

HISTOCHEMISTRY, THE PRESENCE OF CARBOHYDRATES, ALKALINE AND ACID PHOSPHATASE ENZYMES, IN THE ADRENAL GLAND TISSUE DURING PRENATAL DEVELOPMENT IN DROMEDARY CAMEL

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ABSTRACT

The current study's goals are to investigate the foetal adrenal gland's histochemistry. Twenty-five foetuses at various developmental stages were used for conventional histological and histochemical techniques. Alkaline phosphatase activity was discovered in the same locations as PAS positive diastase-digested material. Neuroblasts, blood vessels, the adrenal capsule, the cells of the zona glomerulosa, the innermost part of the zona fasciculata and the foetal zone were all shown to have both alkaline phosphatase and PAS positive diastase resistant material. The blood vessels and a few isolated cells in the cortex and medulla were found to have the enzyme acid phosphatase. In conclusion, although it has unique characteristics, the histochemistry of the developing camel foetal adrenal gland was generally comparable to that of the foetal adrenal glands of other domestic animals.

Key words: Adrenal gland tissue, alkaline and acid phosphatase, camel, histochemistry, prenatal development

One of the most crucial endocrine organs for survival is the adrenal glands (Bielohuby, 2007). The adrenal cortex and the adrenal medulla are two distinct organs with distinct origins, roles and physical traits that come together during embryonic development. They originate from many germ layers. The coelomic epithelium gives rise to the cortex, while the neural gives rise to sympathetic ganglion cells which is the source of medulla cells (Dudek, 2011; Kim *et al*, 2009).

The function of the adrenal glands is crucial throughout foetal development. In order for the liver, lung, thyroid gland and gut to functionally mature and prepare for life outside the uterus, the foetal adrenal gland is essential for maintaining intrauterine homeostasis (Liggins, 1976).

The present study was therefore planned to investigate the histochemistry of the adrenal gland's constituents (enzymes and carbohydrates).

Materials and Methods

The adrenal gland was collected in the present study from 25 foetuses at different stages of development.

Small pieces of tissue up to 3 mm thick were fixed for 24 hours in cold (-4°C) acetone for paraffin processing method and then dehydrated with acetone at room temperature (two changes, each change for 1 hour), cleared in two changes of chloroform for half to one hour each, impregnated with paraffin wax at (56°C) for 15 -30 minutes in the vacuum oven, sectioned at 5µ, flattened on luke warm water bath and mounted on albumenised slides.

Carbohydrates

PAS positive diastase digested material (glycogen)

For detection of glycogen, the tissues were fixed either in the cold gender fluid (saturated aqua's picric acid in 95% ethanol, glacial acetic acid and formalin) for about 4 hours (Culling,1974) or in formal saline. After the routine processing, the sections were stained with PAS (periodic acid with Schiff reagent).

PAS positive diastase resistant material

For mucopolysachrides PAS with diastase control the sections were incubated in 1% diastase solution or saliva at 37°C, for one hour

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for the differentiation between glycogen and mucopolysaccharides.

Enzymes

Alkaline phosphatase

Alkaline phosphatase was detected using Gomori's calcium phosphate technique (Culling, 1974; Durary and Wallington, 1980). This method involved incubating the sections in the substrate for 3 to 6 hours, washing them in distilled water, putting them in an aqueous solution of cobalt nitrate for 5 minutes, then moving them to a fresh 1% solution of yellow ammonium sulphide for 1 minute, washing them under running water, counter-staining them with 0.1% safranin in 0.1% acetic acid, dehydrating, clearing and mounting them in a synthetic medium.

Acid phosphatase

The lead nitrate method (modified from Gomori, 1950) was used and it consisted of 0.05 acetate buffer at PH (5) and 0.53g lead nitrate and 3% solution of sodium β glecrophosphate. Sections were incubated in the prepared solution for one hour, washed briefly and transferred to 1% ammonium sulphide for 1-2 minutes, washed and counterstained with 1% aqueous eosin followed by washing in tap water and then in distilled water and mounted in glycerol jelly.

Results

1. PAS positive diastase digested material (Glycogen)

First trimester

During the early stage of the first trimester, a strong reaction for PAS was found in the blood vessels, in the accumulation of cells which formed the primordia of the adrenal cortex and in the migrating neuroblasts. In the middle stage of the first trimester, the reaction was found in the boundary tissue of the adrenal cortex and a moderate reaction in the capsule and the foetal zone but the zona fasciculata showed weak or negative reaction. The reaction was observed in the neuroplasts, blood vessels and nerve fibres in the developing medulla.

Second trimester

The developing trabeculae as well as the capsule and subcapsular artery plexus showed a significant reactivity in the early stages of the second trimester. The chromaffin cells had moderate to strong reactions, the developing zona fasciculata showed weak reactions, the foetal zone and medulla showed strong

reactions and as people aged, the zona glomerulosa, blood vessels and nerve fibres also showed strong reactions (Fig 2 and 3).

Third trimester

The developing trabeculae as well as the capsule and subcapsular artery plexus showed a significant reactivity in the early stages of the second trimester. The chromaffin cells had moderate to strong reactions, the developing zona fasciculata showed weak reactions, the foetal zone and medulla showed strong reactions and as the developing age, the zona glomerulosa, blood vessels and nerve fibres also showed strong reactions.

PAS positive diastase resistant material (Mucopolysaccharides)

First trimester

The accumulation of cells from the primordia of the adrenal cortex, the capsule and the blood vessels showed a mild reactivity to the diastase-resistant substance. The foetal zone had a weak to moderate reactivity, but the medulla, with the exception of the blood vessels, displayed a negative reaction.

Second trimester

The zona fasciculata displayed a negative reaction, whereas the capsule, trabeculae, zona glomerulosa, blood vessels, nerve fibres and chromaffin cells displayed a faint response (Fig 6).

In general, the response was intensified as compared to the previous periods. The zona fasciculata displayed a negative reaction, whilst the capsule and trabeculae displayed a moderate response. Chromaffin cells, blood arteries and nerve fibres, all responded favourably (Fig 7 and 8).

2. Enzymes

1. Alkaline phosphatase

First trimester

The migrating neuroblasts in the mesentery and adrenal tissue demonstrated a strong positive response to alkaline phosphatase activity during the early stages of development, as did blood vessels. The cells in the foetal zone displayed a moderate response (Figs 9 and 10).

Second trimester

The growing zona glomerulosa, the blood vessels and the vascular plexus of the subcapsular area, all showed a significant response. Alkaline phosphatase also demonstrated a robust positive

reaction on the capsule's exterior. The zona fasciculata's innermost portion displayed a moderate to strong reactivity for alkaline phosphatase, but its outermost part displayed a faint positive reaction. With the exception of nerve fibres and blood vessels, which demonstrated a significant reactivity to alkaline phosphatase, the medullary tissue had a mild to negative reaction (Fig 11).

Third trimester

Alkaline phosphatase activity was strongly positive in the zone glomerulosa. Alkaline phosphatase also showed a robust positive reaction in the blood vessels, nerve fibres and septae boundaries (Fig 12).

Alkaline phosphatase exhibited a moderate reaction in the zona fasciculata's outermost section and a weak to negative reaction in its innermost part. The alkaline phosphatase reaction was mild in the foetal zone and strongly positive in the sinusoids and nerve fibres (Fig 13).

Table 1. Illustrates the degree of alkaline phosphatase reaction in different foetal adrenal gland components through gestation period.

Zone	Gestation period		
	FIRST	SECOND	THIRD
Foetal zone	+++	++	++
Innermost region of zona fasciculata	-	++	+
Outermost region of zona fasciculata	-	+	++
Zona glomerulosa	__	+++	+++
Capsule	±	++	++
Medulla	__	±	±
Hilus	+++	+++	+++
Blood vessel in general	+++	+++	+++
Nerves fibres in general	+++	+++	+++

Key of table: +++ strong, ++ moderate, + weak, ± weak to absence, _negative.

2. Acid phosphatase

With the exception of blood vessels and a few isolated cells that displayed a mild reaction, the adrenal gland tissue during the three trimesters had a negative reaction for the acid phosphatase enzyme (Fig 14).

Discussion

Goats' adrenal capsule and parenchyma were PAS positive starting on day 42 of pregnancy and the reaction's intensity increase as the foetus grew older (Fowden *et al*, 1998; Ashoke *et al*, 2011). The

current study supported earlier findings that a substantial reactivity to PAS was shown in the blood vessels, in the migration of neuroblasts and in the accumulation of cells that formed the primordia of the adrenal cortex in the early stages of the first trimester. According to Bielanska-Osuchowska (1989), the developing cells of the zona glomerulosa, as well as the cortical and medullary cells, were mildly positive for PAS in the foetal pig. These reports are consistent with the current study.

The zona reticularis, trabeculae and capsule of a full-term goat foetus were all highly PAS positive. The zona fasciculata and medulla had a moderate reaction, but the zona glomerulosa displayed a modest reactivity (Ashoke *et al*, 2011). The current study also showed that the capsule, subcapsular vascular plexus and developing trabeculae all exhibited robust reactions in the early stages of the second trimester. The foetal zone exhibited a significant reaction, whereas the growing zona fasciculata displayed a modest reaction.

According to Ashoke *et al* (2011), the acid mucopolysaccharide reaction in goat foetuses was first observed once they were 73 days old and it was weak in the cortical cells and moderate in the capsule. The reaction was discovered earlier in the current study than in the foetal goat.

The accumulation cells of the capsule, blood arteries and primordia of the adrenal cortex showed a modest response to diastase-resistant material. With the exception of the blood vessels, the medulla displayed a negative reaction, while the foetal zone displayed a moderate response.

As foetal age increased, cortical cells exhibited a robust response to acid mucopolysaccharide, but medullary cells displayed a moderate response. In young foetuses, the cortical and medullary cells had a positive response to glycogen; however, as pregnancy progressed, the cortical cells' intensity diminished. According to Ashoke *et al* (2011), goat foetuses' future cortex cells showed a significant buildup of glycogen starting on day 122. The current study showed that as development progresses, the reactivity between glycogen and mucopolysaccharides intensifies.

According to Ashoke *et al* (2011), the goat foetus's developing cortical cells exhibited robust alkaline phosphatase activity up to 84 days, moderate activity up to 122 days and further increased toward term.

These authors found that the decreased activity of alkaline phosphatase between 84 and 122 days of

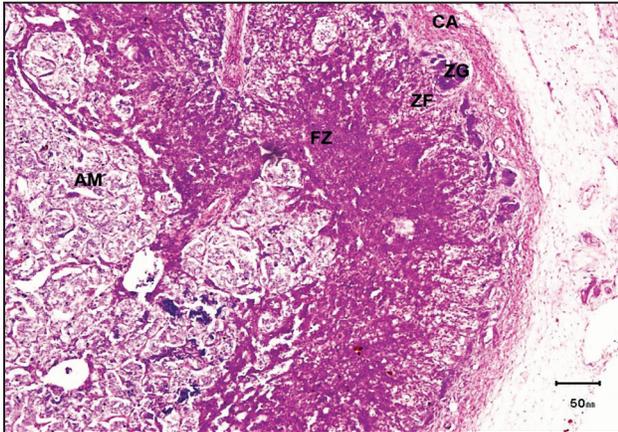


Fig 1. Photomicrograph illustrating a strong PAS- positive diastase digested material in foetal zone (FZ) and zona glomerulosa (ZG) while the capsule (CA), zona fasciculata (ZF) and the adrenal medulla (AM) showed moderate to weak reaction. From a foetus of 63 cm CVRL (238 days of age). PAS technique diastase digested material X 40.

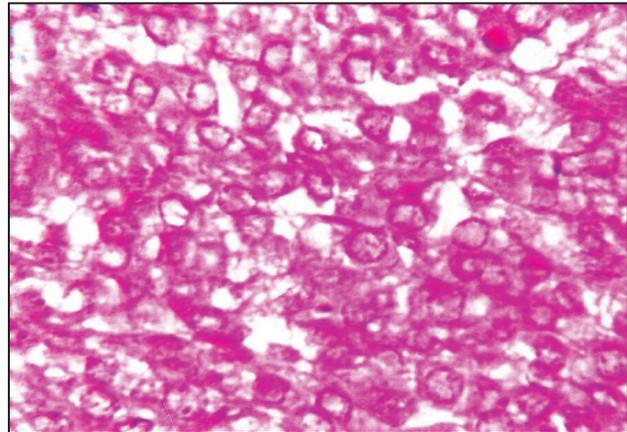


Fig 2. Photomicrograph showing a strong PAS- positive diastase digested material in the cells of the foetal zone from a foetus of 72 cm CVRL (262 days of age). PAS technique diastase digested material X 1000.

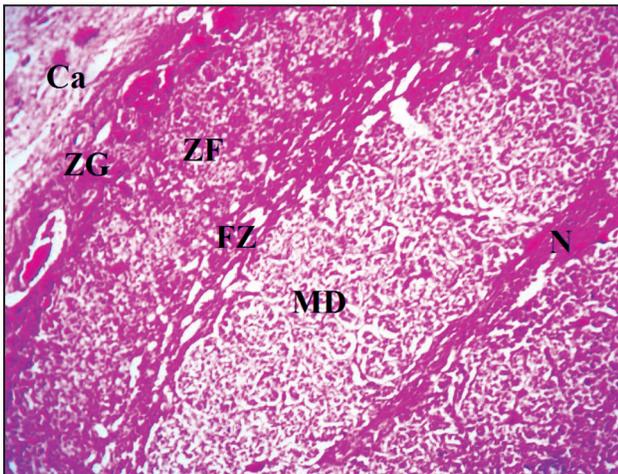


Fig 3. Photomicrograph from a foetus of 78 cm CVRL (278 days of age) demonstrating a strong PAS- positive diastase digested material in the zona glomerulosa (ZG) and nerve fibres within the medulla (N) while the capsule (Ca), zona fasciculata (ZF) and the adrenal medulla (MD) showed a moderate reaction. PAS technique diastase digested material. X 100.

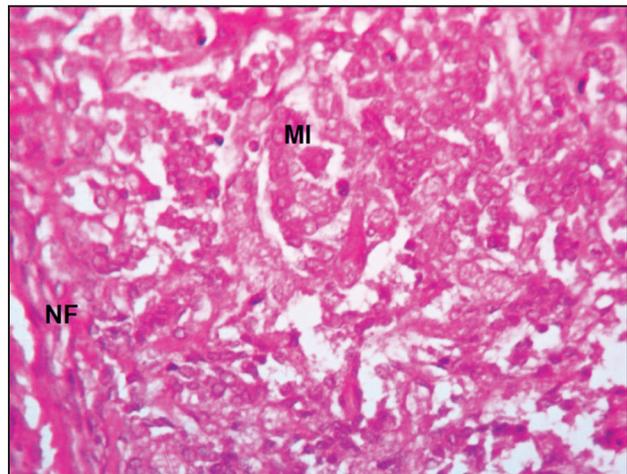


Fig 4. Photomicrograph demonstrating a strong PAS- positive diastase digested material in nerve fibres (NF) within the medulla, while the medullary islets (MI) showed moderate reaction. From a foetus of 78 cm CVRL (278 days of age). PAS technique diastase digested material X 400.

foetal life was caused by the cortical zones' decreased activity during this time, as seen in goats.

Similar to Bielanska-Osuchowska's (1989) findings in the foetal pig, this investigation found weak alkaline phosphatase activity in the subcapsular zone and none in the migratory cells.

Goat foetuses from 58 days to full term showed a moderate level of acid phosphatase activity in the cortical cells directly beneath the capsule. Throughout foetal development, medullary cells exhibited high levels of enzyme activity. Up to 122-day-old foetuses had very little or no enzyme activity in other cortical

cells. After that, these cells showed a modest level of activity until term.

Acid phosphatase activity was generally higher in medullary cells than in cortical cells (Ashoke *et al*, 2011). According to the prenatal investigation, the camel foetal adrenal gland's blood arteries were the only places where the acid phosphatase reaction was detected.

According to Osman *et al* (1976) and Osman (1984), the alkaline phosphatase enzyme is typically found where PAS-positive diastase-resistant material may be seen and it may play a part in the movement

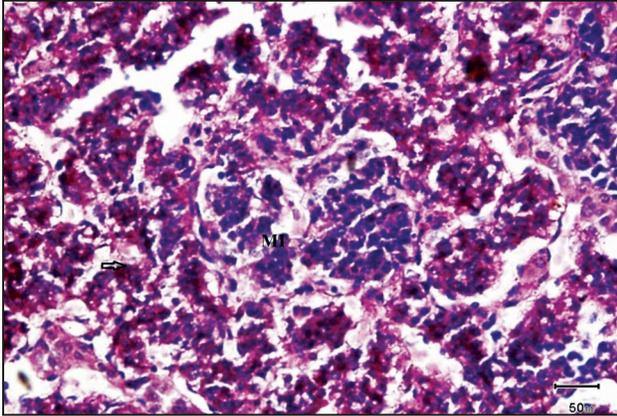


Fig 5. Photomicrograph from a foetus of 80 cm CVRL (260 days of age). Note the strong PAS- positive diastase digested material in the chromaffin cells(arrow) within the medulla, medullary islets (MI). PAS technique without diastase X 100.

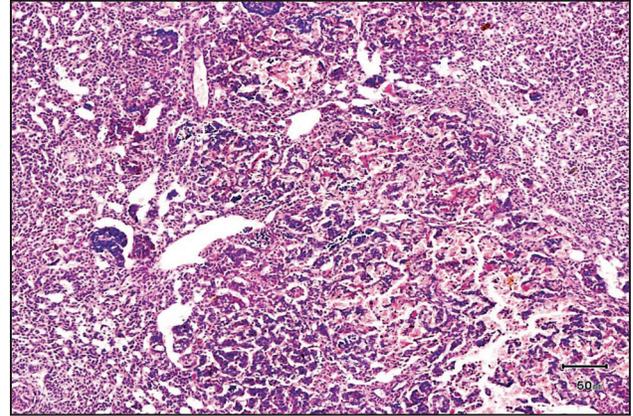


Fig 6. Photomicrograph illustrating a weak PAS-positive diastase resistant material in the adrenal medulla in a foetus of 40 cm CVRL (175 days of age). PAS technique with diastase, X 40.

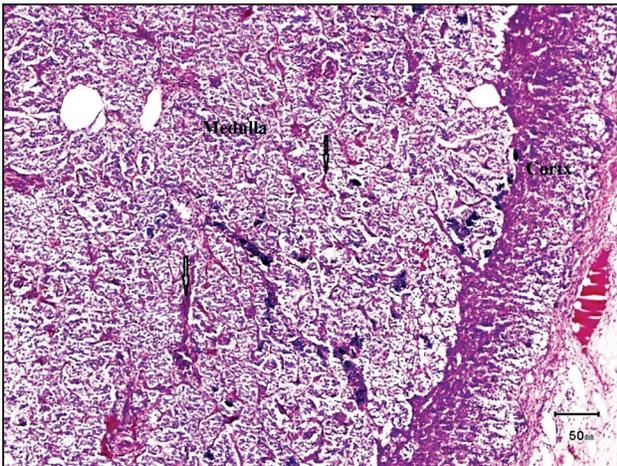


Fig 7. Photomicrograph demonstrating moderate PAS-positive diastase resistant material in the cortex and in the nerve fibres (arrows) and connective tissue of the adrenal medulla from a foetus of 63 cm CVRL (238 days of age). PAS technique with diastase. X 40.

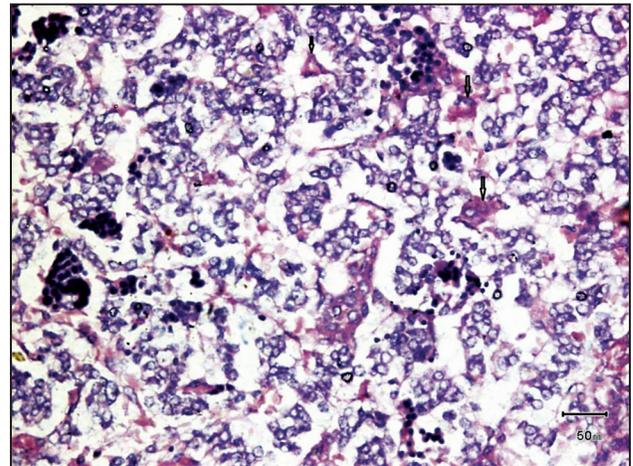


Fig 8. Photomicrograph demonstrating a weak PAS-positive diastase resistant material in the adrenal medulla in general but a moderate reaction was found in the ganglion cells (arrows) of a foetus of 75 cm CVRL (270 days of age). PAS technique with diastase, X 100.

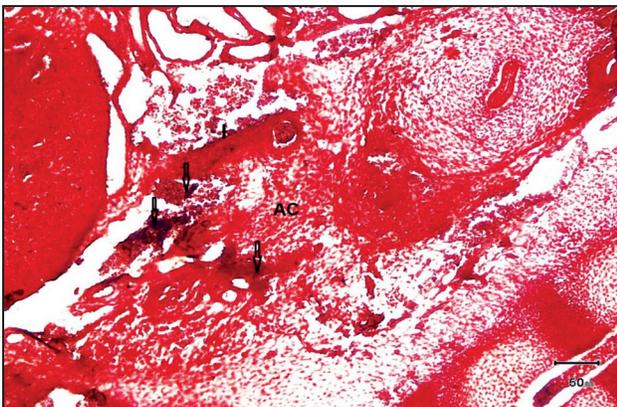


Fig 9. Photomicrograph showing a strong alkaline phosphatase activity in the primordia of the adrenal cortex (AC) (arrows) in a foetus of 2 cm CVRL (71 days of age). Gomori and Lillie technique, X40.

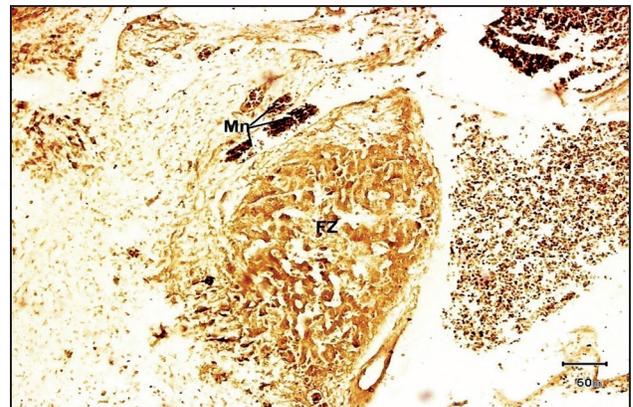


Fig 10. Photomicrograph demonstrating a strong alkaline phosphatase activity in the migrating neuroblasts (Mn) and moderate reaction in the foetal zone (FZ) of the adrenal cortex in a foetus of 5.6 cm CVRL (80.8 days of age). Gomori and Lillie technique, X40.

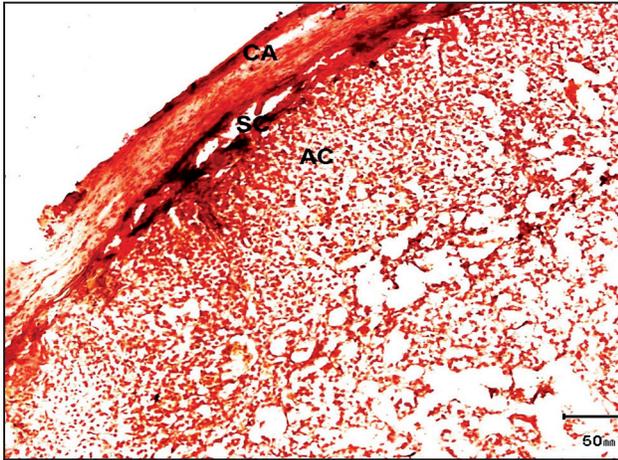


Fig 11. Photomicrograph illustrating a strong alkaline phosphatase activity (Black colour) in the Adrenal capsule (CA) and sub capsular arterial plexus (SC). AC: adrenal cortex in a foetus of 65 cm CVRL (243 days of age). Gomori and Lillie technique, X100.

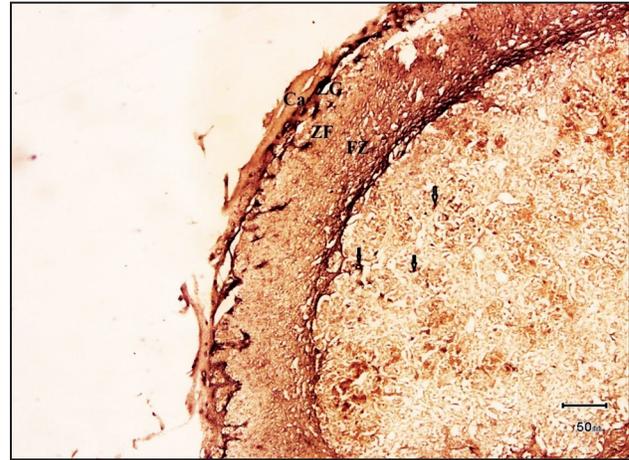


Fig 12. Photomicrograph showing a strong alkaline phosphatase activity in the capsule (Ca), zona glomerulosa, (ZG) and foetal zone (FZ) (black colour) and a moderate reaction was found in the medulla (arrows) in a foetus of 92 cm CVRL (317 days of age). Gomori and Lillie technique, X100.

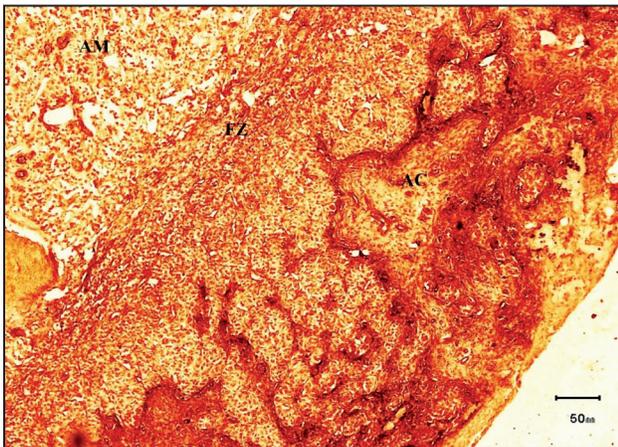


Fig 13. Photomicrograph demonstrating a strong alkaline phosphatase activity in adrenal cortex (AC) (brown to black colour), moderate in the foetal zone (FZ) and weak in the adrenal medulla (AM) from a foetus of 101 cm CVRL (342 days of age). Gomori and Lillie technique, X100.

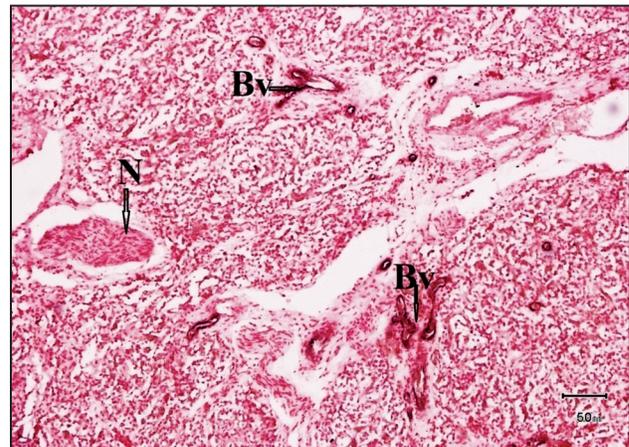


Fig 14. Photomicrograph illustrating a strong acid phosphatase activity in the medullary blood vessels (BV) while the nerve fibres (N) showed negative reaction from a foetus of 78 cm CVRL (278 days of age). Gomori and Lillie technique, X40.

of substances. According to Verma and Guraya (1967), alkaline phosphatase activity locations are highly vascularised, suggesting a direct physiological link between the enzyme and organ component blood vascularity.

Alkaline phosphatase therefore plays a crucial role in facilitating the transport of materials across the cellular membranes in the vertebrate's ovaries and may be involved in the transfer of nutrients, secretory materials, or waste products across the cellular membranes of blood vessels and surrounding tissues. The findings of the aforementioned writers are consistent with the current investigation.

It was opined that the activity of the acid phosphatase enzyme in individual germ cells can be caused by the cell's apoptotic process.

Conclusion

Glycogen was found in the blood vessels, in the accumulation of cells which formed the primordia of the adrenal cortex and in the migrating neuroblasts. In the middle stage of the first trimester, the reaction was found in the boundary tissue of the adrenal cortex, moderate reaction in the capsule and the foetal zone but zona fasciculata showed weak or negative reaction. In the developing medulla, the reaction was

found in the nerve fibres, blood vessels and in the cytoplasm of the neuroplasts.

- A weak reaction of the diastase resistant material was found in the accumulation of cells of the primordia of the adrenal cortex, the capsule and blood vessels in the foetal zone. The medulla showed negative reaction except in the blood vessels. During the second and third trimesters, the capsule, trabeculae, zona glomerulosa, blood vessels, nerve fibres and chromaffin cells showed a weak reaction, while the zona fasciculata showed a negative reaction.
- During the first trimester, the neuroblasts and the blood vessels showed a strong positive reaction for alkaline phosphatase, while the cells of the foetal zone showed a moderate reaction. During the second and third trimesters, a strong reaction was found in the developing zona glomerulosa, the blood vessels and in the arterial plexus and the outer surface of the capsule. The outermost region of the zona fasciculata showed a weak positive reaction while the innermost region of zona fasciculata showed moderate to strong reaction for alkaline phosphatase.
- The medullary tissue showed a weak to negative reaction for alkaline phosphatase except the nerve fibres and the blood vessels which showed a strong reaction for alkaline phosphatase.
- The acid phosphatase activity was absent in the developing adrenal glands during the different stages of development except in the blood vessels and in sporadic cells in the cortex and medulla.

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Ethical Statement

This study was done according to the guidelines of the Declaration of Helsinki.

Data Availability

The data generated during the study can be requested from the corresponding author.

Conflict of Interest

The authors declare no conflict of interest.

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