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Morphological and histological structure of the cerebellum of turkey at the different levels of age (comparative study)

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ABSTRACT

This study aimed to investigate the structural development of the brain and cerebellum in local turkeys from hatchling (1 day) to adulthood (2 months, 3-7 months, and adult age). Following euthanasia, the skulls were dissected to extract the cerebellum, which were then rinsed and preserved in 10% neutral buffered formalin for histological analysis. The morphology revealed two cerebellum lobes, Histological examination identified three layers in the cerebellar cortex across all age stages. In the first month, delicate meninges and a few glial cells accompanied small to medium-sized neurons and numerous blood capillaries. As the turkeys aged, there was a notable increase in glial cells and larger, with more developed meninges. Additionally, cavitation's around neurons and nerve bundles were observed, particularly at one year of age. The cerebellum comprised two layers: an outer cortex with a pial meningeal membrane, consisting of a molecular layer with small nerve cells and glial cells, and a Purkinje layer that increased in size with age. The internal granular layer was broad, filled with small nerve cells and glial cells. Overall, the study concluded that the brain and cerebellum of turkeys exhibit well-developed nerve and glial cell layers, similar to those found in mammals, reflecting significant structural changes as they mature.

1. Introduction

The cerebellum of the turkey and other poultry is determined histologically by the gray matter of cortex and white matter of medulla. The brain is connected with the cerebellum (Abbas, *et al*., 2012), so integration for performing the information of functional activity is carrying out (Wael and Joseph, 2021) for example the cerebellum is the coordination center for equilibrium and precise movements and other wise, the brain is elegant for the sensory input and motor activity output (Sunil *et al*., 2019).

The development of brain parts and cerebellum were studied by (Mania *et al*., 2020; Ruth, 1959) when examined the embryonic period in details and followed up for other stages of period life focusing including the structural and biological aspects (Muhammad and Fatima, 2025).
(Rehammer *et al*., 2003) attributed that local breed chicken brain is formed by three units, cerebrum, cerebellum and medulla oblongata and this investigation was resemble to that of (Peng and Feng, 2010) during the studying of different species of poultry and ostrich , when demonstrated that cerebellum of poultry is formed by two cerebellar hemispheres which are pear shaped and the layers of cerebellum cortex were three, otherwise (Bubo *et al*., 2014) mentioned that the cerebellar cortex was formed by three layers, molecular, purkinje and granular layer.

The present study was dealing with local breed of turkey at different stages of life for assessment the histological structure of cerebellum.

Materials and Methods

The present technique of histological methods was done according to (Kim *et al*., 2023), The specimens from the cerebellum were obtained and washed with running water for ten minutes to remove the blood clot and tissue debris. The specimens of .05cm thickness were immersed in 10% formalin for 24 for fixation, after that transferred to 70% ethanol alcohol, 80%, 90% and 100% to changes for dehydration and 15 minutes for each step.

The specimens were put in xylene for 30 minutes for clearing, after that, they put in paraffin wax at 60 C in oven two changes, three hours for each step.

The specimens were put in plastic casts and blocked with wax.

The specimens were sectioning by rotary manual microtome at thickness 6µm.

The ribbons of tissues were manipulated on the glass slides under the presence of adhesive egg albumin, the slides with tissues were put in xylene for 20 minute, then absolute alcohol for 10mintues, 90% alcohol, 80% and finally 70% alcohol for 5 minutes for each. Staining with Harris Hematoxylin for 10mintues. Running water 10. minutes and Eosin Y for 3 minutes. Then 2 dips in acid alcohol. 70%, 80% ,90% alcohol two minutes for each and finally in xylene two changes, 5mintutes for each. The slides were loaded with DPX and put cover slides on it. The whole tissues were put in Dry Cabinet at 40ºC for draining and finally examined under light microscope manipulated with digital camera (AM Scope).

Results

The molecular layer of cerebellar cortex was containing few small nerve cells . The purkinje cells layer had few atrophied purkinje cells . The granular layer was containing great number of small nerve cells and glial cells, the medulla of cerebellum had network of nerve fibers with few glial cells in between (fig.1).

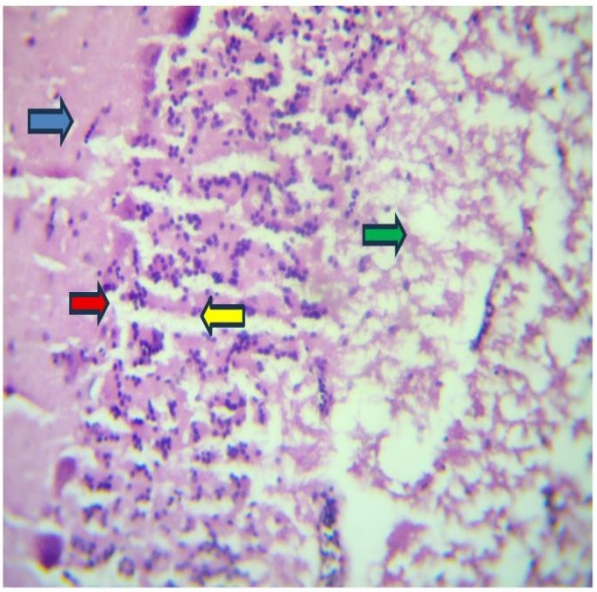


Figure (1). Molecular layer of cerebellar cortex (blue arrow), atrophy of purkinje cells (red arrow), extensive granular cells layer (yellow arrow), network of nerve bundles of medulla (green arrow) (H&E X40).

The molecular layer of cerebellum was invested by pial meningeal membrane . The molecular layer was containing few small pyramidal nerve cells. The purkinje cells layer had purkinje cells which had certain the atrophic purkinje cells , surrounded by few glial cells, granular layer was containing groups of small

nerve granular cells intermingled with small glial cells. The medulla was demonstrated as scattered nerve bundles (fig. 2).

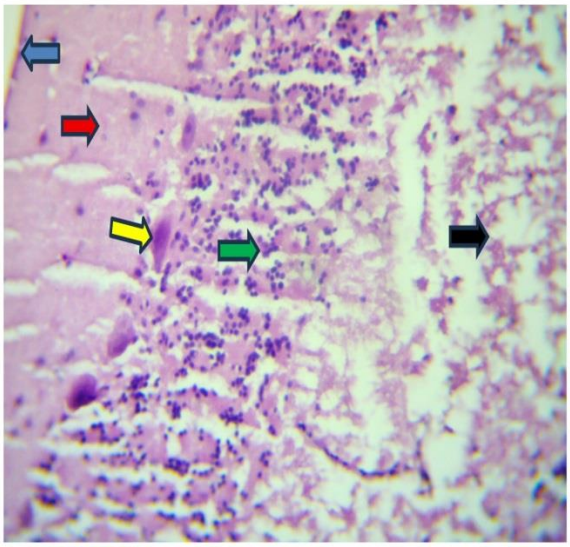
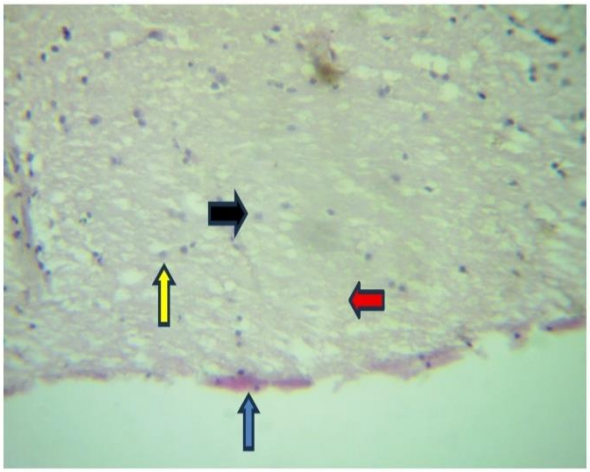


Figure (2). Cerebellar cortex and medulla, pial cerebellar meningeal membrane (blue arrow), molecular layer with small nerve cells (red arrow), purkinje cells (yellow arrow), extensive granular layer (green arrow),network nerve bundules of medulla (black arrow) (H&E X40).

The cerebellum cortex was surrounded by degenerated meninges which had blood clot, the cortex of br cerebellum ain demonstrated as foamy appearance for the nerve bundles with scattered glial cells and small nerve cells (fig. 3).



Figure(3). Pial membrane with degeneration and blood clot (blue arrow), foamy appearance of cortical layer (red arrow), small nerve cells(yellow arrow),surrounded by glial cells (black arrow) (H&E X40).

The cerebellar meningeal membrane was invested to the molecular layer of cortex, and the molecular layer had few scattered glial cells around the crakes of nerve bundles, the purkinje cells layer was formed by many purkinje cells with presence of atrophied purkinje cells in certain region of this layer. Granular layer was wide occupied by multiple small granular nerve cells associated with glial cells. The medulla was present as network of nerve fibers(fig. 4).

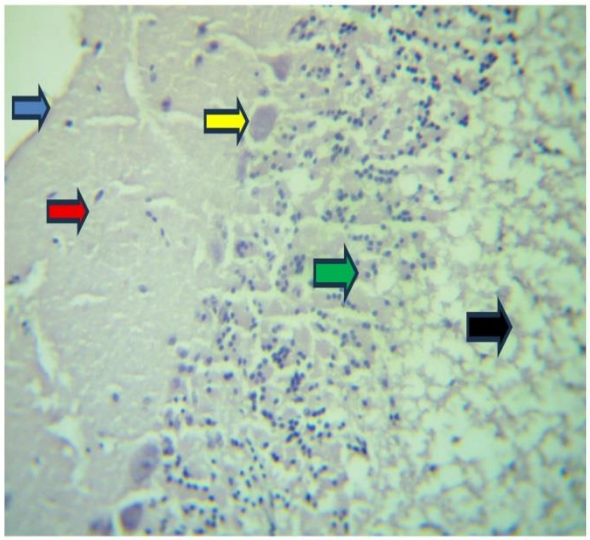


Figure (4). Cerebellum, cerebellar meninges (blue arrow), molecular layer with small nerve cells red arrow), purkinje cells layer with atrophied cells (yellow arrow), granular layer (green arrow), network of nerve bundles of medulla (black arrow) (H&E X40).

Cerebellum (1 day- 2 months)

The cerebellar cortex was invested by cerebellar pial meningeal membrane, and beneath that membrane, there was the molecular layer which was broad and formed by great number of nerve fibers with glial cells and the scattered small pyramidal nerve cells, the purkinje cell layer was containing few large purkinje cells, the granular layer was containing great number of small pyramidal cells near by the medulla (fig. 5).

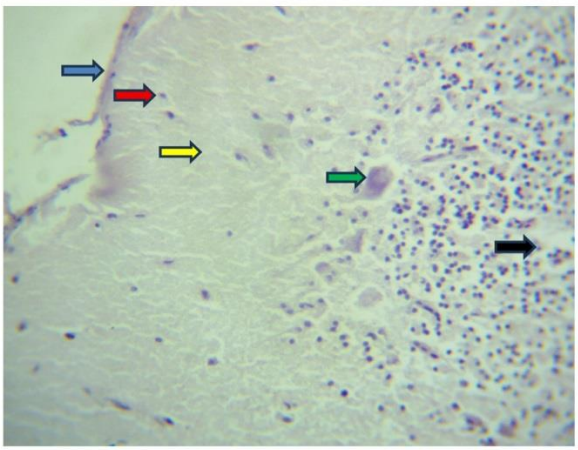


Figure (5). Cerebellar cortex. Cerebellar meningeal membrane (blue arrow), molecular layer with small

pyramidal cells (red arrow), nerve bundles (yellow arrow) purkinje cells of few numbers (green arrow) granular layer (black arrow) (H&E X40).

Purkinje cells were present which were few in number and located in between the molecular layer and granular layer which was demonstrated with small and medium sized pyramidal nerve cells associated with glial cells (fig. 6).

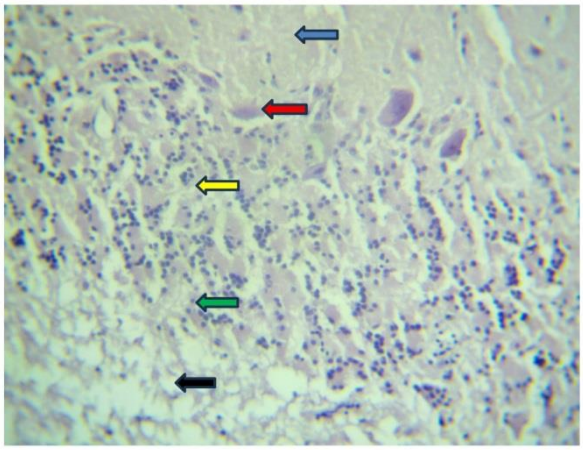


Figure (6). Molecular layer (blue arrow) , few number of purkinje cells (red arrow) small pyramidal nerve cells of granular layer (yellow arrow), glial cells (green arrow), foamy appearance of medulla (black arrow) (H&E X40).

The pial cerebellar meningeal membrane was extended in between the folia of cerebellar cortex, associated with blood vessels and certain white blood cells. The molecular layer had few scattered small pyramidal cells and glial cells (fig.7).

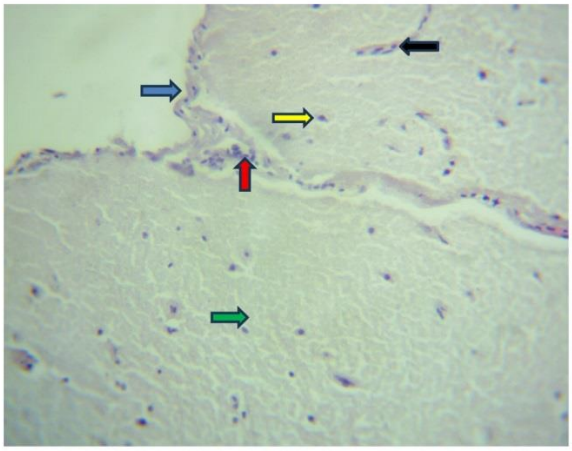


Figure (7). Cerebellar meningeal membrane(blue arrow), meningeal blood vessels (red arrow), small pyramidal nerve cells (yellow arrow), glial cells (green arrow), micrblood capillaries (black arrow) (H&E X40).

Cerebellum (3 – 7 months)

The cerebellar cortex was consist of molecular layer which formed by bundles of nerve fibers with few small pyramidal nerve cells with scattered glial cells, purkinje cells layer was containing few cells, surrounded by glial cells, the granular layer was demonstrated with great number of small nerve cells which extended to the border of medulla (fig.8).

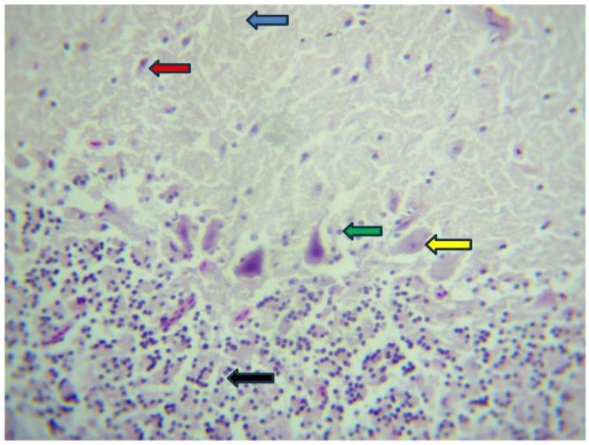


Figure (8). Cerebellar cortex, molecular layer with nerve bundles (blue arrow), small pyramidal cells (red arrow), purkinje cells (yellow arrow), glial cells (green arrow), granular layer with great number of pyramidal granular cells (black arrow) (H&E X40).

Cerebellar cortex demonsterated the presence molecular layer with nerve bundles, associated with small pyramidal nerve cells, glial cells and micro blood capillaries, the purkinje nerve cells were of great size, present in one row.

The granular layer was containing profuse number of small neurons with glial cells (fig. 9).

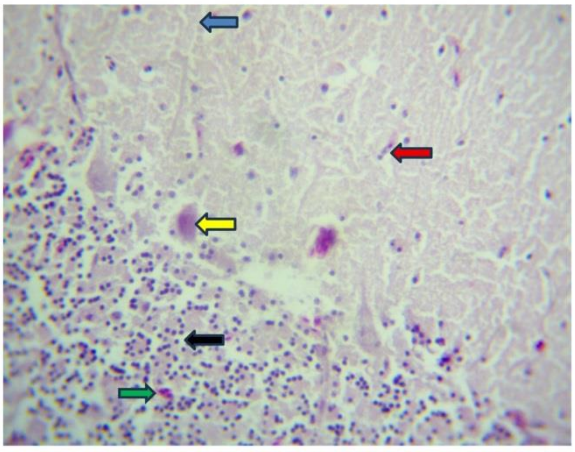


Figure (9). Molecular layer of cerebellar cortex with small pyramidal cells (blue arrow), glial cells (red arrow), purkinje cells (yellow arrow), small pyramidal cells (green arrow), of granular layer , great glial cells number (black arrow) (H &E X40).

The medulla of cerebellum was demonstrated with nerve bundles and glial cells were embedded in between the nerve bundle, great blood vessels was containing blood clot, the periphery of medulla was formed by the molecular layer (fig. 10).

The cortex of cerebellum was formed by three layers, the outer layer was molecular layer, formed by glial cells and few pyramidal small nerve cells. The second layer was formed by series of purkinje cells surrounded with few glial cells, the granular layer was containing great number of small pyramidal cells (fig.11).

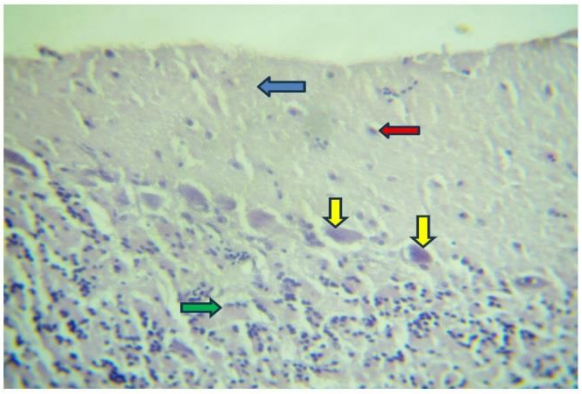


Figure (11). Molecular layer, nerve bundles (blue arrow), pyramidal nerve cells (red arrow) series of purkinje cells (yellow arrow), granular cells of inner layer of cortex (green arrow) (H&E X40).

Discussion

Investigation on local turkey concerned with structural arrangement are few. The present study facilitate the understanding of brain and cerebellum tissues. The brain of birds and other poultry are not differ away from the brain mammals (Stephan *et al*., 1986) and there for can serve as an excellent comparison for fundamental understanding the principle of cerebellum structure and function of turkey.

The cross inspection of cerebellar cortex of turkey demonstrated, that was located in the head and was well protected by the bones of the cranium and the brain was consisting of cerebrum, cerebellum, optic vesicles, small olfactory lobe and medulla oblongata, these parts were in agreement with (Michael *et al*., 2024) in the white crested polish chicken.

The first stage of life of turkey (1 day -2 months) after hatching showed the delicate pial meningeal membrane. This membrane is protective for the brain tissue, cerebellum and spinal cord, the blood supply to the brain is arriving via meninges, in this case the profuse vascularization of the brain at first stage of life in poultry could be imperfect meningeal coat, this fact was documented in the present study and referred this concept by (Frahm and Rehkamper, 1998) in the multiple glial cells were present and produced at this early stage of life in turkey which consider as a process of carrying out the whole activities of nervous system including, phagocytosis by microglial cells, myelination by oligodendrocytes also protoplasmic astrocytes which were demonstrated around the micro- blood vessels, associated with Blood Brain Barrier, this fact was in agreement with (Ribatti *et al*., 1993) when he suggested that the glial cells as supporting cells were numerous in different stages of avian life, but the neurons are established in its number without increase in number, this concept was in agreement with (Kawabe *et al*., 2017) in most mammals.

The present study indicated the presence of many vacuoles in the cortex and medulla of 1st two months of life in turkey, these are reflecting the delicate bundles of myelinated nerve fibers, so arranged as spongy appearance in the cortex and medulla surrounded by (Mehihorn and Capser, 2020). However the other stages of life particularly the advanced livestock referred to be presence of those vacuoles and cavities around the nerve granular cells and glial cells, these are interpreted by (Bogdan *et al*., 2012). When attributed these phenomena to be aging of animals resulting degenerative changes to certain bundles of nerve fibers and even neuronal damage which demonstrated delict in the shape of certain neurons.

The cerebellar cortex of early stages of life for turkey was demonstrating the scattered small nerve cells in the molecular layer, surrounded by few scattered glial cells, this result was in agreement with (Wright *et al*., 2021; Pal *et al*., 2003) when suggested that this layer reflect the connection with purkinje cell layer via climping fibers, responsible for coordination the fine movements and stabilization of the body during walking and flying for the avian family. Purkinje cells layer was arranged in one row, those cells were the greatest size cells among the other neurons of brain and even in other system of body, purkinje cells are polydendrtic cells associated with molecular layer through climping fibers and synapsing with the cells of internal granular cells by descending fibers. Also, there is horizontal fibers control the reflex behavior of the animals, the cerebellum is highly developed and consist of larger connected vermis and two small lateral lobes (Horalskyi *et al*., 2024). The other

development of cerebellum is possibly connected with the control of movement and the precise timing during flight, 4th ventricle is not extended to the cerebellum, so it will be solid tissue (Arends, 1991; Adrew *et al*., 2006).

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الشكل المظهري والتركيب النسجي للمخيخ في الديك الرومي عند مستويات مختلفة من العمر (دراسة مقارنة)

بدر ختلان حميد

فرع التشريح والأنسجة ، كلية الطب البيطري ، جامعة تكريت ، تكريت ، العراق

الملخص

صممت الدراسة الحالية لبيان التطور لتركيب المخيخ في الديك الرومي المحلي ,عينات المخيخ للرومي اخذت بأعمار عند (1 يوم – 2 شهر)، (3- 7 اشهر) ومرحلة البلوغ عند سنة واكثر.

ذبحت الحيوانات مع مراعات القتل الرحيم بها وتم فصل المخ عن المخيخ بلطف بعد فتح الجمجمة ثم وضعت العينات تحت الماء الجاري لمدة 10 دقائق لإزالة الدم وحطام الخلايا. تم اجراء التقنية النسجية ابتداءاً بوضع العينات بمحلول الفورمالين المتعادل 10% لمدة 12 ساعة ثم تبعته خطوات التمرير الأخرى للكحولات والزايلين وشمع البرافين للحصول على شرائح نسجية تلون بالهيماتوكسلين والايوسين وتم تصوير انسجة العينات بالمجهر الضوئي.

اظهر الفحص العياني المخيخ وجود فصي المخيخ ، البصلات العينية، ، النخاع المستطيل والفص الشمي، نتائج الفحص النسجي بينت وجود ثلاث طبقات لقشرة المخيخ في كل مراحل العمر، عند الشهر الأول تبين وجود سحايا غشائية رقيقة مع خلايا ساندة دبقية محدودة العدد إضافة الى خلايا عصبية صغيرة ومتوسطة الحجم مع ظهور شبكة اوعية دموية متعددة في القشرة. بتقدم العمر لوحظ وجود زيادة باعداد الدبقيات في كل طبقات القشرة هرمية كبيرة احيطت بنطاق فجوي، غشاء السحايا ظهر أكثر استقراراً ووجود تفجي وتكهف حول العصبونات في القشرة والنخاع للمخيخ . التكهفات والتفجي حول العصبونات ازداد اكثر عند عمر سنة ، احتوى نسيج قشرة المخيخ على خلايا عصبية صغيرة وبعض الخلايا الدبقية والمتواصلة مع الطبقة الثانية في القشرة والمؤلفة من خلايا بركنجي كبيرة الحجم خاصة عند تقدم العمر أصبحت اكبر حجماً ، الطبقة الثالثة تألفت من الخلايا الحبيبية الداخلية وبدت اكثر سعة واكثر عدداً في خلاياها العصبية الصغيرة والساندة مع وجود الياف عصبية مختلفة متواصلة مع نخاع المخيخ المحتوي على الياف عصبية نخاعينية واحتقان الاوعية الدموية فيه، ولوحظ كثرة الخلايا الدبقية وانتشارها في كل طبقات المخيخ (القشرة والنخاع) وفي مراحل العمر المختلفة.

يستنتج من هذه الدراسة بأن المخيخ في الديك الرومي محتوي على الطبقات المختلفة والحاوية على العصبونات والخلايا الدبقية والتي ظهرت بشكل متطور مع تقدم العمر وطبقات المخيخ كانت مناظرة لما موجود في مخيخ اللبائن.

الكلمات المفتاحية: علم الأنسجة، علم التشريح المجهري، تركي، المخيخ، التطور.