

**MORPHOLOGICAL STUDY OF ADRENAL GLAND IN  
PRENATAL AND POSTNATAL PERIODS OF DOMESTIC  
RABBIT  
(ORYCTOLAGUSCUNICULUS)**

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**ABSTRACT**

The developmental study of adrenal gland has been done on the local rabbit fetuses and neonate, which including a morphological and histological description of the adrenal gland. The study revealed that the differentiation of the adrenal gland began in the rabbit between twenty two and twenty four days from pregnancy as adrenal cortex and medulla and relatively completed it is histologically pictures at twelve days old. Also the study showed that the adrenal cortex composed of three zones: zonaglomerulosa or the zonaarcuata , the zona fasciculate and the zonareticularisglomerulus, while the medulla have dark and light cells. The purpose of our study was to provide a more complete quantitative description of the histomorphology of the adrenal gland in rabbits during prenatal and postnatal periods.

**INTRODUCTION**

Domestic rabbits in Iraq are descended from the European rabbits (*Oryctolagus cuniculus*). The length of gestation in rabbits is 30-32 days, ovulation occurs about 10-12 hours postcoitus. Implantation in rabbits begins on days 7<sup>th</sup> or 8<sup>th</sup> , duration of estrous prolongs about 28 days (1,2,3).

The mammalian adrenal gland composed of two morphologically and functionally distinct components, mesodermal portion which form the cortex and ectodermal portion which form the medulla. These two portions combined within a common connective tissue capsule during the embryonic development (4,5,6).

During the fifth week of development in human the mesothelial cells between the root of mesentery and the developing gonads begin to proliferate and penetrate

the underlying mesenchyme, here they differentiate into large acidophilic organs which form the primitive cortex of the adrenal gland, then these cells to develop the definitive cortex after birth the fetal cortex regress rapidly except for its outermost layer which differentiate into the reticular zone. The adult structure of the cortex is not achieved until puberty ,while the fetal cortex is being formed cells originating in the sympathetic system invade its medial aspect when they are arranged in cords and clusters. These cells give rise to medulla (7).

The adrenal cortex is divided into three zones: either zonaglomerulosa or zonaarcuata, the zona fasciculata and zonareticularis. The zonaglomerulosa is found in humans and bovines where as the zonaarcuata is described in horses, carnivores and pigs (6,8). In birds , reptiles and amphibians, the internal cells of adrenal gland are not arranged in zones of distinct cell types (9). The adrenal gland in duck is encapsulated by a layer of connective tissue and consists of two major secretory cell types ,the internal cells and medullary cells (10). The adrenal gland of bottlenose dolphin presented zonaarcuata which contains columnar cells arranged in the form of arches but in the striped dolphin this zone is replaced by zonaglomerulosa containing rounded clusters of polygonal cells .In both species the zona fasciculata consists of radially oriented cords of polygonal cells whereas in zonareticularis cells are arranged in branching and anastomosing cords. The adrenal medulla contains dark and light cells, the gland is surrounded by a thick connective tissue capsule(11). In late stage of gestation ,sheep fetuses ,the adrenal cortex showed cortical zonation but three zones were not easily demarcated at their junctions(12). The adrenal gland of postnatal rats showed three zones , zonaglomerulosa was well developed and the radial columns in zonafasciculata and zonareticularis became more distinct in day 13 postnatal rats, and the medulla appeared distinctly on day 9 of postnatal life(13).

## **MATERIALS AND METHODS**

The study was performed on forty rabbits fetuses and neonates, twenty fetuses from uteri of the pregnant local breed does where collected and twenty neonates rabbits where used in estimated ages, five fetuses prepared for every stage beginning from (24, 26, 28, 30) days of gestation and neonates in (4, 8, 12, 16) days after birth. All fetuses' ages were estimated according to the days assumed to have elapsed from copulation (14). The crown-rump length (CRL) was measured for corrections (table

1), while the neonates ages were estimated according to the birth time (15). The body weight was recorded for each prenatal fetus by using sensitive balance before sacrifice of the fetuses. The mean weight at each stage is summarized in the table (2).

Routine procedure according to (16) has been done to prepare the samples for staining with (H&E) .

## **RESULTS**

***Fetuses in 24 and 26 days of gestation:*** The adrenal gland appeared a clear demarcation line between adrenal cortex and medulla and the adrenal gland was enclosed by a thin capsule of connective tissue and there was epitheloid cells located beneath the capsule (Fig.1). The cortex presented uncompleted differentiating zonaglomerulosa which occupy a thin layer located directly under the capsule of the gland. The cells of this layer appeared undifferentiated and in small ovoid groups of cells with large nuclei. The zonafasciculate appear located direct under zonaglomerulosa as undifferentiating layer and is still mixed with zonareticularis (Fig.2). The adrenal medulla appeared smaller than the cortex containing undifferentiating mesenchymal cells and visible central vein and also medullary cells in this stage become consolidated in the gland's centre (Fig.1).

***Fetuses in 28 and 30 days of gestation:*** The adrenal gland appeared more differentiated cortex and its capsule was thicker than in the previous stage, it was containing blood capillaries with visible demarcation line between adrenal cortex and medulla (Fig.3). The adrenal cortex could be distinguish into two zones, the first a narrow zone located in periphery of adrenal gland known as zonaglomerulosa as cellular groups more clear and arched in shape (Fig.4) and the second wide zone called zonafasciculate and other zones of about the same thickness as the fasciculate and of similar cells in different directions but in this period there was no demarcation between zonafasciculata and zonareticularis.

***Neonatal rabbits in 1 day and 4 days after birth :*** The adrenal gland appeared solid, flattened body or reniform in shape located craniomedial to kidney usually in large quantity of fat about on a level with the anterior end of the kidney of its respective side. The adrenal gland is white in color surrounded by a tough fibrous capsule and the right one being anterior in their location to the left adrenal gland and also the right adrenal gland is often more elongated than the left which is generally

oval or reniform(Fig.5).Histologically, the adrenal capsule was defining in shape and composes of elongated spindle cells in shape of connective tissue with elongated and dark nuclei . The adrenal cortex composed of zonaglomerulosa, zona fasciculate and finally zonareticularis. The zonareticularis begin differentiration from zona fasciculate at four day old neonate rabbits. The zonaglomerulosa presented small cells with few vacuoles and gradually the cells organized and differentiated into small balls and arcs supported by connective tissue trabeculae, while zonafasciculata start to separate from zonareticularis in this stage and it composed of dark and light cells, and lipid globules were extremely fewer than zonaglomeruolsa. The adrenal medulla appeared definitive containing light and dark cells (Fig.6).

*Neonatal rabbits in 8 and 11 days after birth* :Adrenal cortex divided into three clear zones on the basis of difference in arrangement of the parenchymal cells which are: **zonaglomerulosa**: composed of cell groups which were small and arranged in radiating columnar rows which located under the capsule forming small arches and supported by connective tissue trabeculae extend from capsule toward the medulla. Between the cellular groups there were capillary blood-vessels and few lipid globules in different sizes (Fig.7).**zonafascicuclate**is the intermediate and broadest of three zones of adrenal cortex and presented irregular cell groups of long cuboidal or columnar cells arranged in narrow cords separated by connective tissue strands containing capillary sinusoids and this layer characterized by foamy appearance (Fig.7).**zonareticularis** is the innermost zone of adrenal cortex located between zonafascicuclate and adrenal medulla which appeared more clearly differentiated than the previous stage. It composed of an irregular network of cords and clumps of cells with numerous wide capillary sinusoids and the cells had spherical darkly staining pyknotic nuclei and acidophilic cytoplasm had few lipid vacuoles(Fig.7, 8).Adrenal medulla is the central portion of adrenal gland composed of ovoid groups of light cells. The medulla also contain sympathetic ganglion cells seen scattered single or in groups(Fig.7, 8).

**Table (1): Shows Crown rump length of embryo in different ages before birth.**

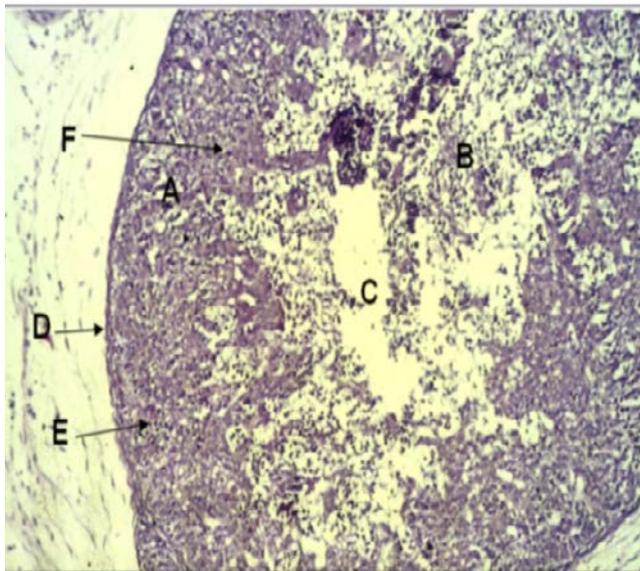
parameter Period	crown-rump length /mm
24	49.082±0.735 A
26	66.757±1.243 B
28	80.708±2.197 C
30	90.628±2.346 D

Values represent mean ±S.E  
Different capital letters mean significant (P≤0.05) results between different age pups.

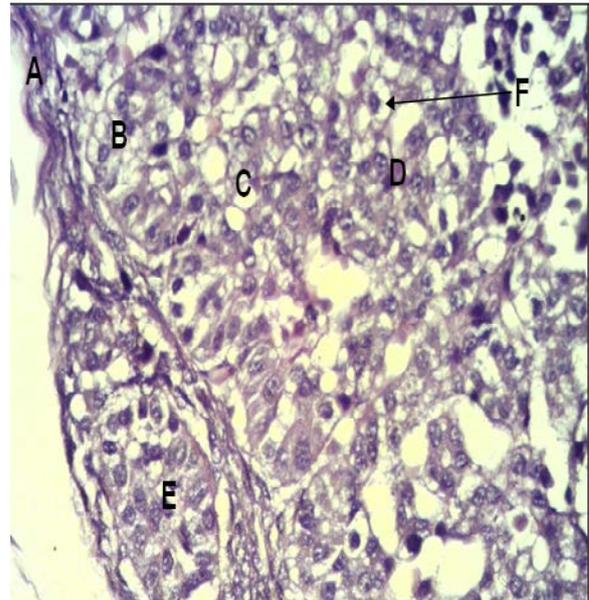
**Table (2): Shows body weight of fetus in different ages before birth.**

parameter period	Weight of fetuses / gm
24	13.17±0.316 A
26	17.087±0.721 B
28	25.225±1.205 C
30	35.500±1.426 D

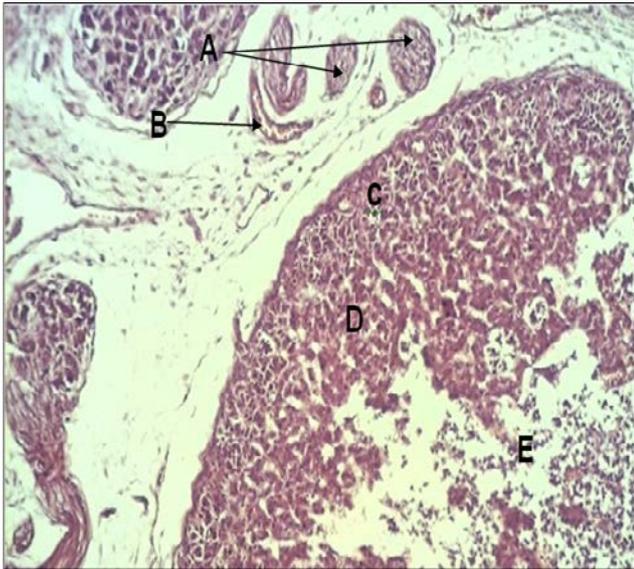
Values represent mean ±S.E  
Different capital letters mean significant (P≤0.05) results between different age pups.



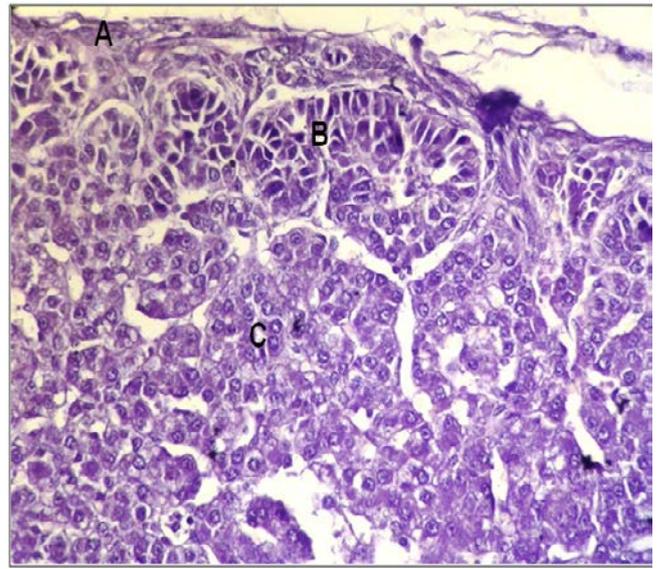
**Fig.(1)** show adrenal gland in 24 day rabbit fetus (cross-section) A-cortex, B-medulla, C-central vein, D-capsule, E-zonaglomerulosa, F-zona fasciculate. (H&E. 4 X )



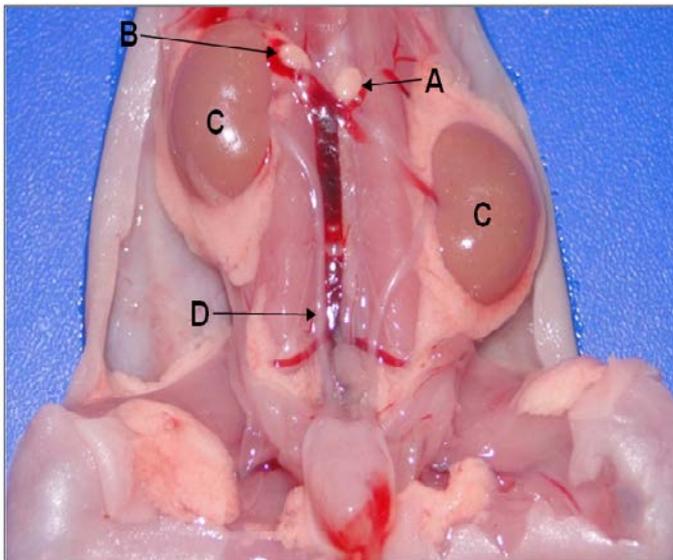
**Fig.(2):** Showsadrenal gland in 26 day rabbit fetus (cross-section) A-capsule, B- zonaglomerulosa, C- zona fasciculate, D- capsule E-epitheloid cells, F-droplet vacuoles (H&E. 40 X )



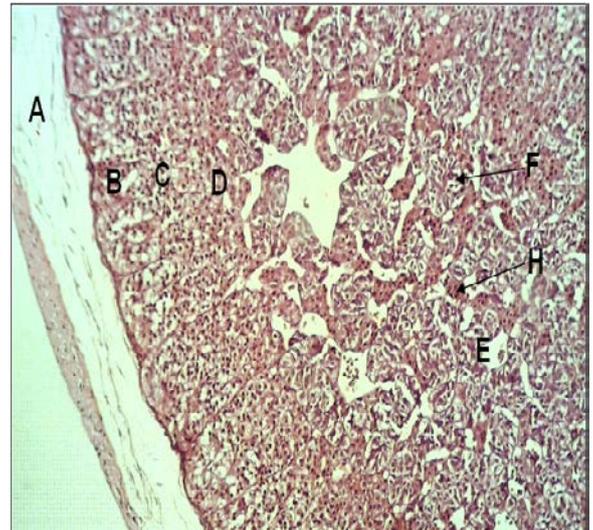
**Fig.(3)** show adrenal cortex and medulla in 28 day rabbit fetus (cross-section) A-unmyelinate nerve fibers B-capillaries C-zonaglomerulosa D-zona fasciculata and reticularis E-medulla (H&E. 10 X )



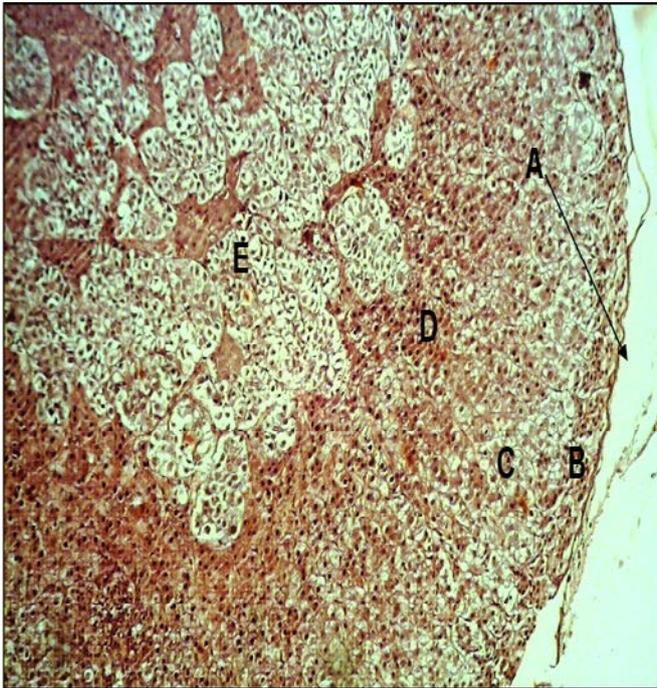
**Fig.(4)** show adrenal cortex in 30day rabbit fetus (cross-section) A-capsule B-zonaglomerulosa C-zona fasciculata (H&E. 40 X )



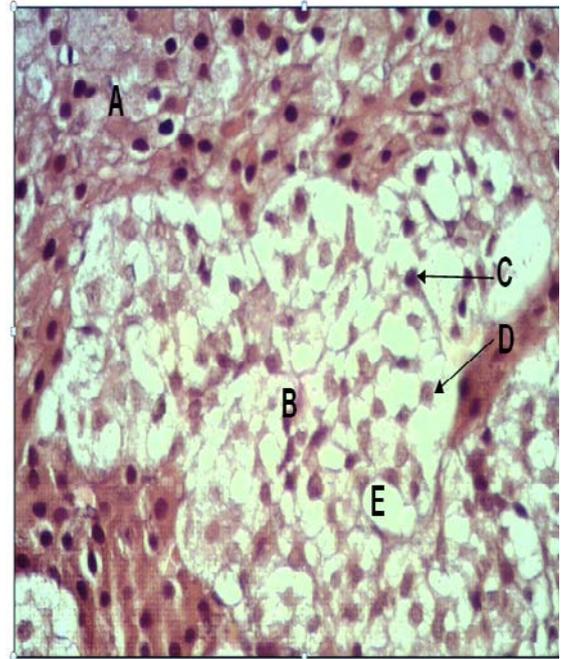
**Fig.(5):** Shows anatomical location of adrenal gland in one day old rabbit neonate A-left adrenal gland B-right adrenal gland C-kidney D-ureter.



**Fig.(6):** Shows adrenal gland at 4 day old rabbit neonate (cross-section) A-capsule B-zonaglomerulosa C- zona fasciculata D-zonareticularis E-adrenal medulla F-light cells H-dark cells (H&E. 10 X )



**Fig.(7):** Shows adrenal gland in 12 day old rabbit neonate (cross-section) A-capsule B-zonaglomerulosa C- zona fasciculata D-zonareticularis E-adrenal medulla (H&E. 10 X)



**Fig.(8):** Shows zonareticularis and adrenal medulla in 12 day old rabbit neonate (cross-section) A-zonareticularis B-adrenal medulla C- dark cell D-light cell E-central vein (H&E. 40X)

## DISCUSSION

The adrenal gland of rabbit fetuses from 24 to 26 days of gestation appeared clear demarcation between cortex and medulla but uncompleted differentiation of zonaglomerulosa, and zona fasciculata was still mixed with zonareticularis, this result agreed with (12) who mentioned that in the late stage of gestation, the adrenal gland of sheep fetuses showed no clear demarcation between the zones junctions. In this study, the adrenal cortex of 30 days of gestation rabbit fetuses appeared more differentiation and its zones who showed the three zones of adrenal gland in rats were clear in postnatal period. Adrenal cortex appears three zones at four days old neonate rabbits and become clear at twelve days old rabbits. This results unlike to that in mice (14) who mentioned that the zonation of the cortex occurs soon after birth. Birds are unique among homeothermic vertebrates, in which cortical and medullary tissues of the adrenal gland are found always intermingled and the zonation of the internal

tissue into sub-capsular zone and inner zone was not evident in the adrenal of three days-old Japanese quail (15).

The adrenal cortex during postnatal stage in present study compose of three zones: zonaglomerulosa, zonafasculate and zonareticularis. This results disagreed with (16) who mentioned that the in mice a functional zonareticularis is absent, instead, the so called X-Zone can be found during certain postnatal developmental stages in mice and (14) who mentioned that the mice and rats do not have a functionally distinct zonareticularis due to the lack of 17 $\alpha$ -hydroxylase expression in the adrenal gland. Therefore, adrenals from mice as well as other rodents are devoid of the secretion of adrenal androgens.

Our results showed that the zonaglomerulosa compose of cell groups which are small and arrangement in radiating columnar rows which located under the capsule from small arches. This results agreement with (17) who mentioned that the parenchymal cells of zonaglomerulosa (zone multiformis) in the carnivore, horse, and pig are columnar and arranged into arcs and (18) in mice who mentioned that the zonaglomerulosa is the thinnest layer consisted of packed groups of columnar or pyramidal cells forming arcades of cell columns. In contrast to (17) in horse who seen the columnar cells are especially tall, while in ruminants the zonaglomerulosa contains polyhedral cells that form irregular clusters or cords and finally in chicken who state that the adrenal glands are enclosed within a capsule of dense connective tissue. Unlike mammals, the parenchyma is not organized into a distinct cortex and medulla. Instead, it is composed of intermingled cortical (interrenal) tissue and medullary (chromaffin) tissue.

The zona intermedia is not well developed in rabbit and most species except in horses, dogs and cats where it is conspicuously present between the z. glomerulosa and the z. fasciculata. The cells in this zone may be reserve cells that can develop into cells of the zonafasciculata. (19).

Our results of adrenal gland in rabbit fetuses and neonate in contrast with the histological and anatomical description of adrenal gland in fish, amphibians and many reptiles where the adrenal tissues of fish, amphibians and many reptiles are significantly different. The adrenal glands (some times referred to as inter-renal or intrarenal tissue) of most fish are embedded in the cranial kidneys, where they surround the larger blood vessels and may be intermixed with hematopoietic tissue.

There are two kinds of adrenal cells: medullary or chromaffin cells and sympathetic nerve-like paraganglion cells. Amphibians possess discrete paired adrenals composed of three major cell types: chromaffin (medullary) cells that are of neuroectodermal origin; (cortical) cells; and stromal cells that are of mesodermal origin. In reptiles the paired adrenals usually lie immediately adjacent to the kidneys or in some instances where the kidneys are located within the pelvic canal, they lie medial to the gonads. Chromaffin or medullary cells that secrete catecholamines; and pale staining cortical steroidogenic cells (19).

There is clear line between adrenal cortex zones in rabbits. This is in accordance with the findings in buffalo (20) and in contrast with (21) who presented that the demarcation between the zona fasciculata and zona reticularis in goat was lacking. The spongy appearance of zone fasciculata because of dissolution of the lipid droplets during routine histological preparation. This is in accordance with (17) in domestic animals.

The pyknotic appearance of nuclei due to the apoptosis phase. This resembles to the theory of migration that recorded the zona reticularis as the graveyard of the cortical cells. This is in agreement with (22) who stated that the cortical cells originate in the outermost layers of the cortex and directed downward to die there.

## دراسة شكلية للغدة الكظرية في الفترة المحيطة بالولادة للأرانب المحلية

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### الخلاصة

أجريت الدراسة على مراحل تطور الغدة الكظرية في أجنة ومواليد الأرانب المحلية، والتي تضمنت وصف شكلي ونسجي للغدة الكظرية. أظهرنا لدراسة ان تمايز الغدة الكظرية يبدأ في الفترة ما بين اليوم الثاني والعشرين والرابع والعشرين من الحمل كقشرة ولب وتكمل صورتها النسيجية في اليوم الثاني عشر بعد الولادة. كذلك أظهرت الدراسة ان القشرة تتكون من ثلاثة انطقة: النطاق الكببي، والنطاق الحزيمي والنطاق الشبكي بينما اللب يتكون من خلايا فاتحة و غامقة. الغرض من الدراسة هو لتوفير وصف تشريحي نسيجي كمي شامل للغدة الكظرية عند الارانب خلال الفترة قبل الولادة وبعد الولادة.

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