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Histomorphological peculiarities of the adrenal gland of the large white Yorkshire pig

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Abstract
The present investigation was carried out on 12 pairs of adrenal glands from recently slaughtered pigs of both genders of 6-12 months age group. Histologically, the adrenals were divided in stroma and parenchyma. Stroma consisted of capsule and trabeculae. Collagen and reticular fibres were present at the capsule. Parenchyma composed of the cortex and medulla. Cortex was divided into three portions according to their cell arrangements in zona glomerulosa, zona fasciculata and zona reticularis. Reticular fibres were present throughout frame work of the adrenal glands. The Medulla was divided in the inner and outer parts. Inner medulla had polyhedral cells where as outer medulla had columnar shaped cells. Collagen fibres were also present at the medullary area. Patches of medulla were seen at some cortex area.

Keywords: Adrenal gland, large white Yorkshire pig

Introduction
The Large White Yorkshire, breed of swine was produced in the 18th century by crossing the large Indigenous white of Northern England with the smaller, fatter, white Chinese pig. Throughout the world, the secondary consumption of pig farming is the production of pig skin, bristles and manure (Banerjee, 2013) [8].

In India, the population of pig is 9.06 million according to 20th livestock census of India. It ranks fifth in the world. The adrenal gland is one of the most important organ because it plays a significant role in the body activities and essential for the maintenance of whole life. The adrenal gland is called the suprarenal gland because it is situated on the cranio-medial aspect of the kidney. It regulates many physiological functions both in foetal and postnatal life (Hill, 2007) [15]. The adrenal gland is an indispensable organ that takes part in the formation of hypothalamic pituitary adrenal axis (HPA) termed as body's stress system. HPA mainly controls the levels of cortisol and other important stress related hormones (Hu and Funder, 2006; Pippal and Fuller, 2008) [29]. Each adrenal gland has two distinct structures, the outer adrenal cortex and inner medulla, both of which produce hormones. The adrenal gland has two distinct functional units which are of different embryological origins; a mesodermal cortex and a neural crest ectodermderived medulla. A connective tissue capsule surrounds each gland. Comparative anatomical records reveal that the nephric origin of adrenocortical cells is a general feature of all vertebrates (Wrobel and Suss, 1999) [37]. The adrenal cortex is subdivided into three (Dellman, 1993) [11] or four (Bacha and Wood, 1990) [4] distinct zones of epithelial cells. The outermost zone is called zona glomerulosa in ruminants, and is formed of irregular clusters and cords of cells in the horse, donkey, pig and carnivores. This zone is called zona arcuata because the cells are arranged into arcs (Banks, 1993; Prasad and Sinha, 1981) [9]. The zona fasciculata, the widest zone of the adrenal cortex arc consists of radially arranged cords of cuboidal or columnar cells. The foamy appearance of the cells is caused by the presence of numerous vacuoles, when lipid is removed in processing. The zona reticularis consists of cells disposed as freely anastomosing cords. The cells are roughly the same in morphological features as the cells of the zona fasciculata, but their nuclei and cytoplasm have darker staining. There are two types of chromaffin cells in the adrenal medulla. The granules of epinephrine cells are smaller and less electron-dense compared to norepinephrine cells (Coupland and Weakley, 1968) [10]. The norepinephrine cells contain a large spherical nucleus while chromaffin cells contain a full complement of cytoplasmic organelles. The cortex produces aldosterone, cortisol and androgens which are responsible for the regulation of blood pressure, electrolyte balance, glycogen and lipid metabolism, and estrogen biosynthesis, respectively. It is known that the cells situated at the periphery of the medulla produce

adrenalin and, therefore, are called A cells; the cells in the central part of the medulla secrete noradrenalin (norepinephrine) and are denoted N cells (Hullinger and Andrisani, 2006) [17]. Both adrenaline and nor adrenaline provoke a quick response on diverse organs in stress situation. The study aimed to investigate structure of the adrenal glands of pig to justify the importance and essentiality in the body. The better knowledge of morphological norms and the causes of their variations are essential not only for a better understanding of physiology but also for a correct diagnosis and prognosis of diseases. The detailed description of the adrenal gland of pig has received little attention. However, paucity of available literature on the mature adrenal gland of swine has prompted this research work, which will contribute to the future research on its functions and pathology of adrenal gland structures and will provide a theoretical basis for ongoing studies.

Materials and Methods

For histomorphological and histochemical studies, the samples was fixed in 10% formalin for routine staining and in bouin's fluid for special staining 24-72 hours. After fixation, washing, dehydration, clearing, embedding in paraffin wax done and then preparation of block. Five to six micron thick sections was cut by using rotary microtome then mounting of the section on albuminized slides and drying of section and then stain for general histomorphological and histochemical observation.

Following staining methods were used.

1. Ehrlich's Hemotoxylin and Eosin
2. Van Gieson's method for Collagen fibres
3. Masson's trichrome for Collagen fibres
4. Weigert's method for elastic fibres
5. Verhoeff's stain for elastic fibres
6. Gomori's method for reticulum for reticular fibres
7. Selective demonstration method by silver orcein and aniline blue for collagen, elastic and reticular fibres.
8. Gomori's method for chromaffin granules.

Results and Discussion

Histological Examination

The present study showed that the adrenal gland was divided in two parts stroma and parenchyma which simulated the observation of Hullinger (1978) [18] in the dog and Kour *et al*. (2017) [21] in Bakerwali goat.

Capsule

The finding of present study showed that capsule was mainly formed by the collagen and reticular fibres. The findings were in agreement with the report of Prasad and Yadava (1972) [30] in Indian buffalo, Panchal *et al*. (1998) [27] in sheep. Nagpal *et al*. (1991) [24] in camel and Baishya *et al*. (1998b) [7] in mithun and yak reported the similar findings as predominance of the collagenous fibres over reticular and elastic fibres. In disagreement to present study, Stokoe (1959) [34] found the abundance of elastic fibres in capsule of sheep, However, contrary to this collagen fibres were observed in abundance in the outer layer of the capsule of Indian buffalo in the findings of the Prasad and Yadava (1972) [30]. Nabipour *et al*. (2008) [23] in camel and Paul *et al*. (2016) [28] in the black bengal goat also found the connective tissue fibres in the capsule. In present study, there were accessory adrenal cortical nodules in the capsule which were surrounded by the connective tissue fibres containing the zona glomerulosa cells in the centre. It was in accordance with the reports of the Smollich (1967) in cattle, Prasad and Yadava (1972) [30] in buffalo calf, Nagpal *et al*. (1991) [24] in camel, Panchal *et al*. (1998) [27] in sheep, Sanyal *et al*. (2005) [32] in goat, Nama *et al*. (2009) [25] in sheep and Kour *et al*. (2017) [21] in Bakerwali goatThe trabeculae were reported in the capsule in present study which was formed by the collagen and reticular fibres. These finding resembled with the study of Sohal and Chaturvedi (1962) [33] and Prasad and Sinha (1981) in buffalo. The trabeculae reached to the zona fasciculata and medulla at some area. This was in uniformity with the findings of Nama *et al*. (2009) [25] in the sheep and Kour *et al*. (2017) [21] in Bakerwali goat.

Cortex

The present study revealed that there were three zones in the cortex which were zona glomerulosa, zona fasciculata and zona reticularis. This was in conformity with the observations of Prasad and Yadava (1974) in buffalo, Ganguly and Ahsan (1978) in goat, Dellman (1993) [11] in ruminants, Svendson *et al*. (1998) in minipig, Sanyal *et al*. (2005) [32] in goat, Nama *et al*. (2009) [25] in sheep, Nwaogu and Francis (2009) [26] in Kano brown goats. Whereas, Holmes (1961) [16] in ferret, Sohal and Chaturvedi (1962) [33] and Prasad and Yadava (1974) in Indian buffalo, Baishya *et al*. (1998a) [6] in mithun and yak, Fujioka (1956) in pig, Bacha and Bacha (2000) [3] in ruminants and Ye *et al*. (2017) in the bactrian camel observed the intermediate zone between zona glomerulosa and zona fasciculata.

Zona glomerulosa

The zona glomerulosa was the outer most zone of cortex, had the cuboidal cells containing the irregular clusters with ovoid or spherical nucleus reported the similar findings with Sohal and Chaturvedi (1962) [33] in buffalo, Prasad and Sinha (1984), Dellman (1993) [11] in domestic animals, Panchal *et al*. (1998) [27] in sheep, Sanyal *et al*. (2005) [32], Nama *et al*. (2009) [25] in sheep and Nwaogu and Francis (2009) [26] in Kano brown goats. While Al-Bagdadi (1969) in camel observed that zona glomerulosa was divided into the dorsal and ventral portion by a thick band of fibrous connective tissue. Prasad and Sinha (1984) in buffalo described that cords of cells were connected at the capsule end by arch of cells which was not reported in the present study. The present study was in contradiction with the observation of Nagpal *et al*. (1991) [24] in camel and Baishya *et al*. (1998a) [7] in mithun and yak who revealed that the clusters of glomerular cells were separated by the sinusoids. Trautman and Fiebiger (1957) [36] in horse, Prasad and Sinha (1984) and Dellman (1993) [11] in horse called this zone as zona arcuata. Such kind of differentiation in zones were not reported in present study. Some cells of zona glomerulosa were surrounded in the trabeculae. This finding resembled to that of Panchal *et al*. (1998) [27] in marwari sheep who observed that some cortical cells of zona glomerulosa were encapsulated in the capsule. In the present study, the reticular fibres were found in the zona glomerulosa along with bundles of the collagen fibres which surround the clusters of cells. These observations were in close harmony with the finding of Sohal and Chaturvedi (1962) [33], Prasad and Sinha (1972) [30] in buffalo and Nama *et al*. (2009) [25] in sheep.

Zona Fasciculata

Zona fasciculata was the largest layer of the cortex part of adrenal gland. This finding of the present study was in

conformity with the reports of Prasad and Yadava (1974) in buffalo, Prasad and Sinha (1981) in domestic animals, Baishya *et al*. (1998) [6] in mithun and yak, Svendsen *et al*. (1998) [35] in minipig, Bacha and Bacha (2000) [3] in ruminants, Sanyal *et al*. (2005) [32], Nwaogu and Francis (2009) [26] in goat. The zona fasciculata cells were oval and round in shape arranged in radial columns directed towards the medullary zone. This observation was in consonance with the reports of Fujioka (1956) in pig, Prasad and Yadava (1974) in buffalo, Ganguly and Ahsan (1978) in goat, Dellman (1993) [11] in ruminants, Baishya *et al*. (1998a) [6] in mithun and yak, Nwaogu and Francis (2009) [26]. However, Nagpal *et al*. (1991) [24] in camel, Sanyal *et al*. (2005) [32] in goat and Ye *et al*. (2017) in bactrian camel reported that the cells of fasciculata layer were polygonal.

Svendsen *et al*. (1998) [35] in minipig also reported that the zona fasciculata was composed of large rectangular cells arranged in vertical columns 2 to 3 cells wide separated by capillaries. Nagpal *et al*. (1991) [24] in camel and Hullinger (1978) [18] in dog noticed that zona fasciculata was easily divided into inner and outer regions of parenchyma contrary to the observations found in present study. The reticular fibres were present in the zona fasciculata arranged along with the columns of cells. This finding resembled with the reports of Sohal and Chaturvedi (1962) [33], Nama *et al*. (2009) [25] in Marwari sheep and Kour *et al*. (2017) [21] in Bakerwali goat.

Zona Reticularis

The zona reticularis was the second widest zone in the adrenal cortex and deepest among other three zones in cortex. The findings were in agreement with the report of Ganguli and Ahsan (1978), Svendsen *et al*. (1998) [35] in minipig, Bacha and Bacha (2000) [3] in ruminants, horse and pig, Kour *et al*. (2017) [21] in Bakerwali goat, Ye *et al*. (2017) in Bactrian camel. Contrary to this, Prasad and Sinha (1981) in domestic animals, Sanyal *et al*.(2005) [32] in goat, Nama *et al*.(2009) [25] in sheep observed that the zona reticularis was the narrowest among three cortical zones. The cells of fasciculata were arranged as irregular anatomosing network that turned to become zona reticularis so there was no clear demarcation between these two zones. The cells were polyhedral in shape. These observations were in close harmony with the reports of Prasad and Yadava (1974) in buffalo, Nagpal *et al*. (1991) [24] in camel, Baishya *et al*. (1998b) [7] in mithun and yak, Svendsen *et al*. (1998) [35] in minipig, Nwaogu and Francis (2009) [26] in Kano brown goats, Nama *et al*. (2009) [25] in sheep, Kour *et al*. (2017) [21] in Bakerwali goat, Ye *et al*. (2017) in Bactrian camel. The collagen and reticular fibres were present in the zona reticularis. It was also reported by Fujioka (1956) in pig, Kour *et al*. (2017) [21] in Bakerwali goat.

Medulla

In present study, the medulla and zona reticularis were not clearly divided by regular arrangement of cells. This observation was in conformity with the findings of Prasad and Yadava (1973) [31] in Indian buffalo, Nagpal *et al*. (1991) [24] in camel, Nama *et al*. (2009) [25] in sheep. The medulla was the central part of the adrenal gland, consisted of two layers as outer and inner medulla surrounding the central vein. The outer zone had columnar shaped cells and inner zone had the polyhedral cells. The findings of present study was in agreement with the Smollich (1967) in different species of animals, Prasad and Yadava (1973) [31] in Indian buffalo, Gelberg *et al*. (1979) in horse, Fazekaz (1996) [13] in antelope, Baishya *et al*. (1998b) [7] in mithun and yak, Panchal *et al*. (1998) [27] in Marwari sheep, Jelinek and Konecny (2010) in cattle, Kour *et al*. (2017) [21] in Bakerwali goat. The peripheral part of the medulla consisted of the long axes cells in which nucleus was situated at the corner of the cells it was also observerd in horse by the Gelberg *et al*. (1979). The medulla was composed of epitheloid cells called chromaffin cells which were irregular and pale staining medullary granules, which simulated to the findings of Smollich (1966) in ruminants, Krumery and Buss (1969) [22] in African elephants, Narasimhan and Kamat (1970), Prasad and Yadava (1973) [31], Esther (1978) [12] in swine, Gelberg *et al*. (1979) in horse. They also described that the medullary tissue was comprised of two types of chromaffin cells. This was also explained by Nama *et al*. (2009) [25] that two distinct cells *viz*., adrenaline and noradrenaline generator cells were found in the adrenal medulla of sheep. Qiu *et al*. (2013) in Beagle dog revealed that the majority of the chromaffin cells were located in the medulla, were loosely arranged in clusters and cords.

There were plentiful collagen and reticular fibres in the fibro-architecture of the adrenal medulla. The bundles of the collagen fibres along with reticular fibres surrounded the adrenal medullary cells. This observation was in consonance with the report of Krumery and Buss (1969) [22] in African elephant, Prasad and Yadava (1973) [31] in the Indian buffalo, Gelberg *et al*. (1979) in horse, The medullary patches were seen at some area of cortex. These observations were also noticed by the Kour *et al*. (2017) [21] in bakerwali goat.

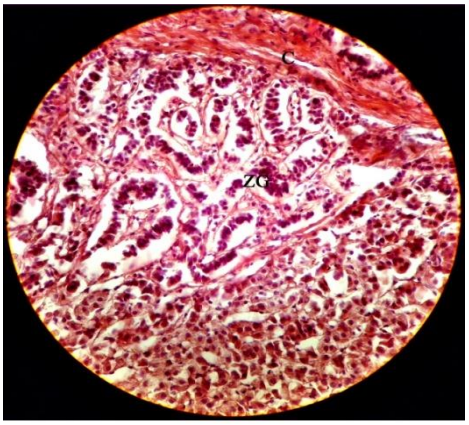

Fig 1: Photomicrograph of adrenal of pig showing collagen fibres in capsule and zona glomerulosa. C - Capsule, ZG - Zona glomerulosa (H&E stain, 400X)

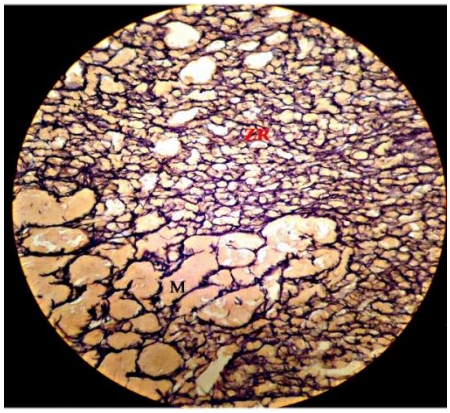

Fig 2: Photomicrograph of adrenal of pig howing the reticular fibres in zones of cortex and medulla. ZR - Zona reticularis, M - Medulla. (Gomori's stain for reticular fibres, 400X)

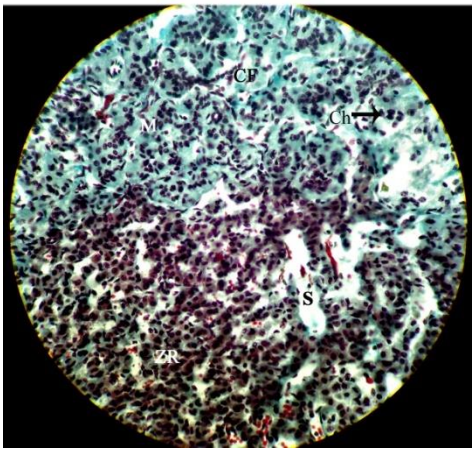

Fig 3: Photomicrograph of adrenal of pig showing the zones of cortex and medulla showing presence of collagen fibres. ZR - Zona reticularis, CF - Collagen fibres M - Medulla, Ch - Chromaffin cells, S- Sinusoids. (Masson's trichrome stain, 400X)

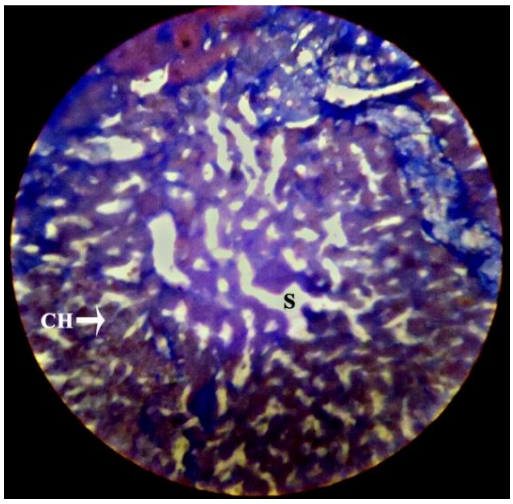

Fig 6: Photomicromicrograph of adrenal of pig showing the chromaffin cells in medulla. Ch - Chromaffin cells, S - Sinusoids, M-Medulla. (Gomori's stain for chromaffin cells, 1000X)

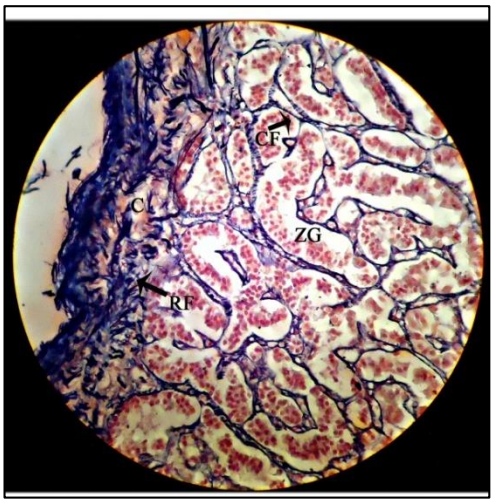

Fig 4: Photomicrograph of adrenal of pig showing the capsule and cortex zone. C - Capsule, ZG - Zona glomerulosa, CF - Collagen fibres, RF-Reticular fibres. (Selective demonstration of elastic, reticulum and collagen by silver orcein and aniline blue, 400X)

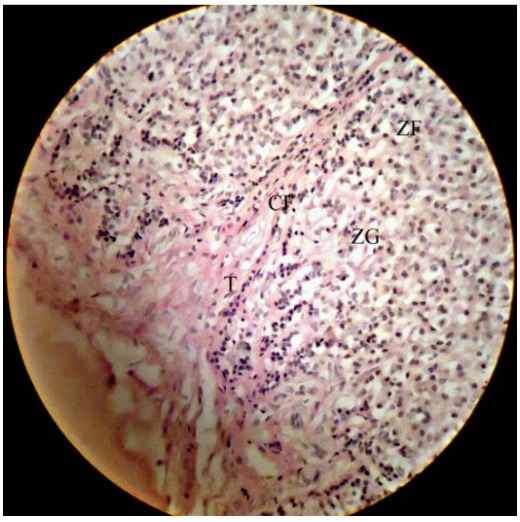

Fig 7: Photomicrograph of adrenal of pig showing trabeculae and zones of cortex. ZG - Zona glomerulosa, ZF - Zona fasciculata, T-Trabeculae, CF-Collagen fibres. (Van Gieson's stain, 400X)

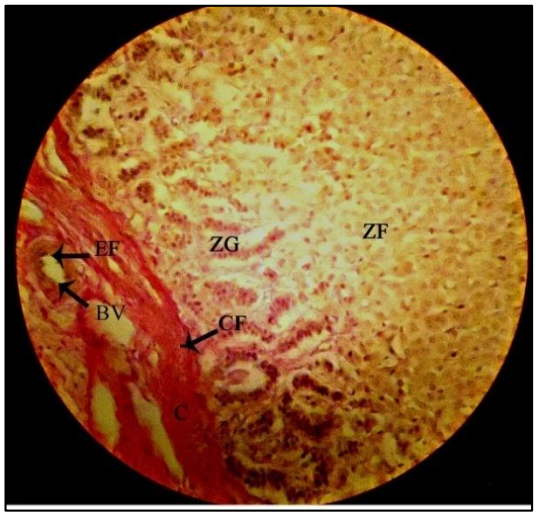

Fig 5: Photomicrograph of adrenal of pig showing the collagen fibres and elastic fibres in capsule and cortex. ZG - Zona glomerulosa, ZF - Zona fasciculata, CF - Collagen fibres, EF - Elastic fibres, C-Capsule, BV-Blood vessel. (Verhoeff's stain, 400X)

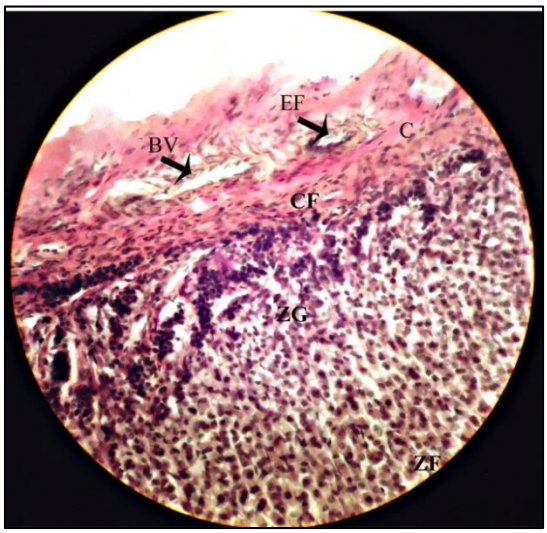

Fig 8: Photomicrograph of adrenal of pig showing the capsule and zones of cortex. C - Capsule, ZG - Zona glomerulosa, ZF - Zona fasciculata, EF- Elastic fibres, CF - Collagen fibres, BV - Blood vessel (Weigert's stain, 400X)

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