

Histological Study of Adrenal Gland in Male and Female Rabbits

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Abstracts: The aim of this study was to investigate the histomorphometric properties of the rabbit adrenal gland. Five adult male and five adult female New Zealand white rabbits were used in the experiment. The animals were deeply anesthetized with ketamine and after fixation via intravascular perfusion, the right adrenal glands were dissected and used for histological preparation. Results showed that the zona glomerulosa is a relatively narrow zone in which the arrangement of the cords is such that the cells are in arcuate groups. The zona fasciculata, the broadest zone, is composed of cell cords coursing parallel to one another in a radial direction toward the medulla and the zona reticularis is composed of a network of cell cords. The mean volume of the adrenal gland is significantly greater in female rabbits than in males. This difference is due to apparent differences in the volume of the zona glomerulosa, zona reticularis and zona fasciculata which are all significantly greater in females than in males ($p < 0.05$). In all three histological regions of the gland, the average cell volume is also significantly greater in female rabbits in comparison to those in males ($P < 0.05$). In all three examined regions of the adrenal cortex, the average nuclear volume is greater in female rabbits in comparison to males, but the differences are not statistically significant.

Key words: Adrenal gland • Rabbit • Histomorphometric

INTRODUCTION

Mammals typically possess two adrenal glands, one located superior to each kidney. The adrenal cortex of adult mammals which forms the bulk of the gland may be subdivided by means of histological criteria into three well-defined regions: zona glomerulosa, zona fasciculata and zona reticularis. These regions are arranged as concentric shells surrounding the adrenal medulla [1]. The outermost zone is called zona glomerulosa in ruminants and primates [2] and in a few rodents [3, 4]. In most other domestic species including dogs, cats, horses and pigs, the parenchyma forms archades just beneath the capsule. Consequently, in those animals this portion of the cortex is referred to as the zona arcuata. The next portion of the cortex is named the zona fasciculata. The secretory cells of the zona fasciculata have a foamy appearance as a result of the presence of numerous lipid droplets and because of that morphological trait are called spongiocytes [2]. This zone is the widest zone of the adrenal cortex consisting of radially arranged cords of

cuboidal or columnar cells [5, 6]. The cells of the zona fasciculata end internally next to the innermost zone of the cortex, the zona reticularis. As the name implies, the parenchyma of the zona reticularis appears to anastomose and form reticulated chains of cells [2]. In the available literature, there is a lack of detailed histomorphometrical data characterizing the morphometric properties of the adrenal gland in both sexes in the rabbits. The aim of this study was therefore to compare these properties of the adrenal gland in male and female rabbits.

MATERIALS AND METHODS

Five adult male (3.3-3.7 kg body weight) and five adult female (3.1-3.5 kg body weight) New Zealand white rabbits bred in our colony were used in the experiment. Animals were maintained on normal rabbit pellets and water *ad libitum*. After two weeks, the animals were deeply anesthetized with ketamin and fixed by intravascular perfusion via the left ventricle with buffered formalin. The right adrenal glands of all animals were

dissected and were immediately immersed in 10% buffered formalin. Paraffin-embedded sections were cut at 5 μm and stained with haematoxylin-eosin. Photomicrographs were taken with a camera (Canon) attached to a light microscope. Micrometric measurements and volume were carried out with ocular micrometer. All stereological results were statistically evaluated by Student t-test. Results are reported as mean \pm SEM with a significance level of 0.05.

RESULTS

Morphological data obtained from this study showed that in both sexes the adrenal cortex can be divided into three zones according to differences in the arrangement of its cells: an outer zone, the glomerulosa; a middle zone, the fasciculata; and an inner zone, the reticularis. The zona fasciculata is the broadest of the three. The zona glomerulosa is a relatively narrow zone in which the arrangement of the cords is such that the cells are in arcuate groups. The arches are apparently more wider in female rabbit in comparison to males.

The cells tend to be columnar and they have spherical nuclei. A few lipid droplets may be found in the cytoplasm, but they are sparse in comparison with the droplets in the zona fasciculata. The zona fasciculata, the broadest zone, is composed of cell cords coursing parallel to one another in a radial direction toward the medulla. The cords are usually only one or two cells in width. The secretory cells are generally cuboidal or polyhedral in shape and they are sometimes binucleate. The nuclei appear more vesicular than those of the glomerulosa, with less dense chromatin. The cells are relatively large.

Since the lipids are dissolved by the usual technical procedures, the cytoplasm has a spongy appearance.

The zona reticularis is composed of a network of cell cords. The cells are generally smaller than those of the fasciculata and they frequently have deeply staining nuclei. The cytoplasm has relatively few lipid droplets in comparison with the fasciculata and the droplets vary greatly in size.

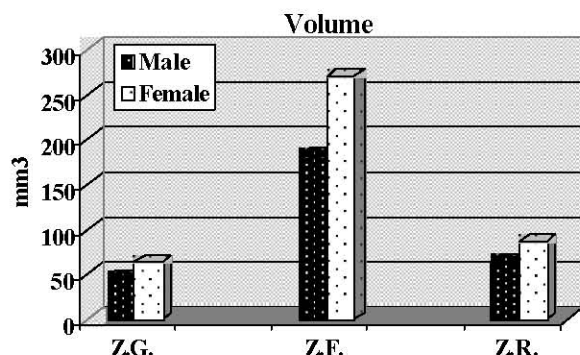
Data obtained from micrometric measurements of the adrenal gland in both sexes are shown in table 1.

DISCUSSION

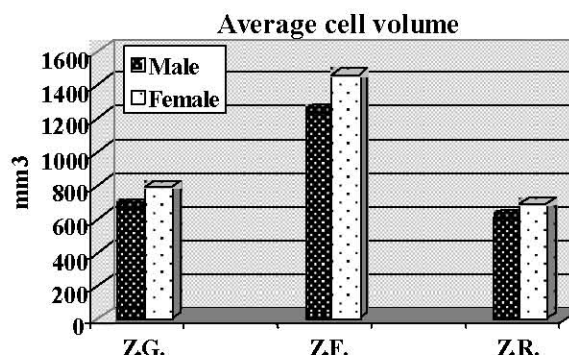
The adrenal cortex is composed of two [3], three [1, 2], or four [7] distinct area of epithelial cells. The outermost zone is called zona glomerulosa in ruminants and primates [2] and in a few rodents [3, 4]. In most other domestic species including dogs, cats, horses and pigs, the parenchyma forms archades just beneath the capsule. Consequently, in those animals this portion of the cortex is referred to as the zona arcuata. The next portion of the cortex is names the zona fasciculata. The secretory cells of the zona fasciculata have a foamy appearance as a result of the presence of numerous lipid droplets and because of that morphological trait are called spongiocytes [2]. This zone is the widest zone of the adrenal cortex consisting of radially arranged cords of cuboidal or columnar cells [5, 6]. The cells of the zona fasciculata end internally next to the innermost zone of the cortex, the zona reticularis. As the name implies, the parenchyma of the zona reticularis appears to anastomose and form reticulated chains of cells [2]. Results obtained from the present investigation showed that in both male

Table 1: Average micrometric measurements in μm of the adrenal components

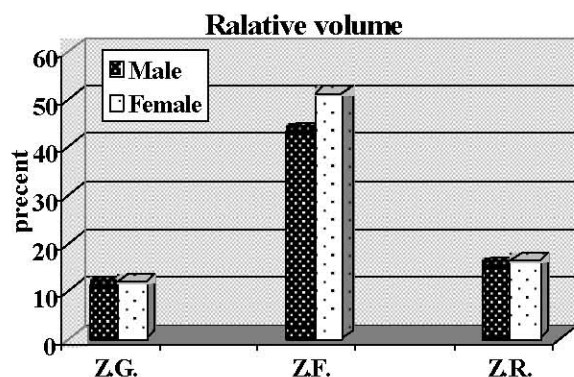
Parameters	zones	Male	Female
Volume (mm^3)	The entire gland	420 \pm 40	530 \pm 44
	Zona glomerulosa	47.04 \pm 5.12	64 \pm 6.14
	Zona fasciculata	182.28 \pm 32.46	270 \pm 44.22
	Zona reticularis	64.68 \pm 8.28	86.22 \pm 10.24
Relative volume (%)	Zona glomerulosa	11.20 \pm 0.82	12.07 \pm 2.04
	Zona fasciculata	43.40 \pm 3.76	50.94 \pm 6.22
	Zona reticularis	15.44 \pm 0.92	16.26 \pm 1.22
Average cell volume (mm^3)	Zona glomerulosa	678 \pm 52	790 \pm 44
	Zona fasciculata	1240 \pm 60	1460 \pm 38
	Zona reticularis	610 \pm 28	690 \pm 32
Average nuclear volume (mm^3)	Zona glomerulosa	144 \pm 12	168 \pm 22
	Zona fasciculata	128 \pm 10	144 \pm 18
	Zona reticularis	112 \pm 8	122 \pm 12



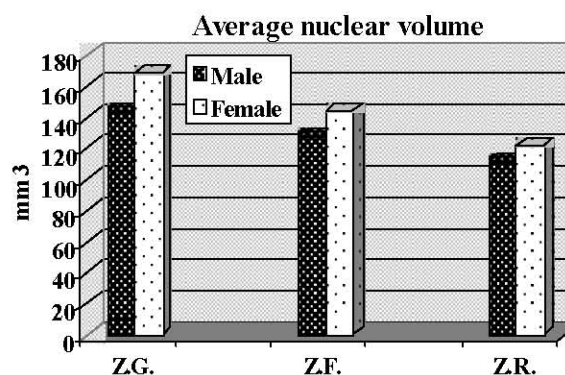
Graph 1: Mean volume of the zona glomerulosa (Z.G.), Zona fasciculata (Z.F.) and zona reticularis (Z.R.) in male and female rabbits.



Graph 3: Mean cell volume of the zona glomerulosa (Z.G.), Zona fasciculata (Z.F.) and zona reticularis (Z.R.) in male and female rabbits.



Graph 2: Relative volume of the zona glomerulosa (Z.G.), Zona fasciculata (Z.F.) and zona reticularis (Z.R.) in male and female rabbits



Graph 4: Mean nuclear volume of the zona glomerulosa (Z.G.), Zona fasciculata (Z.F.) and zona reticularis (Z.R.) in male and female rabbits.

and female rabbits, the adrenal cortex is clearly subdivided into three zones: zona glomerulosa, zona reticularis and zona fasciculata. The cells of the outermost zone (zona glomerulosa) have been arranged in separate arcs in male and female rabbits. It is also important to be noted that the arches of the zona glomerulosa are wider in female rabbits in comparison to males.

Results obtained from the present investigation also showed that the mean volume of the adrenal gland is significantly greater in female rabbits than in males. This difference is due to apparent differences in the volume of the zona glomerulosa, zona reticularis and zona fasciculata which are all significantly greater in females than in males. Malendowicz *et al.* [8] stated that the adrenal glands of mature female rats were heavier than those of males; however they stated that this is almost exclusively due to conspicuous differences in the volume of cells of the zona fasciculata and zona reticularis. They also stated that the volume of the mitochondrial and lipid droplet compartments, as well as the surface area per cell

of mitochondrial cristae and smooth endoplasmic reticulum tubules, were markedly higher in the zona fasciculata and zona reticularis cells of female animals. They suggested that the sexual dimorphism of the rat adrenal cortex may depend upon the inhibitory action of testosterone and the stimulatory effect of estradiol on the hypothalamo-hypophyseal-adrenal axis. Therefore it can be stated that the differences seen between two sexes regarding to volume of the abovementioned zones of the adrenal glands in the present investigation may be due to the different physiological effects of steroidal sex hormones on histomorphological properties of the gland. The adrenal glands of the female rats are also larger than those of the male rats but the reverse is true in the hamster [9]. Dohm *et al.* [10] also stated that the adrenal cortex of the female rats is consistently larger than that of the male. The zona reticularis of the normal female hamster is much less well developed than in the male; its cells are smaller and the zone atrophied [11-13].

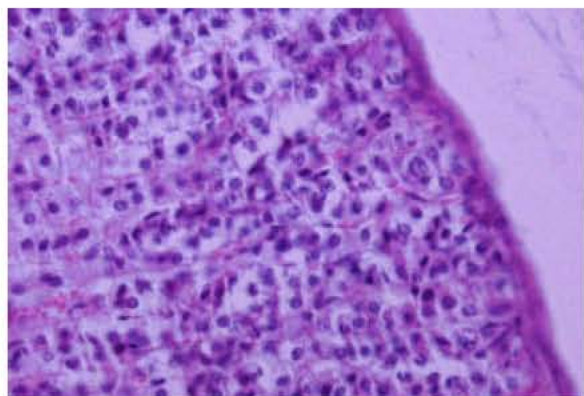


Fig. 1: Light photomicrograph of the zona glomerulosa in male rabbits. (H&E, $\times 200$).

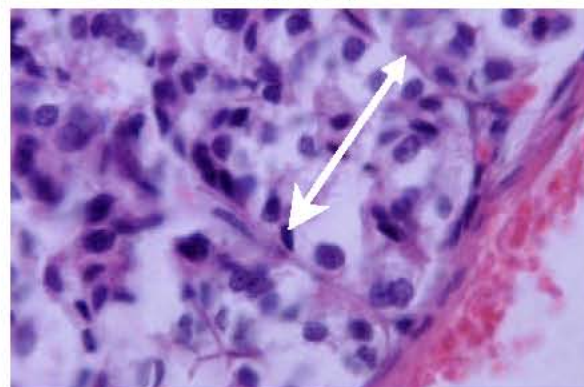


Fig. 2: Light photomicrograph of the zona glomerulosa in male rabbits. Note that the cells of this zone have an apparent arcuate arrangement (two headed arrow); (H&E, $\times 400$).

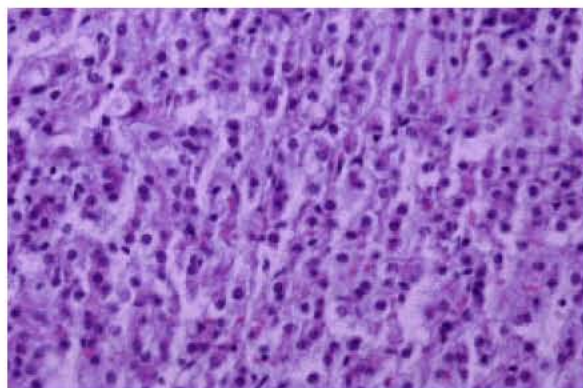


Fig. 3: Light photomicrograph of the zona fasciculata in female rabbits. (H&E, $\times 200$).

Data obtained from the present investigation also showed that in all three histological regions of the gland, the average cell volume is also significantly greater in female rabbits in comparison to those in males. Stereological studies on the adrenal cortex of unilaterally

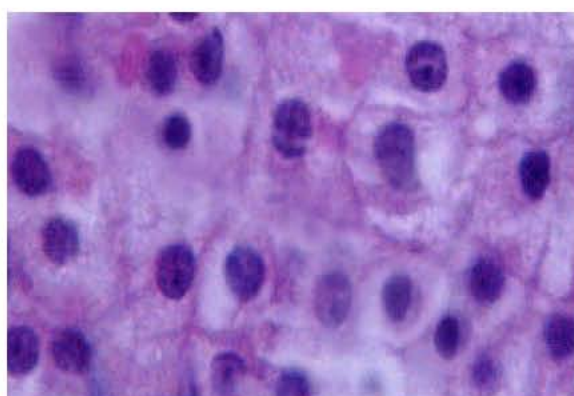


Fig. 4: Light photomicrograph of the zona fasciculata in female rabbits. Note that this zone is composed of cell cords coursing parallel to one another in radial direction. (H&E, $\times 1000$).

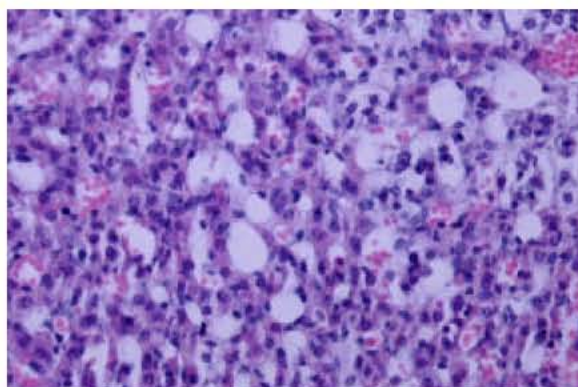


Fig. 5: Light photomicrograph of the zona reticularis in male rabbits. (H&E, $\times 200$).

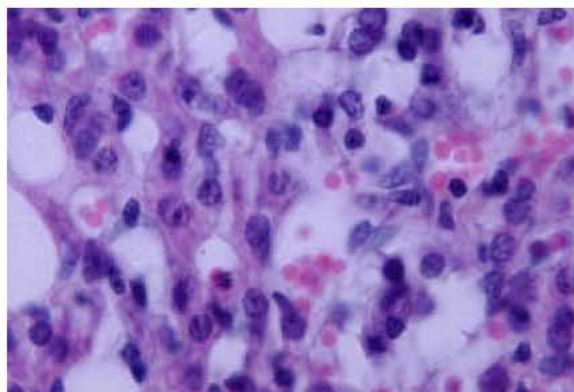


Fig. 6: Light photomicrograph of the zona reticularis in male rabbits. Note that this zone is composed of a net work of cell cords with deeply staining nuclei; (H&E, $\times 400$).

adrenalectomised hamsters have revealed differences in adrenal compensatory hypertrophy in male and female animals. The operation evokes an increase in the average

volume of the zona fasciculata in the male and of the zona reticularis cells in the female [14]. Kasprzak *et al.* [15] stated that mitotic index of the zona reticularis cells is higher in female rats as compared to males; however, there is no difference in the remaining zones of the cortex. They also stated that neither orchidectomy nor testosterone has an effect on mitotic index of the zona glomerulosa and zona fasciculata cells. In the zona reticularis, orchidectomy increases this index, an effect reversed by testosterone. Neither Ovariectomy nor estradiol administration has an effect on mitotic index of the zona glomerulosa nor zona fasciculata, while in the zona reticularis estradiol lowers this index if compared with control and gonadectomised rats. They also added that total number of mitoses per equatorial section of the gland is similar in male and female rats. Orchidectomy increases this value, an effect reversed by testosterone.

Data obtained from present investigation also showed that in all three examined regions of the adrenal cortex, the average nuclear volume is greater in female rabbits in comparison to males, but the differences are not statistically significant.

ACKNOWLEDGMENTS

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