Original Article

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Light Microscopic Study of Developing Cortex of Fetal Adrenal Gland

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Abstract

In this study, we observe the developing cortex of the fetal adrenal gland in one hundred normal Human fetuses (71 males and 29 females) over a period of two and half years in various stages of development. Subsequently, we correlate the light microscopic studies of the fetal adrenal gland with respect to its development and functional activity. Moreover, the width of definitive and fetal cortices is noted and its ratio for the respective gestational age is calculated. The ratio of the measurements of width of definitive cortex and fetal cortex remains constant i.e. 1:4 at all stages of development; consequently, it can be suggested that the definitive cortex is continuously proliferating and adding cells to fetal cortex where the cells become differentiated and functional. After functioning for some time, the fetal cortical cells must be degenerating. Further, the fetal cortex comprises 80% of adrenal cortex till birth. The cells of the fetal cortex show vacuolated cytoplasm indicating a steroidogenic activity as early as 15-16 weeks of gestation and acts as an endocrine gland throughout the gestational period. Finally, we affirm and compile the development of the cortex in various gestational stages.

Keywords: Definitive Cortex; Fetal Cortex; Zona Glomerulosa; Zona Fasciculata.

Introduction

The adrenal glands were first recognized as organs distinct from the kidneys by Bartholomeo Eustachius in 1563. Subsequently, Emil Huschke first differentiated the cortex from medulla anatomically [1]. Next, the three concentric zones of the cortex were described by J. Arnold [2]. Although, the growth and differentiation of human adrenal cortex has been a subject of much investigation in recent years, the formation of fetal adrenal cortex and its subsequent invasion by neural precursors of medulla was described by Rudolph Albert Von Kolliker in 1861 [3].

Investigations carried out previously on the morphogenesis of the fetal adrenal cortex have led to conflicting views with respect to the development and functional activity of the two zones composing this endocrine organ. According to the first theory, the two zones of the fetal adrenal cortex develop separately from each other [4]. However, the findings of a second study suggested that there is a gradual development of the two zones from one original zone of early embryonic gland [5].

Ross et al, in 1962, described the electron microscopic structure of adrenal gland and suggested that permanent cortex or outer zone might represent a germinative zone where as the ultra-structure of fetal or inner zone might reflect a functional activity.

Materials and Methods

The present study was carried out on 71 male and 29 female fetuses in various stages of development having crown-rump length ranging from 7 cm to 36 cm. The fetuses were obtained from medical termination of pregnancy and spontaneous abortions from a tertiary care hospital in Mumbai.

Preservation

In order to minimize post-mortem changes, the

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fetuses were immediately preserved by injecting formalin into the abdominal cavity. Subsequently, they were immersed in formalin solution. For identification, numbered tags starting from 1 were tied to their wrists.

Normal growth of the fetus was ensured by taking different external measurements and ascertaining various parameters.

Dissection and Fixation

The fetuses were dissected by taking vertical abdominal incision. Subsequently, the adrenals were removed along with the kidneys by blunt dissection. Immense care was taken during the separation of the right adrenal from the liver. The left adrenals were kept in small locked plastic pouches to differentiate from the right. Finally, the adrenals were stored in small bottles and were numbered.

Next, the large adrenals were cut into pieces with sharp razor blade. The capsule was not damaged in the process.

Finally, the tissues were fixed by immersing in Sublimate-Salt solution for 24–36 hours.

Dehydration

First, the tissues were immersed in 50% alcohol solution for 6–8 hours. Next, they were transferred to 70% alcohol and allowed to stay in it overnight. Subsequently, the tissues were bathed in 90% alcohol for one hour and finally they were subjected to absolute alcohol for duration of one hour each. Lastly, the tissues were dried using filter paper.

Clearing and Embedding

Post dehydration, the tissues were subjected to two–three xylene changes until they turned transparent.

Once the tissues were impregnated with xylene, they were embedded in melted paraffin at 58°–60° for duration of 5–6 hours. The paraffin blocks containing tissues were made using L-shaped molds. These blocks of paraffin were then numbered accordingly. Care was taken during the preparation of the tissues to ensure that the respective numbering was maintained.

Sectioning

The numbered blocks of paraffin containing tissues were sanctioned by microtome steel blade to a thickness of 4–8 μm. The sections were floated on warm water and transferred to glass slides which were rubbed with a drop of albumin. A drop of 70% alcohol was put on a glass slide to ensure spreading of the section.

Subsequently, the slides were numbered accordingly with diamond marker and kept in over for a few seconds to fix the section on slide.

Slide Staining

Next, the slides were stained with Hematoxylin and Eosin stain. After the slides were stored in Koplin jar, the following procedure was undertaken:

1. First, two changes of xylene were given for a short period of time. Next, the slides were successively bathed in descending grades of alcohol starting from absolute alcohol (three momentary changes as absolute alcohol I, II, III) to 90% and 70% alcohol.
2. The Koplin jars were kept under running water for 5–10 minutes. Then, the slides were stained with Hematoxylin for 10–15 minutes. After checking the intensity of staining under the microscope, the Koplin jars were kept under running water for 15 minutes,
3. Subsequently, Eosin was used as an acid dye for staining the slides for 3–5 minutes.
4. After the Eosin staining, the slides were transferred from absolute alcohol I to II and III and then kept in xylene.
5. Using DPX mount, the coverslips were added. The slides were then cleaned and readied for the microscopic study.

The slides were then examined under the microscope to study the developing cortex of fetal adrenal gland under various stages. Histological slides of adrenal glands from each group were photomicrographed using trinocular Nikon microscope.

Measurements of width of definitive and fetal cortex were taken using an eyepiece grid. The adrenals were then studied under a light microscope and the width of the fetal cortex was measured. The materials used for the measurement are as follows:

1. Dispensing weight balance
2. Transparent scale
3. Thread
4. Divider
5. Grid in eyepiece.

Finally, we calculate and record the ratio of width of definitive and fetal cortex.

Results and Discussion

The ratio of definitive to fetal cortex is calculated and listed in Table 1.

Weeks 12–14

The adrenal cortex is distinctly developed into two zones: outer definitive cortex and inner fetal cortex.

Fetal cortex is differentiating into fascicular pattern. In terms of nucleus–cytoplasm ratio and staining characteristics of nucleus and cytoplasm, the cells of the definitive cortex appear to be immature whereas those of fetal cortex seem matured.

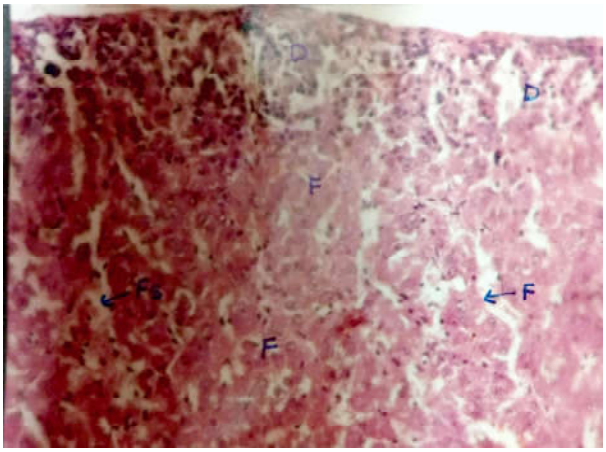

Fig. 1.1: Depicting definitive cortex (D) and fetal cortex (F) and the sinusoids in fetal cortex (Fs)

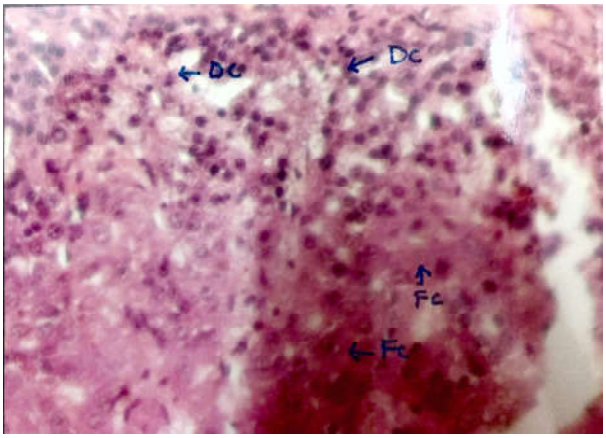

Fig. 1.2: Cells of definitive cortex (DC) and fetal cortex (FC) showing nuclei

Weeks 15–16

Cells of the definitive cortex have started differentiating into mature cells. Density of cells decreases with the appearance of blood vessels. This suggests rudimentary glomerulosa.

Next, the fascicular pattern of the fetal cortex is distinct and clear. Dilated sinusoids are arranged between the cell cords. The cells of the fetal cortex exhibit a spongy appearance.

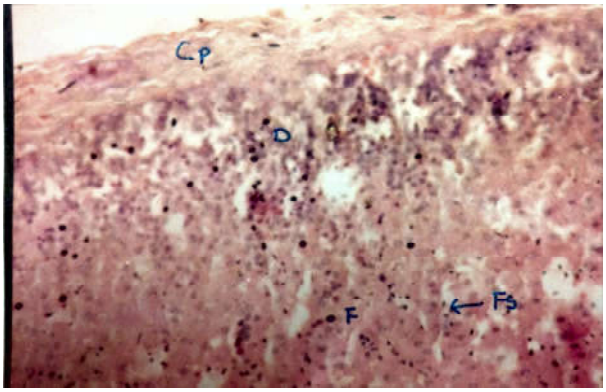

Fig. 2.1: Depicting capsule (Cp), fetal cortex (F), definitive cortex (D), and dilated sinusoids in fetal cortex (Fs)

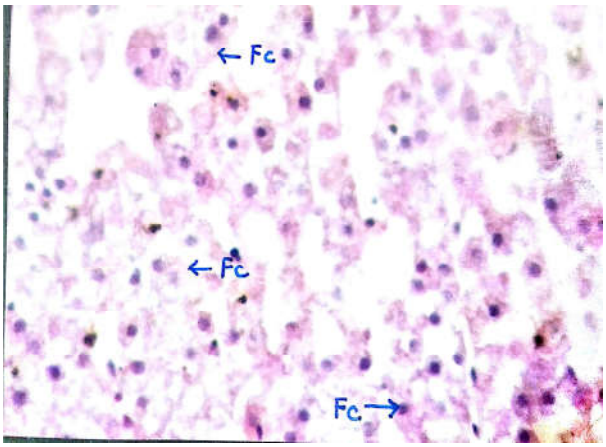

Fig. 2.1: Cells of fetal cortex (Fc) showing spongy appearance.

Weeks 17–18

No changes are observed in the definitive cortex. Towards medulla fascicular arrangement of fetal cortical cells is broken into reticular network. It may be the indication of degeneration of fetal cortical cells.

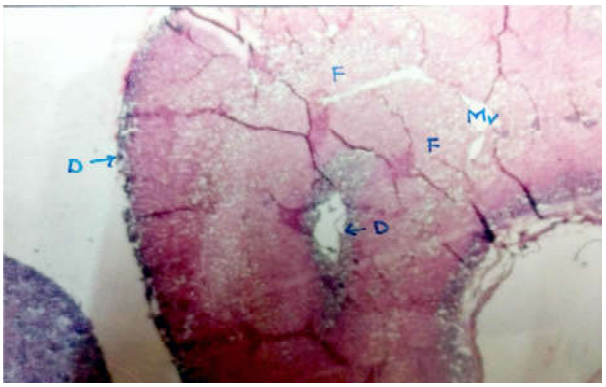

Fig. 3.1: Fetal cortical cells (F) broken in reticular network and appearance of medullary vein

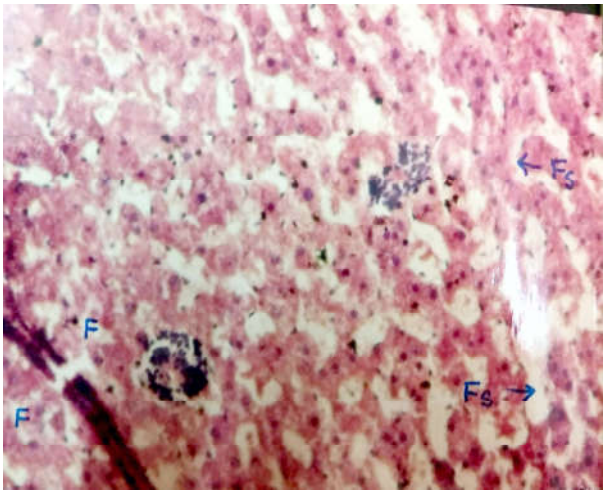

Fig. 3.2: Sinusoids in fetal cortex (Fs) and fetal cortex (F)

Weeks 19–20

This stage exhibits the appearance of a transitional zone between fetal and definitive cortex. The cells of this zone are of intermediary stage, i.e. they are neither functional as cells of fetal cortex nor undifferentiated as the cells of definitive cortex. This suggests a migration of stem cells from the definitive cortex into fetal cortex where they proliferate and differentiate to begin function.

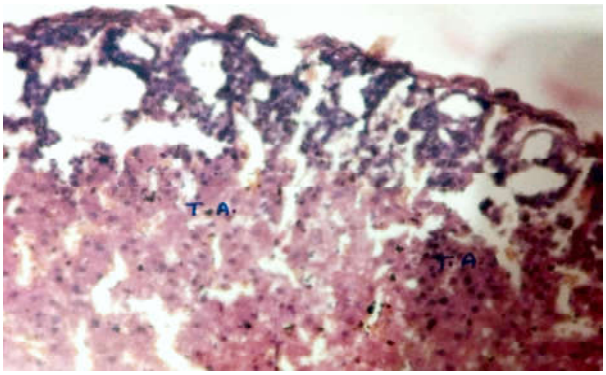

Fig. 4.1: Appearance of a transitional area (TA) between fetal and definitive cortex

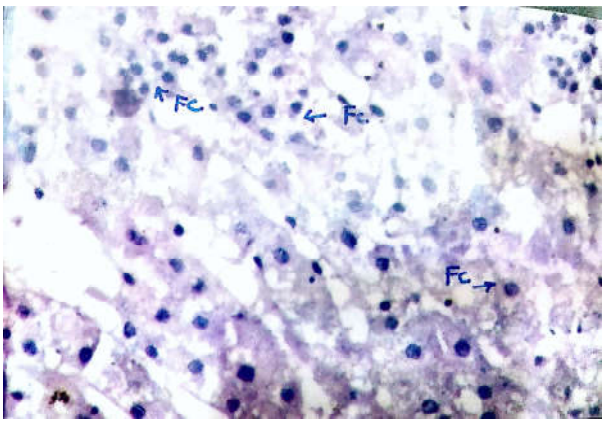

Fig. 4.2: Marked spongy appearance of fetal cortical cells.

The fetal cortical cells show marked spongy appearance indicating that these are highly functional.[6] Therefore, this indicates that the cortex not only degenerates but also functions as a steroidogenic organ.[7]

Weeks 21–22

The definitive cortex does not reveal any changes. However, the cells of the transitional area of the fetal cortex are enlarged indicating that the cells are maturing. Spongy appearance is well marked in fetal cortex.

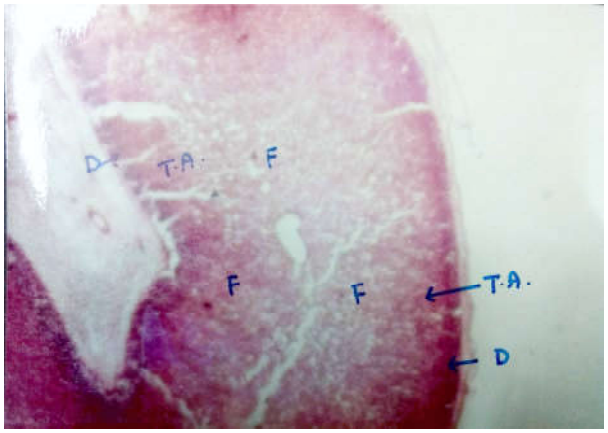

Fig. 5: Enlarged fetal cortical cells (F) in the transitional area (TA)

Weeks 23–28

Cortex does not reveal any changes.

Weeks 29–30

Marked basophilic staining of definitive cortex is decreased. The cells appear differentiated. Also, there appears to be a decrease in the density of cells. The cells of the transitional area of fetal cortex have started functioning, as depicted by their vacuolated cytoplasm.

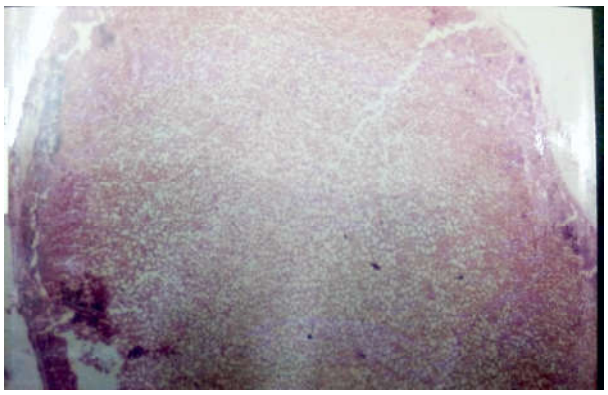

Fig. 6.1: Marked basophilic staining of definitive cortex decreased

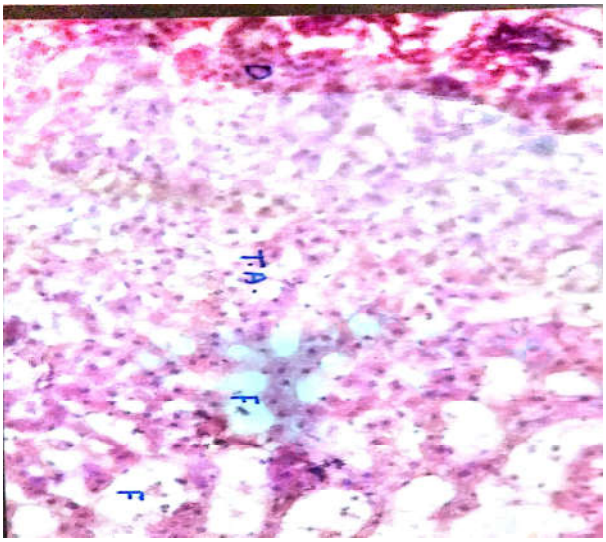

Fig. 6.2: Cells of transitional area (TA) show vacuolated cytoplasm

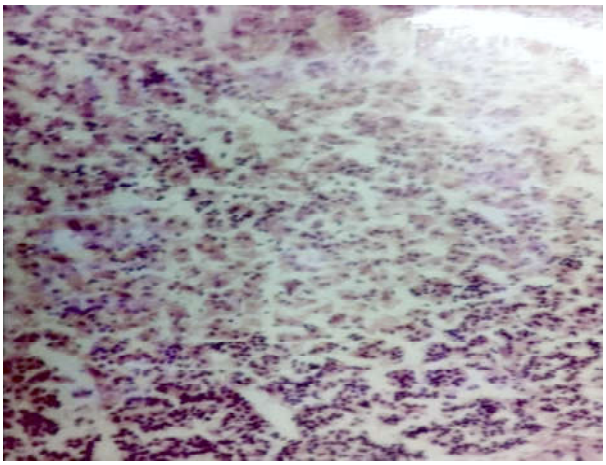

Fig. 6.3: Decrease in density of cells

Weeks 31–35

No significant changes are observed.

Weeks 36–38

In this stage, we observe a striking feature, i.e. the differentiation of definitive cortex into zona glomerulosa and zona fasciculata. The staining of this zone is totally different from what was observed in previous stages. The small dark basophilic cells of definitive cortex have transformed into large lightly staining cells arranged into zona glomerulosa and zona fasciculata.

The cells of zona glomerulosa are arranged in ovoid groups as observed in an adult mature cortex. Next, the cells in zona fasciculata are arranged in fascicular pattern with sinusoids between them. The cells are large and eosinophilic with vesicular nucleus and vacuolated cytoplasm suggestive of liquid droplet accumulation.

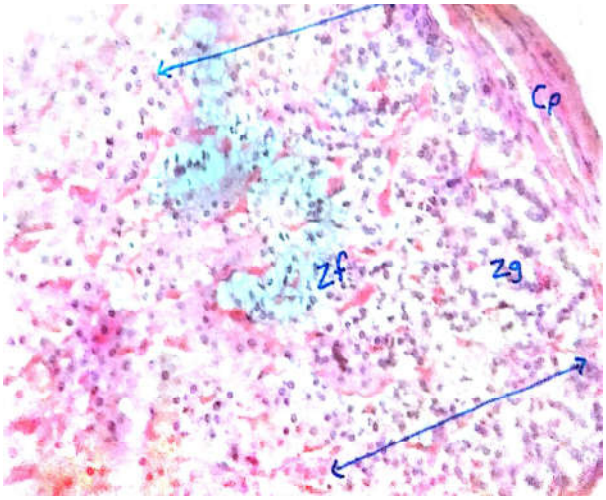

Fig. 7.1: Depicting capsule (Cp), zona glomerulosa (Zg) and zona fasciculate (Zf)

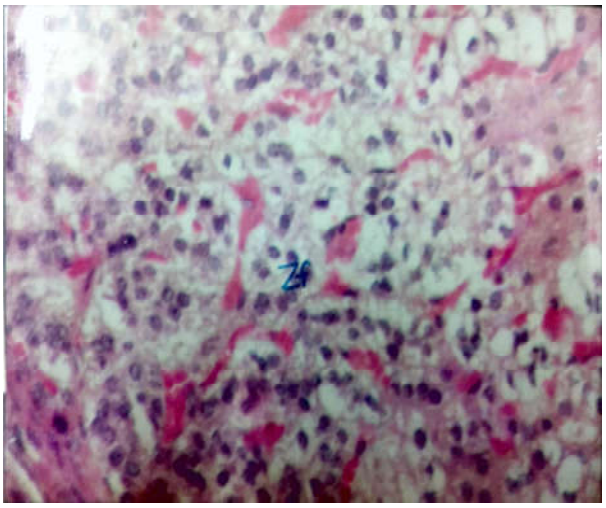

Fig. 7.2: Zona fasciculate (Zf) cells are large with vacuolated appearance

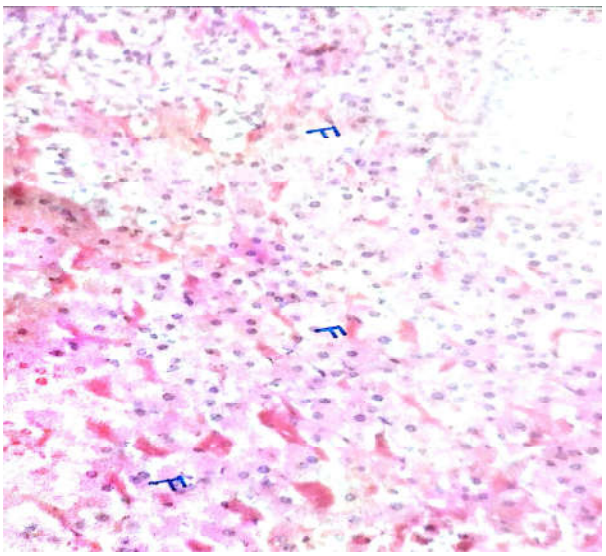

Fig. 7.3: No further differentiation in Fetal Cortex

Table 1: Ratio of definitive to fetal cortex

Gestational Age (Weeks)	Definitive Cortex	Fetal Cortex	Ratio of Definitive to Fetal Cortex
12–14	20	80	1:4
15–16	12.5	50	1:4
17–18	16	60	1:3.7
23–24	15	65	1:4.3
27–28	20	80	1:4
29–30	22.5	90	1:4
36–38	25	102	1:4

Fetal cortex occupies around 80% of adrenal cortex without any further differentiation.

Finally, as depicted in Table 1, the ratio of measurements of width of the definitive cortex and fetal cortex is constant i.e. 1:4 at all stages of development.

This suggests that the definitive cortex is continuously proliferating and adding cells to the fetal cortex where the cells are differentiated and become functional. After functioning for some time, the fetal cortical cells begin degenerating.

Conclusion

In this paper, we observe the development of cortex in fetal adrenal gland. We observe that in early as 12–14 weeks, the adrenal cortex comprises of two distinct zones i.e. definitive cortex and fetal cortex.

The definitive cortex, which is going to develop into permanent adult cortex, has not shown much differentiation till 29-30 weeks of gestation. The definitive cortex is also named as rudimentary zona glomerulosa. As the ratio of the measurements of width of definitive cortex and fetal cortex remains constant i.e. 1:4 at all stages of development, it can be suggested that the definitive cortex is continuously proliferating and adding cells to fetal cortex where the cells become differentiated and functional. After functioning for some time, the fetal cortical cells must be degenerating. Further, the fetal cortex comprises 80% of adrenal cortex till birth. The cells of the fetal cortex show vacuolated cytoplasm indicating a steroidogenic activity as early as 15-16 weeks of gestation and acts as an endocrine gland throughout the gestational period.

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