

Vesicles Stage of Camel Brain Development

Etapas del Desarrollo de las Vesículas Cerebrales del Camello

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SUMMARY: A successive embryonic developmental study was conducted on the brain of twenty eight embryos and fetuses of one humped camel (*Camelus Dromedarius*), whose crown vertebral rump lengths (CVRL) ranged from 9 to 80 mm, collected from the El-Basateen (Cairo) and Belbees (ElSharqya) Slaughterhouse. The current investigation revealed that camel brain was found to consist of fore, mid and hind brains. The fore brain is divided into telencephalon and diencephalon while the rhombencephalon divided into metencephalon and myelencephalon. Flexures appeared between the vesicles are cervical flexure between the rhombencephalon and the spinal cord, cephalic flexure in the mesencephalon and pontine flexure between the metencephalon, and the myelencephalon of the hind brain (rhombencephalon). The cavity of the rhombencephalon is the fourth ventricle, while that of the diencephalon is the third ventricle, and those of the telencephalon are the lateral ventricles but that of mid brain is the cerebral aqueduct. myelencephalon becomes medulla oblongata and metencephalon developed to pons and cerebellum while mesencephalon gives rise to the cerebral crura and anterior and a posterior colliculus. Diencephalon gives the thalamus, hypothalamus, mamillary body, infundibulum and pineal body while telencephalon becomes the cerebral hemispheres and corpus striatum

KEY WORDS: Camel; Embryo; Vesicles; Brain; Development.

INTRODUCTION

Mammalian brain is the most complex organ of all biological systems (Pakkenberg & Gundersen, 1997), its developmental stages are protracted processes that begin in the early gestational weeks with the differentiation of the neural progenitor cells and extends at least through late adolescence, arguably throughout the lifespan (Stiles & Jernigan, 2010). The rostral region of vertebrate neural tubes develops into three morphologically distinct swellings or the primary brain vesicles by differential proliferation of neuroepithelial territories: the forebrain, midbrain, and hind brain (Ishikawa *et al.*, 2012), each of brain vesicles may be composed of several smaller repetitive units known as neuromeres (Nieuwenhuys, 1998). The three primary brain vesicles (forebrain, midbrain, and hindbrain vesicles) subdivided into a series of five secondary brain vesicles (Ishikawa *et al.*).

At the center of the developing brain, series of interconnected cavities were found to form the ventricular system of the brain which is filled with a fluid called cerebral spinal fluid that is completely recycled several times per day. The ventricular system has a number of important functions including cushioning and protection of the brain, removal of

waste material, and transport of hormones and other substances (Brodal, 2010). During brain development, the walls of the ventricles are the site of most neuron production.

Many literatures achieved their research works dealing with prenatal developmental studies of various organs in dromedary camel (Farouk, 2008; Farouk *et al.*, 2012; Osman *et al.*, 2014). The early embryological development of the brain in human and many animals has been reviewed however; no information has been published to date concerning prenatal development of the brain in the one-humped camel. Therefore, the objective of this study was to document the development of brain vesicles stage throughout the early prenatal life in dromedary camel.

MATERIAL AND METHOD

The current study was carried out on twenty-eight camel embryos and fetuses (their CVRL ranged from 9 – 80 mm) collected from El-Basateen (Cairo) and Belbees

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(ElSharqya) Slaughter house. The obtained samples were immersed directly into 10 % neutral buffered formalin. Following fixation, the formalin-fixed specimens were then preserved in 70 % ethyl alcohol. The preserved samples were dehydrated using a graded series of ethanol (75 % up to absolute), subjected to three changes of xylene (I, II, III), then embedded in paraffin wax (melting point = 60 °C). The paraffin-moumt specimens were serially sectioned (sagittaly, longitudinally and transversally cut) at 5 µm thickness. The paraffin sections were subjected to Harris heamatoxylin and Eosin stain (H&E) followed the protocols outlined by Bancroft & Stevens (1996).

The Histological sections were viewed and images were collected with Olympus BX41 research optical photomicroscope equipped with an Olympus DP25 digital camera. The magnification scale bar was reported on the collected photomicrographs.

RESULTS AND DISCUSSION

The early embryonic stages of camel development at 9 mm CVRL stage revealed that the cephalic part of the neural tube is markedly expanded and give rise to the main primary brain vesicles. These divisions of the brain can be distinguished through the formation of accommodated flexures and surface folding in its roof and floor. According to our results in Figure 1, there are two main flexures that constitute the primary brain form. The cranial flexure which is ventral and occurs at the level of midbrain (mesencephalon) separating it from the most fore part of brain (prosencephalon) is known as cephalic flexure. The other one, pontine flexure, is a dorsal flexure that bent brain with the convexity facing downward and located in the most

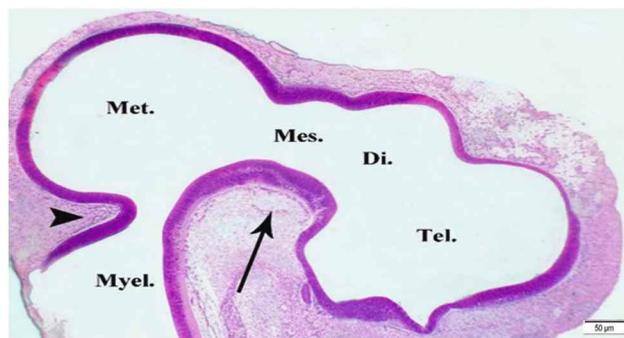


Fig. 1. Photomicrograph of sagittal section of camel embryo (0.9 cm CVRL) showing the main primary vesicles and flexures. Telencephalon (Tel.), Diencephalon (Di.), Mesencephalon (Mes.), Metencephalon (Met.), Myelencephalon (Myel.), arrow represent the cephalic flexure while the arrowhead represents the pontine flexure.

caudal part (hind brain) (rhombencephalon) dividing it into two compartments. In addition to the division of the fore brain into two compartments, these two main flexures configure the brain to be formed from five vesicles. The fore brain gives rise to two divisions; the rostral telencephalon and the caudal diencephalon. Also, the rhombencephalon forms two dilatations as the region caudal to the pontine flexure differentiate into myelencephalon while that rostral to the flexure becomes the metencephalon (Fig. 1).

Each of these brain compartments consists of an outer wall of neural cells enclosing its cavity that named corresponding to its part of brain as follow; Telocaele, diocaele, mesocaele, metacaele, and myelocaele, respectively, which will differentiate later forming the encephalon ventricular system.

These five subdivisions are aligned along the rostral-caudal axis of the embryo and establish the primary organization of the central nervous system (Stiles, 2008). In human, the embryonic patterning affects all brain regions from then forebrain through the spinal column, such that by the end of the embryonic period primitive patterning of sensorimotor regions within the neocortex is established (Bishop *et al.*, 2000), major compartments within diencephalic and midbrain regions have differentiated (Kiecker & Lumsden, 2004; Nakamura *et al.*, 2005), and the segmental organization of the hindbrain and spinal column have been specified (Gavalas *et al.*, 2003). The cavity of the mesencephalon becomes very narrow forming a narrow duct which known as the cerebral aqueduct (aqueduct of Sylvius) [McGeady *et al.* (2006) in domestic animals and Champney (2016) in human] that connects the third and fourth ventricles (Fig. 2). The lateral ventricles communicate with the third ventricle by a foramen which is called the interventricular foramen (foramen of monro) [McGeady *et al.* in domestic animals and Champney (2016) in human].

Derivatives and differentiation of the brain subdivisions:

Myelencephalon: The myelencephalon mainly developed into the medulla oblongata (Fig. 2). Its roof plate appeared as a single layer of neuroepithelial ependymal cells which covered by tela choroidea (vascular mesenchyme and the pia mater). The vascular mesenchyme proliferates and invaginate into the underlying ventricular cavity forming the choroid plexus of the 4th ventricle (posterior choroid plexus). Its cavity (myelocaele) forms the most caudal part of the fourth ventricle (Fig. 2). Same findings were recorded by McGeady *et al.* in domestic animals and Champney in human.

Metencephalon: Like that were recorded by McGeady *et al.* in domestic animals and Champney. In human. The

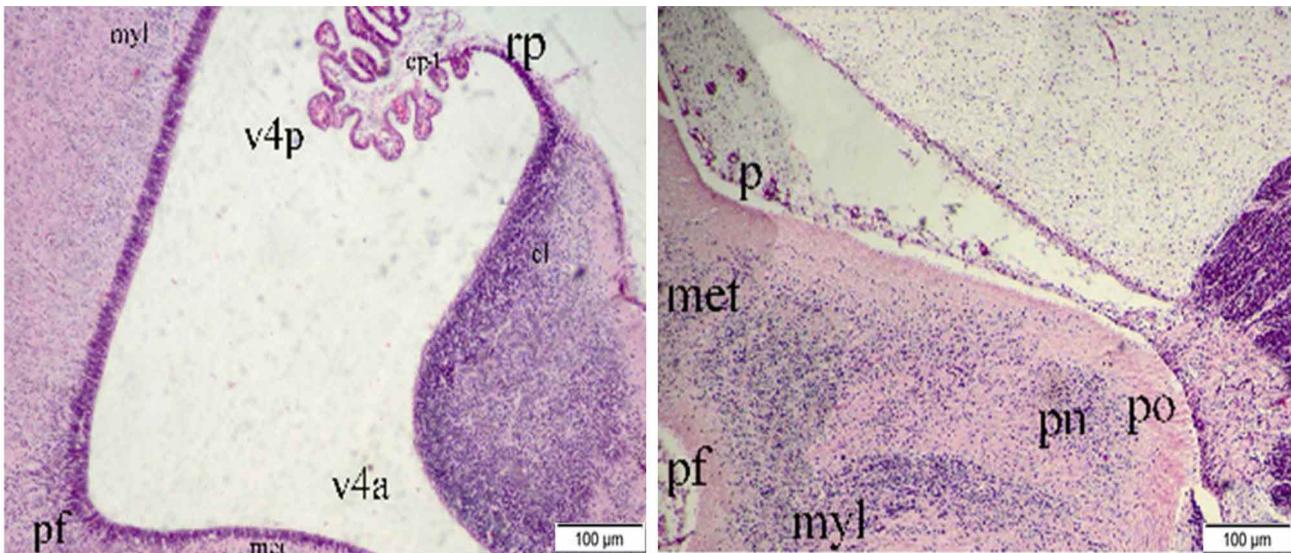


Fig. 2. Photomicrograph of sagittal section of camel embryo (3 cm CVRL) showing: Myelencephalon (Myl), metencephalon (met), pontine flexure (pf), pontine nuclei (pn), pons (po), pia mater (p), anterior part of the 4th ventricle (v4a), posterior part of the 4th ventricle (v4p), roof plate of the myelencephalon (rp), choroid plexus (cp1), developing cerebellum (cl).

current study revealed that the marginal layer of the basal plates of metencephalon in camel embryo undergoes extensive growth ventrally forming a bridge of nerve fibers (pons) that contains the pontine nuclei (Fig. 2). The pontine flexure deepens and the alar plate at the roof of metencephalon proliferate dorsally forming the cerebellar plate which developed to the cerebellum. Its cavity (metacaele) is reduced in size and regarding to formation of the anterior part of the fourth ventricle (Fig. 2).

Mesencephalon: The marginal layer of each basal plate of mesencephalon enlarges and thickened forming the cerebral crura or peduncles (crura cerebri) while the alar plates of the mesencephalon give rise to the anterior and posterior colliculus which appear as two longitudinal elevations separated by a shallow midline depression (corpora quadrigemina) (Fig. 3). As the wall of mesencephalon became much thickened, the mesocaele was reduced to a narrow canal called the mesencephalic

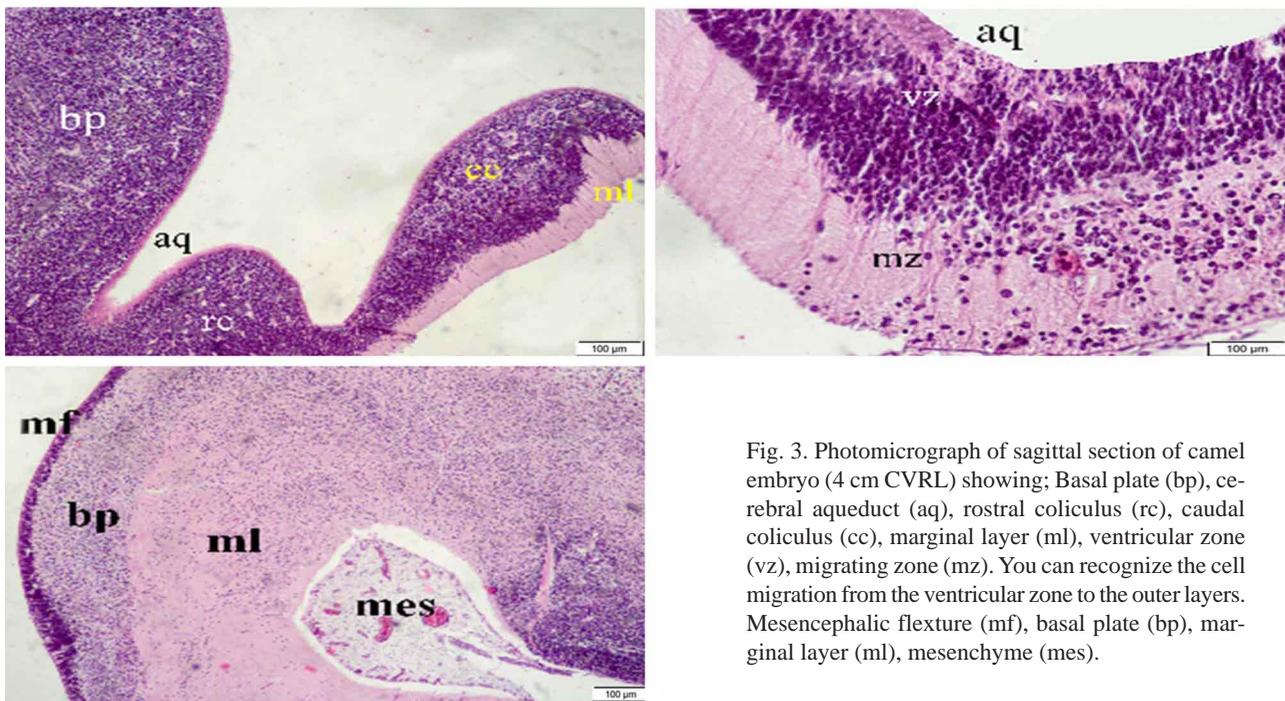


Fig. 3. Photomicrograph of sagittal section of camel embryo (4 cm CVRL) showing; Basal plate (bp), cerebral aqueduct (aq), rostral colliculus (rc), caudal colliculus (cc), marginal layer (ml), ventricular zone (vz), migrating zone (mz). You can recognize the cell migration from the ventricular zone to the outer layers. Mesencephalic flexure (mf), basal plate (bp), marginal layer (ml), mesenchyme (mes).

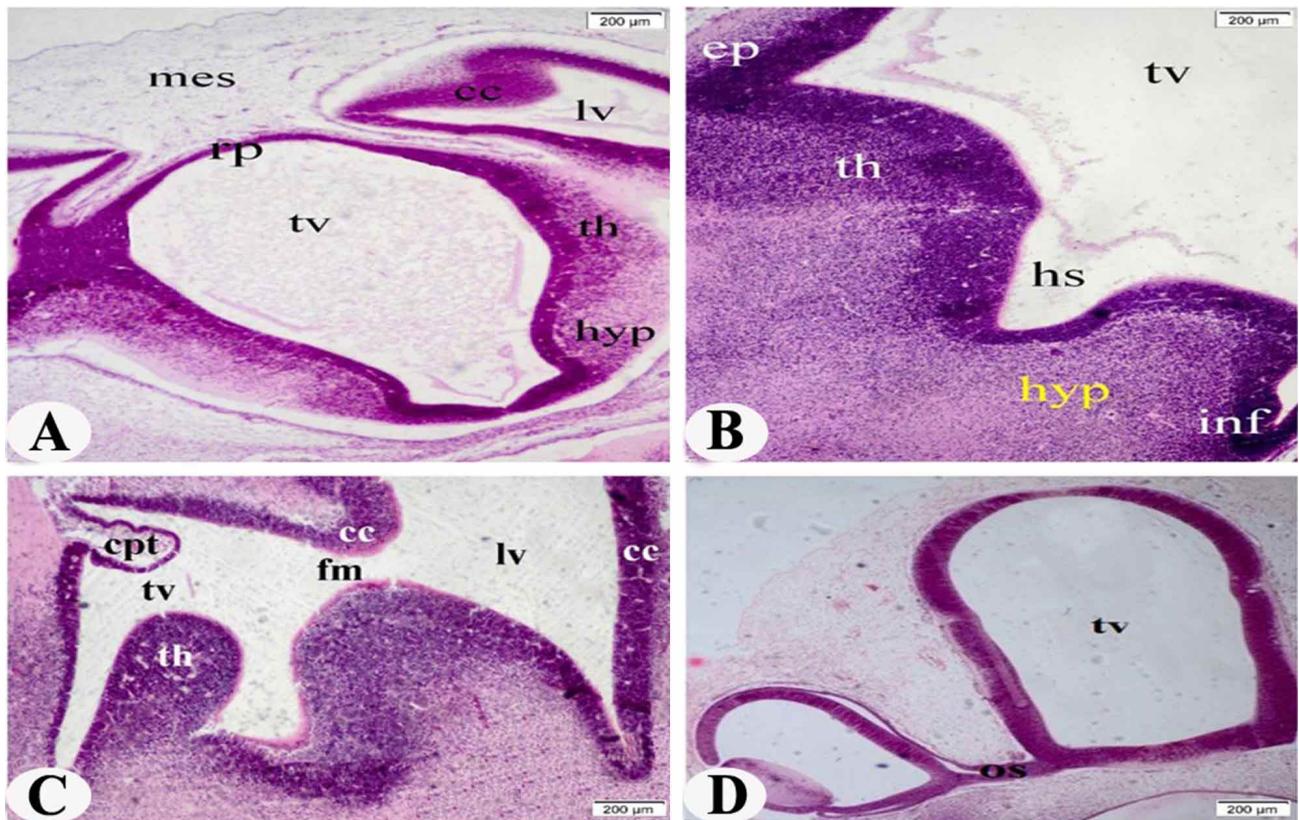


Fig. 4. Photomicrograph of transverse section (A) (2 cm CVRL) & B (3 cm CVRL) and sagittal section (C) & (D) (5 cm CVRL) of camel embryo showing; Cerebral cortex (cc), foramen mono-ro (fm), lateral ventricle (lv), 3rd ventricle (tv), choroid plexus of the 3rd ventricle (cpt), thalamus (th), hypothalamic sulcus (hs), hypothalamus (hyp), epithalamus (ep), infundibulum (inf), mesenchyme (mes), roof plate (rp), and optic stalk (OS).

acquiduct. Such results also had been recorded by same findings were recorded by McGeady *et al.*, in domestic animals and Champney in human. This study declares the cell migration from the ventricular zone to the marginal layer to complete the formation of the corpora quadrigemina (Fig. 3).

Diencephalon: It appears to be consisted of a roof plate and two alar plates and lack the floor and basal plates. The roof plate of the diencephalon appeared thin, formed of a single layer of ependymal cells covered by vascular mesenchyme. Together these layers were pushed into the diocaele inform of a finger like projections and give rise to the choroid plexus of the third ventricle (anterior choroid plexus) (Fig. 4). The alar plates form the lateral walls of the diencephalon which later divided into a dorsal (thalamic) and a ventral (hypothalamic) regions by the hypothalamic sulcus. The thalamus grows projecting gradually into the lumen of the diencephalon till the right and left sides fuse in the midline (Fig. 4). Downward, there is an evagination of the diencephalon, the infundibulum, which gives rise to the stalk and the pars nervosa of the

hypophysis. The diocaele cavity formed the third ventricle. At very early stage of development, the optic vesicles arise as outgrowth from the ventrolateral wall of the diencephalon which differentiated latter as optic stalk and optic cup as shown in Figure (4 d). All these findings were on a line with that of Champney in human and McGeady *et al.*, in domestic animals but the later author mentioned a third mass called the epithalamic mass.

Telencephalon: It appeared to be formed of two lateral vesicles (Fig. 5). The cavities of the two vesicles become the lateral ventricles which are connected to the 3rd ventricle by the interventricular foramen (Fig. 4). The telencephalon expands laterally and caudally (Figs. 4 and 5). The basal part of the hemispheres grows and bulges into the lumen of the lateral ventricle and into the floor of the interventricular foramen forming the corpus striatum (Fig. 5). At the region where the wall of the hemisphere is attached to the roof of the diencephalon, the wall of the telencephalon is thin, formed of a single layer of ependymal cells covered by vascular mesenchyme, and together they form the choroid plexus of the lateral ventricle (Fig. 4)

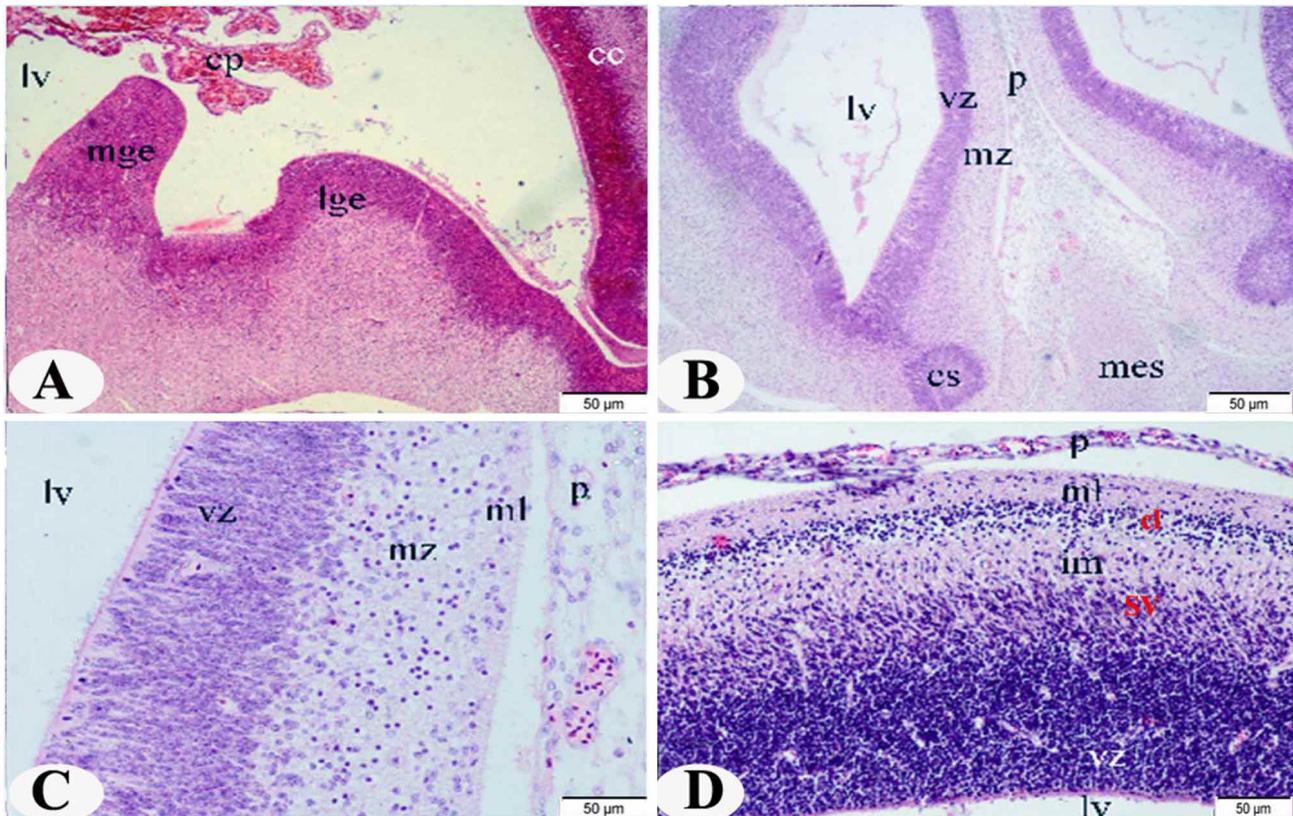


Fig. 5. Photomicrograph of sagittal section (A) and (D) (5 cm CVRL), B (4 cm CVRL), C (1.5 cm CVRL) of camel embryo showing; Cerebral cortex (cc), lateral ventricle (lv), choroid plexus of the lateral ventricle (cp), corpus striatum (cs), medial ganglionic eminence (mge), lateral ganglionic eminence (lge), pia mater (p), migrating zone (mz), ventricular zone (vz), marginal layer (ml), mesenchyme (mes), subventricular zone (sv), intermediate layer (im), cortical layer (cl).

which projects into the lateral ventricle. The telencephalon expands until it covers the lateral aspect of the diencephalon, mesencephalon, and cephalic portion of the metencephalon. The previous results were in accordance with that of Champney in human and McGeady *et al.* in domestic animals.

Neuron formation and migration: The neurons are produced in the ventricular zone (VZ) and migrate radially from the VZ to the developing cortex which consisted at the early stage, 1.5 cm CVRL camel embryo, of development from the primitive layers; ventricular, intermediate zone (IZ), and marginal layer (ml) (Fig. 5). As the development proceeds, the brain becomes larger and thicker and the neurons migrate from inner to outer of the wall as shown in Figure 3. At later stage, 5 cm CVRL camel embryo, this migration of neurons into the developing neocortex resulted in the formation of an orderly 6-layered structure (Fig. 5) which is named as follows; ventricular zone (VZ), sub-ventricular zone (SZ), intermediate zone (IZ), cortical layer (CL), and marginal zone (MZ).

Neuronal migration may occurs by somal translocation that the neurons extend a long basal process attaches to the pial surface, which is the outer surface of the developing brain as that was mentioned by (Miyata *et al.*, 2001) then the nucleus moves through cytoplasm of the basal process. Another way is called radial glial guide that the nucleus of the radial glial cells remains in the VZ, and the basal process forms a kind of scaffolding along which neurons can migrate (Nadarajah & Parnavelas, 2002). It has recently been discovered that the cells that provide the scaffolding, are actually the neural progenitor cells (Noctor *et al.*, 2002; Parnavelas *et al.*, 2002; Weissman *et al.*, 2003). The 3rd type of migration is tangential migration in this region, the medial, lateral and caudal ganglionic eminences (Anderson *et al.*, 2001; Nery *et al.*, 2002) that the neurons use a number of guidance molecules produced in local regions along their migratory route to direct their movement into the cortex (Huang, 2009; Valiente & Marín, 2010). The migration of neurons into the developing neocortex results in the formation of an orderly 6-layered structure like that was reported by Cooper (2008).

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RESUMEN: Se llevó a cabo un estudio del desarrollo embrionario cerebral de veintiocho embriones y fetos de camello jorobado (*Camelus dromedarius*). Las muestras fueron recolectadas en los mataderos de El-Basateen (El Cairo) y Belbees (ElSharqya). La investigación reveló que el cerebro de camello posee un cerebro anterior, medio y posterior. El cerebro anterior se divide en telencéfalo y diencéfalo, mientras que el rombencéfalo se divide en metencéfalo y mielencéfalo. Las flexiones encontradas entre las vesículas son la flexión cervical entre el rombencéfalo y la médula espinal; la flexión cefálica en el mesencéfalo; y la flexión pontina entre el metencéfalo y el mielencéfalo del cerebro posterior (rombencéfalo). La cavidad del rombencéfalo conforma el cuarto ventrículo, la del diencéfalo forma el tercer ventrículo, y las del telencéfalo a los ventrículos laterales. En el cerebro medio, la cavidad corresponde al acueducto cerebral. El mielencéfalo se convierte en médula oblonga y el metencéfalo deriva en puente y cerebelo, mientras que el mesencéfalo da lugar a la crura cerebral y a los colículos anterior y posterior. El diencéfalo origina el tálamo, el hipotálamo, el cuerpo mamilar, el infundíbulo y la hipófisis, mientras que del telencéfalo se originan los hemisferios cerebrales y el cuerpo estriado.

PALABRAS CLAVE: Camello; Embrión; Vesículas; Cerebro; Desarrollo.

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