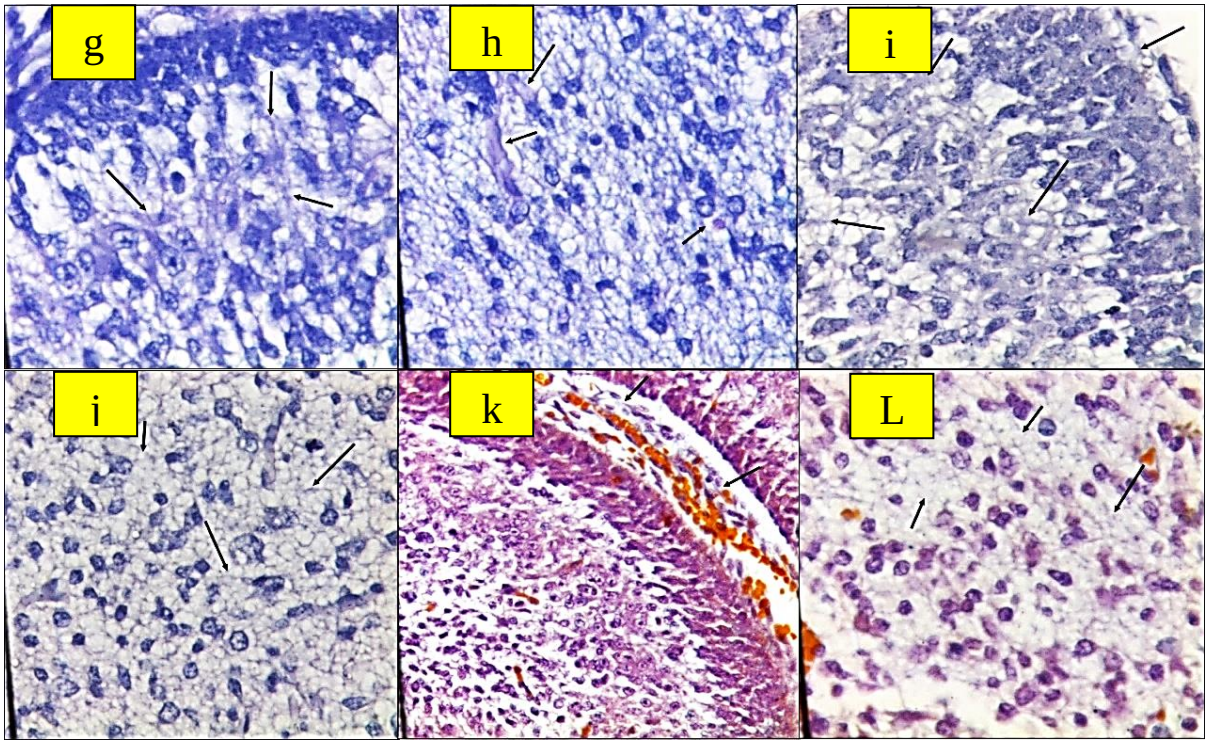
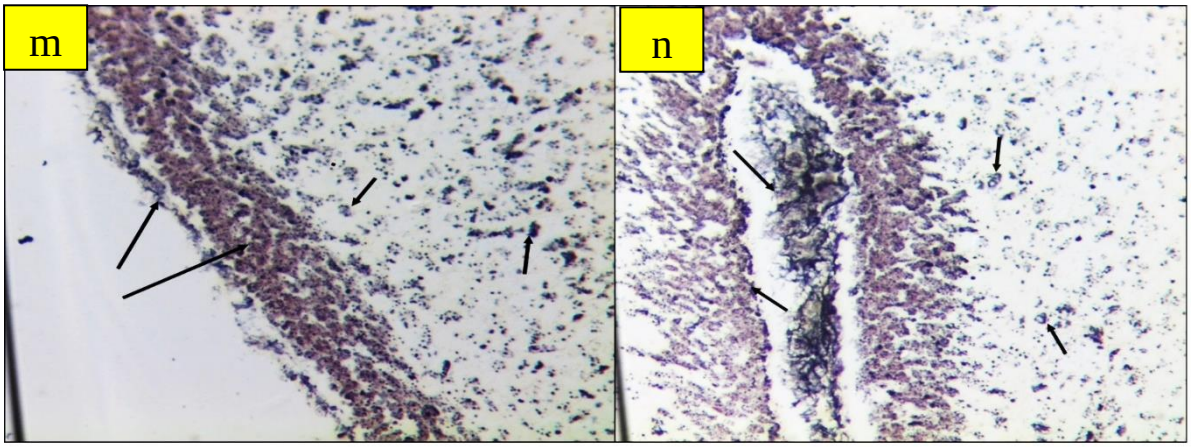


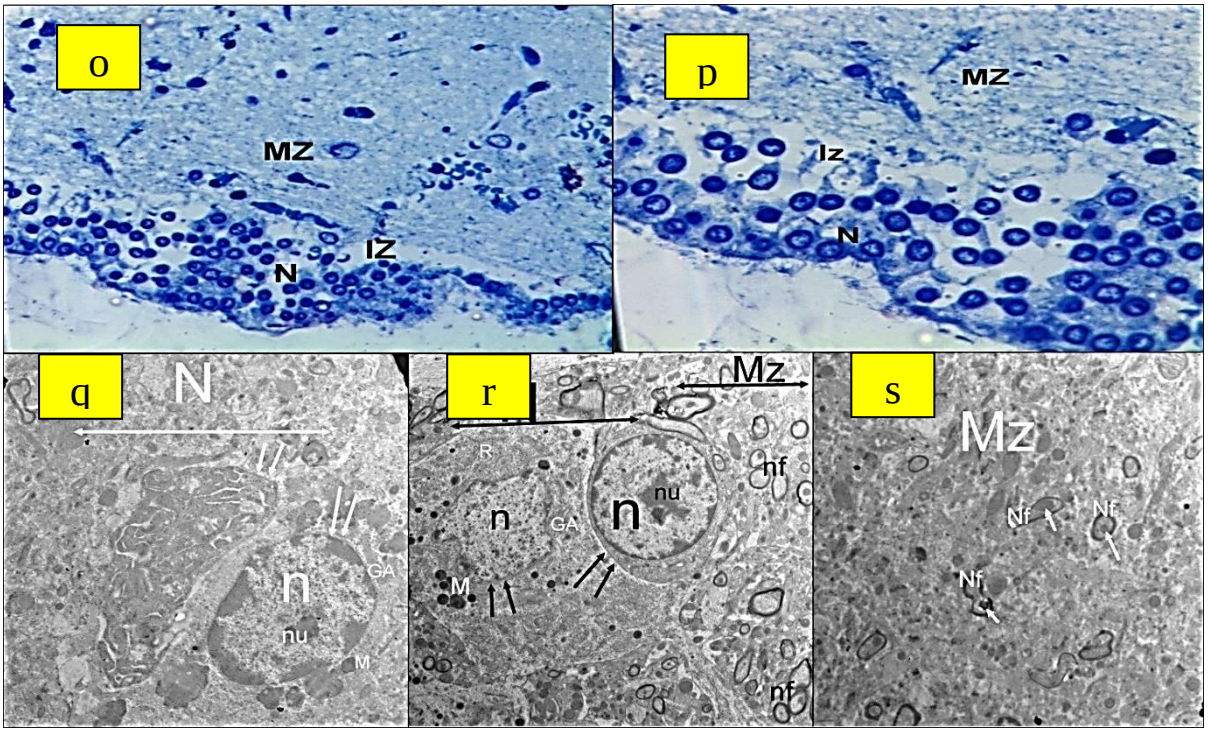
Figure 6. Photomicrograph of the rabbit cerebellum at E 25. (a): H&E stain stereomicroscope×20. (b): showing neuroepithelium (N), intermediate zone (Iz), marginal zone (Mz), white matter (Wm) and dura matter (Dm). (H&E X 40). (c): Higher magnification of (b)**.** (H&E X 200).(d): showing positive reaction to PAS (black arrow). (PAS stain X 400). (e): showing moderate reaction to alcian blue stain. (AB pH 2.5 X 200). (f): Higher magnification of (e)**.** (AB pH 2.5 X 400)**.**



(g): showing AB-PAS positive reaction. (AB pH 2.5 – PAS stains X 400). (h): showing AB-PAS positive reaction (black arrow). (AB pH 2.5 – PAS stains X 400). (i): showing distribution of elastic fibers in mantle layer and dura matter (black arrow). (Weigert′s elastic tissues stain X 400). (j): showing distribution of elastic fibers in mantle layer (black arrow). (Weigert′s elastic tissues stain X 400). (k): showing few &sporadic collagen fibers in dura matter (black arrow). (CT X 400). (L): showing few collagen fibers between marginal layer and white matter (black arrow). (CT X 400)



(m & n): showing distribution of reticular fibers in dura matter and in Egl (black arrow). (GR stain X 200)**.**



(o): A semithin section showing neuroepithelium (N), intermediate zone (Iz), marginal zone (Mz). (TB stain X 200)**.** (p): A higher magnification of (p)**.** (TB stain X 400)**.** Transmission electron micrographs**,** (q): showing neuroepithelium (N)**,** mitotically active cells (white arrows) with large euchromatic nucleus (n) which containing prominent nucleolus (nu). The cytoplasm contains Golgi bodies (GA) and numerous mitochondria (M). (TEM × 1500). (r): showing neuroepithelium (N)**,** marginal zone (Mz), mitotically active cells (black arrows) with large euchromatic nucleus (n) and prominent nucleolus (nu). The cytoplasm contains Golgi bodies (GA), cisternae of rough endoplasmic reticulum (R) and numerous mitochondria (M). Multiple myelinated nerve fibers (NF). (TEM × 1500). (s): showing marginal zone (Mz), myelinated nerve fibers (NF), mitochondria (white arrow). (TEM × 2000).

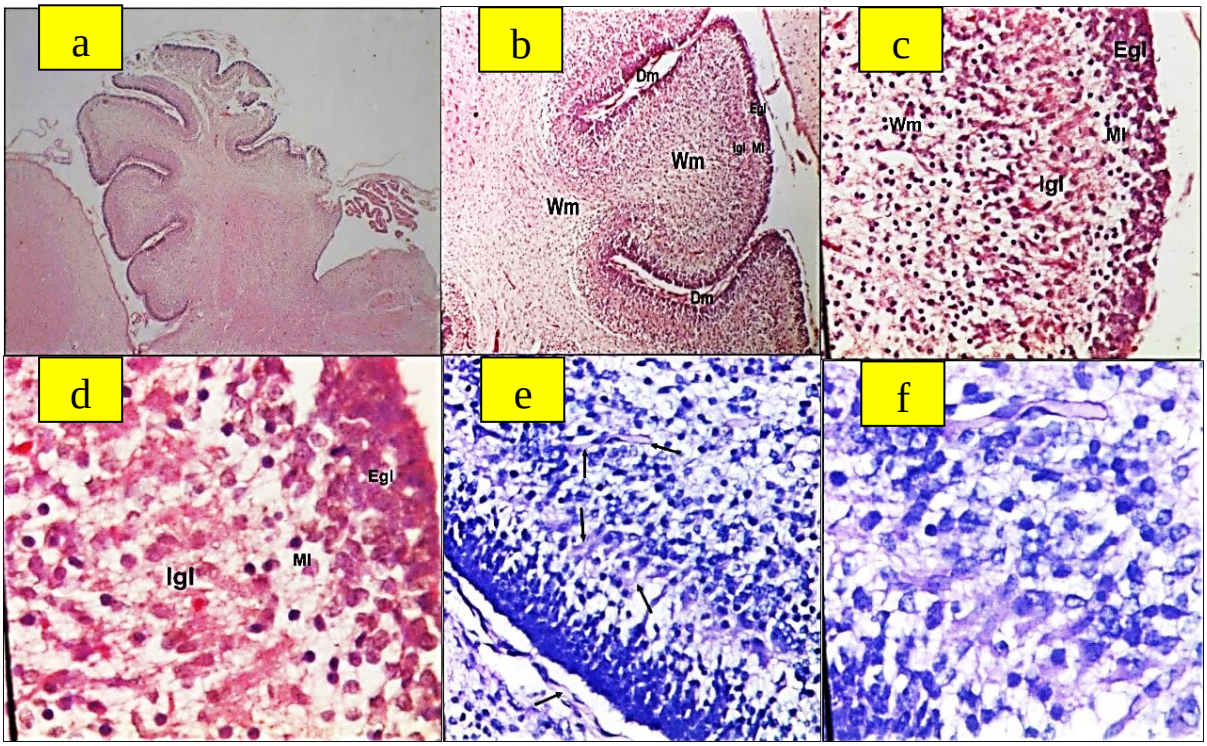
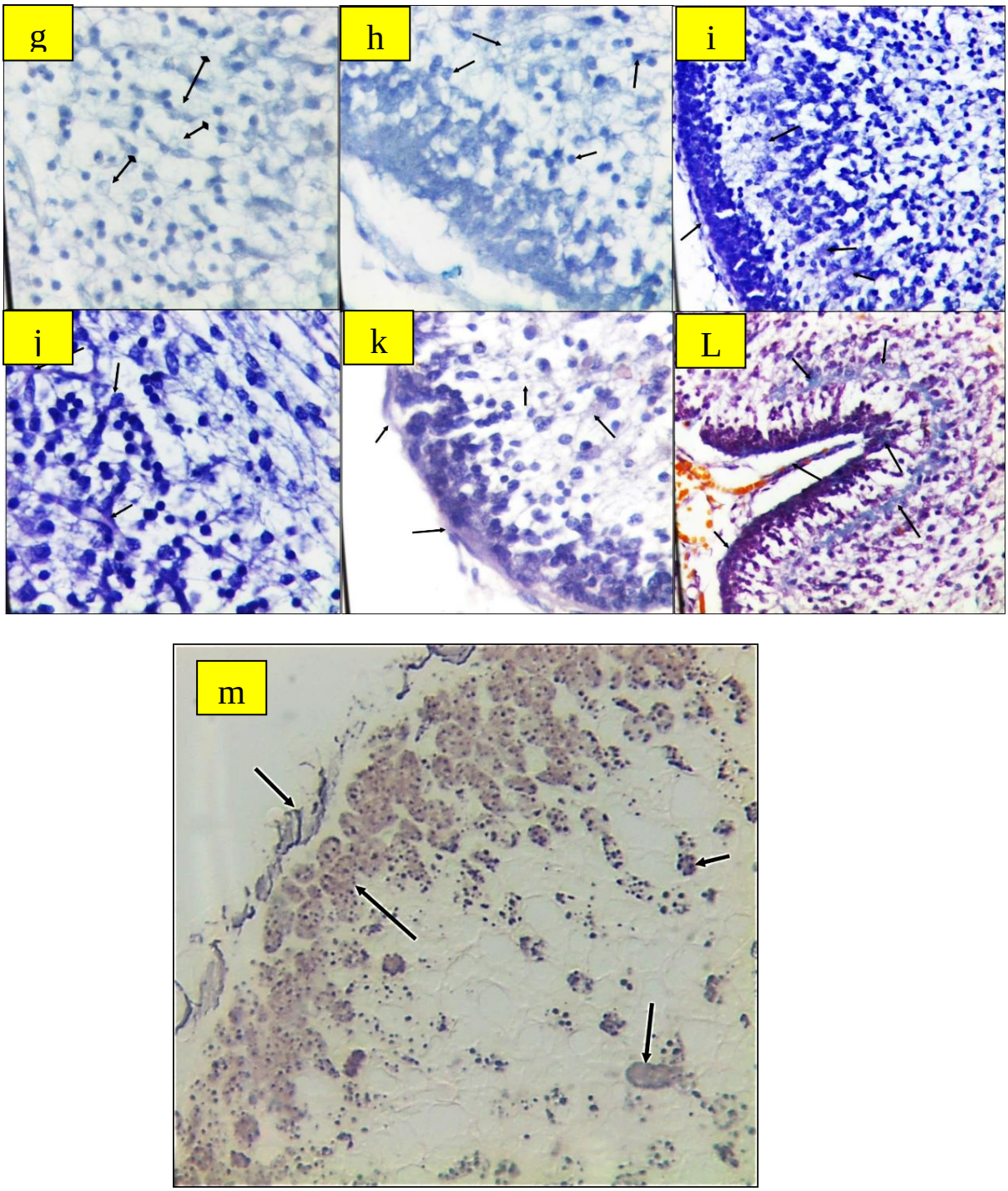
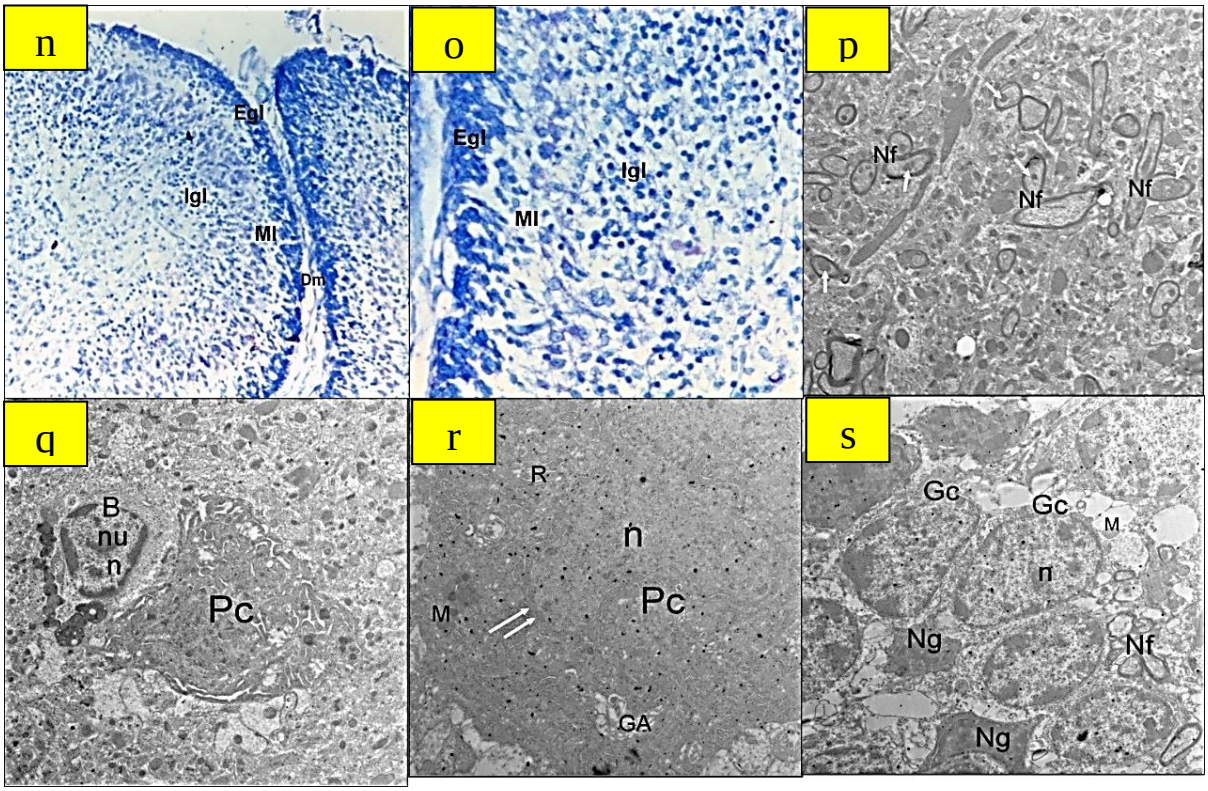


Figure 7. Photomicrograph of rabbit cerebellum at E 30. (a): H&E stain stereomicroscope×30. (b): showing the external granular (Egl), molecular layer (Ml), internal granular layers (Igl), white matter (Wm) and dura matter (Dm). (H&E X 40). (c): showing the external granular (Egl), molecular layer (Ml), internal granular layers (Igl) and white matter (Wm). (H&E X 200). (d): A higher magnification of (c)**.** (H&E X 400). (e): showing positive reaction to PAS (black arrow). (PAS stain X 400). (f): showing positive reaction to PAS (black arrow). (PAS stain X 400).



(g): showing positive acid mucin reaction. (AB pH 2.5 X 400). (h): showing positive acid mucin reaction. (AB pH 2.5 X 400). (i): showing AB-PAS positive reaction. (AB pH 2.5 – PAS stains X 400). (j): showing AB-PAS positive reaction. (AB pH 2.5 – PAS stains X 400). (k): showing distribution of elastic fibers (black arrow). (Weigert′s elastic tissues stain X 400). (L): showing distribution of collagen fibers (black arrow). (CT X 200). (m): showing distribution of reticular fibers in dura matter and Egl (black arrow). (GR stain X 400).



(n): A semithin section showing the external granular (Egl), molecular layer (Ml), internal granular layers (Igl) and dura matter (Dm). (TB stain X 100)**.** (o): A higher magnification of (View p)**.** (TB stain X 200)**.** (p): An electron micrograph showing molecular layer containing multiple myelinated nerve fibers (NF), filled with mitochondria (white arrow). (TEM × 2000). (q): showing the Purkinje cell (Pc) is neighboring a Bergmann astrocyte with nucleus (n), which contains a prominent nucleolus (nu). (TEM × 1500). (r): showing the Purkinje cell (Pc)**,** with large euchromatic nucleus (n). The cytoplasm contains Golgi bodies (GA), rough endoplasmic reticulum (R) and numerous mitochondria (M). (TEM × 3000). (s): showing granule cells (Gc), nuclei (n), mitochondria (M), neuroglial cells (Ng) and the myelinated nerve fibers (Nf). (TEM ×2000)**.**

DISCUSSION:

Many species included rats, mice, and rabbits, have been used in neuroscientific research (Muñoz-Moreno et al., 2013). Proper experimental model must be selected to comprehend specific pathological processes (Konefal et al., 2013; Ming and Song, 2011; Silva et al., 2016).

In this study, we analyzed the development of neurological structures, three primary brain vesicles (prosencephalon, mesencephalon, and rhombencephalon) and cerebellar primordium observed in rabbits at day 10 prenatal embryos of rabbits than in other short-gestation animals such as rats, mice, and pigmy mice. The time of these events is critical and could provide new information on the phylogeny of the target species (Butler & Hodos, 2005).

On day 10 prenatal embryos of rabbits, the rhombic lip developed as posterolateral outgrowth from alar plates of the metencephalon. Many authors have described the rhombic lip as the Rhombencephalic Demilune. Other group of authors had referred to the Rh.D as the "germinal trigone" in their research on the mammalian cerebellum's development during pregnancy (Gilthorpe, 2002; Altman and Bayer, 1985; Al-Salihi, 1995). Other group of authors differed in their

description for the rhombic lip. It was referred to by some as an upper rhombic lip (AL-Salihi, 2011; Chizhikov and Millen, 2013), others described it as rostral rhombic lip (Lin et al., 2001) and another called it anterior rhombic lip (Jensen, 2004). The current study illustrated that the cerebellar primordium (the cerebellar plate) had appeared at 10-day prenatal embryos of rabbits that in contrast with the observations of De Almeida da Anunciaçao et al. (2021).

On 15th day of the prenatal embryos of rabbits, the cerebellar primordium consisted of two distinct germinal zones: ventricular zone and the dorsally located rhombic lip. The primordia of the cerebellum located dorsal to the fourth ventricle. These results agreed with De Almeida da Anunciaçao et al. (2021) in rabbit and Haldipur et al. (2019).

In this study, on 17th day of prenatal embryos of rabbits, cerebellar primordium development was well in advance, and differentiation of cells was seen. The ventricular cavity lined with neuroepithelium layer, Purkinje cell migration wave layer that appeared as a dense layer of long, spindle-shaped cells, deep plexiform layer that appeared as fibrous layer, nuclear migration wave (cellular layer) and another fibrous layer that was extremely superficially dorsal and was called the superficial plexiform layer. This result agreed with (Al-Salihi, 1995; Ahmed et al., 2015). But this result in contrast with the observations of (Altman, 1982; Angela and Abraham, 2013; Butts et al., 2013).

At the 20th and 25th day of the prenatal embryos of rabbits, the current study illustrated that the cerebellar plate was composed of neuroepithelium, mantle layer and marginal layer. Neuroepithelial cells give rise to external granular layer (EGL). EGL is termed as the second germinal layer that is divided mitotically into different types of cells. This layer developed during fetal life as a result of cells migrating from the neuroepithelial layer via the intermediate and mantle layers. The EGL development was a distinct structure in the developing cerebellum that appeared in the late developmental stages of the embryo and persisted during first post-natal period, given rise to neurons of the internal granular layer (IGL), the basket and stellate cells. Some cells of mantle layer migrated to the surface to form the cerebellar cortex, and the remaining cells form cerebellar nuclei. The marginal layer gave rise to white matter (cerebellar medulla) as reported by Lossi et al. (1995) in rabbit. The white matter and deep cerebellar nuclei were observed at E25 Comparably, the development of EGL was also observed in the fetus of goats (Lucy, 2005; Shrivastava et al., 1986; Yong ping et al., 2004) and calves (DeLahunta, 1983). In man, cat and dag this period takes until 3 months after birth (Noden & Lahunta, 1985). In sheep the external germinal layer reaches the maximum thickness of cerebellum's cortex in birth time and become thinner after birth (Ghosh, 2002). In Ovie's fetal, external germinal layer appears around 57 days of pregnancy. And within the limits of day 183 reaches its maximum thickness. In cat and dog, external germinal cells reach to their maximum growth within 7 days of birth and until two weeks after birth, with granular layer neurons starts to decrease in these animals some of external germinal cells last until 3 month (Barbastani, 1990).

On day 30 prenatal embryos of rabbits, our results illustrated that the cerebellum was not fully differentiated. Cortex was comprised of external granular layer, molecular layer, and internal granular layer. External granular layer was polymorphic cells.

Their nuclei were large elliptical in shape. This cell became swollen and spherical in shape due to migration. Molecular layer consisted of little, tiny granular cells with nuclei darkly stained and few cytoplasm with increased branched fibers. The fibers were increased and arranged in groups. Internal granular layer consisted of densely packed tiny granular cells with spherical darkly stained nuclei and few cytoplasm with few fibers. This result agreed with results of Marzban et al. (2007); Ponti et al. (2010); Marzban et al. (2015); Haldipur et al. (2019); De Almeida da Anunciaçao et al. (2021) and Jacobson (1991) whose reported that cells of intermediate zone proliferated to become Purkinje cells, Golgi type II neurons and neurons of the deep cerebellar nuclei.

The ultrastructural examination showed the molecular layer contained multiple myelinated nerve fibers filled with mitochondria. Small sized Purkinje cells appeared, had euchromatic nuclei with apparent nucleoli, the cytoplasm contained rough endoplasmic reticulum, mitochondria, and the Golgi apparatus. The granular layer demonstrated that granule cells contained mitochondria and rounded nuclei with peripheral heterochromatin clumps. There were neuroglial cells and myelinated nerve fibers. These results agreed with results of Mohamed and Mohamed (2018) and Fakhry et al., (2022).

Yamamoto et al. (1996) and Currle et al. (2005) demonstrated that the choroid plexus stimulated development of the cerebellar hemisphere.

The current study showed that the brain development in rabbits occurred in the perinatal period. These results agreed with results of (Van Marthens et al., 1975; Harel et al., 1985) in human. But in rodents, brain developed postnatally, with the majority of myelination occurred after seven days postnatally. (Demir & Demir, 1998; Rasband et al., 1999).

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