



## RESEARCH ARTICLE

### Histomorphometrical Investigations on the Heart, Kidneys and Adrenal Glands in Normal Teddy Goats (*Capra hircus*) Using Image Analysis System

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#### ABSTRACT

This study was conducted to elucidate the effects of age and sex on the histometrical values of blood pressure related organs including heart, kidneys and adrenal glands in teddy goats. The hearts, kidneys and the adrenal glands of 36 teddy goats, divided in three age-groups; kids (6-12 months), adults (13-21 months) and adults (22-24 months), were processed for histometrical studies. Mean diameters, volumes and Intramural connective tissue contents of right atrium, left atrium, right ventricle and left ventricle were recorded from cardiac specimens. Age affected none of the parameters of the heart significantly. Mean diameters and volumes of subcapsular and juxtamedullary glomeruli were recorded from kidney specimens. All parameters of the kidney invariably showed a consistent rise with advancing age. Thicknesses of the *zona glomerulosa*, *zona fasciculata* and *zona reticularis* of the right adrenal gland were measured. Thickness of different zones of the adrenal cortex showed significant ( $P < 0.05$ ) changes with age. It is conceivable from these findings that the development of heart, kidneys and adrenal showed an increase parallel to the advancing age to adjust with the increasing blood pressure due to physiological development process. Sex, however, played a secondary role.

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#### INTRODUCTION

Cardiovascular diseases are the main cause of mortality and morbidity all over the world and remain the focus of intense research efforts (Pourjafar *et al.*, 2011; Bano *et al.*, 2011; Raziq *et al.*, 2012). There is a clear relationship between cardiovascular diseases and chronic kidney disease (Eskridge, 2010). The renin-angiotensinaldosterone system (RAAS) is a major endocrine/paracrine system that regulates blood pressure via angiotensin release and fluid and electrolyte homeostasis via aldosterone release (Cohn, 2007; Rao, 2010). Recently it was discovered that local synthesis of all the RAAS components occurs in target organs and their tissues (the heart, kidneys, vessels, brain tissues) (Shestakova, 2011). Role of *zona glomerulosa* through elevated concentrations of aldosterone are associated with several cardiovascular diseases (Funder and Reincke, 2010; Kopf, 2011) and have been shown to elevate blood pressure, cause left ventricular hypertrophy, and promote cardiac fibrosis (Nappi and Sieg, 2011).

Quantitative morphological data on the blood pressure and blood volume related organs i.e., heart, kidney and adrenal glands is assumed critical for the better understanding of etiology and pathogenesis of heart related diseases. Hence, several studies have been undertaken on the morphological and histological aspects in various livestock species found in western countries. Continuing to this effort the Department of Anatomy, University of Agriculture, Faisalabad, Pakistan has carried out some research work in indigenous species, like buffaloes (Hussain and Qureshi, 2007; Qureshi and Hussain, 2007) and camels (Rehan and Qureshi, 2007). Now, the present study has been particularly focused on the histomorphometrical evaluation of selected inner organs in normal teddy goats with special consideration of sex and progressive age.

#### MATERIALS AND METHODS

A total of 36 clinically healthy teddy goats (*Capra hircus*) of either sex (18 males, 18 females) comprising of

three age groups of equal size viz, kids (6-9 months), adults (13-21 months) and old (22-24 months) were used in this study. The age of animals was determined by dentition. Samples of the heart with pericardium, the left and right kidneys and the left and right adrenal glands along with fat tissue were collected from each animal immediately after slaughter during winter season at the Faisalabad abattoir.

For the microscopic investigation, four 1-2 cm<sup>3</sup> cubes of heart from the right and left ventricular wall and dorsal walls of free right and the left atria were collected. The kidney samples were taken from the mid of the dorsal surface. Likewise, a cross section from an entire adrenal gland mass was collected.

The specimens were marked and fixed in neutral buffered formaldehyde solution within half an hour after slaughter of animals. Some cardiac muscle samples were cut in cross-section to ensure the sectioning of heart muscles at right angles (90°) while others as longitudinal sections to ensure the presentation of muscle cells in longitudinal orientation. The fixed tissues were processed by the Paraffin tissue preparation technique, as described earlier (Bancroft and Gamble, 2008; Shaheen and Akhtar, 2011). These sections were stained with hematoxylin and eosin for morphometric studies.

The microscopic slides of the heart, kidney and adrenal gland were observed at 400x (objective 40x10) for the measurement of cardiac myocyte nuclei, renal glomerular diameter and nuclei of cells of *zona glomerulosa* of the adrenal gland. The measurement from the slides of heart, kidney and adrenal glands were taken by the direct image of the slides by the digital image analysis system. For this purpose image analysis software LUCIA® version 5.0 was used (Laboratory imaging Ltd., Czech Republic).

The measurement of diffusely distributed intramyocardial connective tissue content was measured at 400x magnifications following the point count procedure described earlier (Okada *et al.*, 1988). The count of points, which were present in the connective tissue, was calculated and hence the percentage of connective tissue was determined. All computations were done with the help of a digital image analysis system. Attempt was made to avoid perivascular connective tissue, vacuoles and artifacts etc. In each section analyzed, at least 400 points were counted in one slide. Four slides were prepared from each specimen.

One hundred cell nuclei were randomly measured in each hematoxylin and eosin (H&E) stained slide of heart muscle. For each cell nucleus, length of the longest axis (long cell nucleus diameter), length of its vertical axis (short cell nucleus diameter) were used to work out the volume of nucleus using the following formula as described formerly (Spieler, 1995):

$$V = \pi / 6 a * b^2$$

Where V= volume; a = Short diameter; b = Long diameter. The volume of cardiac myocyte nuclei was measured in  $\mu\text{m}^3$ .

Tissue sections were taken from the cortical and juxtamedullary zones of each kidney for analysis. At least 10 glomeruli were measured in three to four H&E stained slides of each kidney and their mean values were considered for respective animal. Volume and area of the each renal glomerulus were worked out using the following formula as described earlier (Zolnai and Palkovits, 1965):

$$\text{Volume} = \pi / 6 (a * b)^{1.5}$$

$$\text{Area} = a \times b$$

Results thus obtained were expressed in  $\mu\text{m}^3 \times 10^3$ .

Three different H&E stained sections from each sample of adrenal gland were used to measure the thickness of different layers of the adrenal cortex using an ocular and stage micrometer. The results were expressed in  $\mu\text{m}$ . The length and width of each cell nuclei of *zona glomerulosa* of adrenal gland was measured with the help of digital image analysis system. The volume was calculated using the same method as described earlier for the volume of cardiac myocyte nuclei.

**Statistical analysis:** Means ( $\pm$ SEM) were calculated for each parameter in two sexes and three age groups. The means of parameters of heart, kidney and adrenal glands were compared by analysis of variance. Duncan's multiple range (DMR) test was performed for multiple mean comparisons, where necessary. Statistical analysis was performed by using the statistical computer software Minitab (Mtb16) and MSTAT-C. Significance was measured at the 5 percent level.

## RESULTS

Mean $\pm$ SEM of various microscopic anatomical parameters of heart, kidneys and adrenals in both sexes and three age groups of teddy goat (*Capra hircus*) are depicted in Table 1-3. Specimens of histological images subjected to morphometric analysis are presented in Fig. 1, 2 and 3. Statistical analysis revealed that sex specific differences were not significant in any parameters of heart, kidney and adrenals in present study (Table 1, 2 and 3).

Age affected none of the parameters of the heart significantly (Table 1). All parameters of the kidney invariably showed a consistent rise with advancing age. However, in some parameters namely, subcapsular glomerular volume in the left kidney and juxtamedullary diameter of the right kidney these differences were not statistically different. Duncan's multiple range (DMR) test further revealed that the values of all parameters of the kidneys were significantly ( $P < 0.05$ ) higher in old (22-24 m) goats as compared with kids (6-9 m) and adults (13-21 m).

Thickness of different zones of the adrenal cortex showed significant changes with age. Kids (6-9 months old) showed maximum thickness ( $\mu\text{m}$ ) of the *zona glomerulosa* ( $191.36 \pm 14.76$ ) which reduced significantly ( $P < 0.05$ ) in adults ( $136.33 \pm 9.82$ ) but gained a significant increase through old (22-24 months) age ( $157.79 \pm 7.34$ ) although it still remained lower than the kids. The *zona fasciculata* and the *zona reticularis* showed significant increases in thickness in old goats as compared with the kids and adult goats. Nuclear diameter of the *zona glomerulosa* and the volume of nuclei of *zona glomerulosa* cells showed significant ( $P < 0.05$ ) increases with age (Table 3).

## DISCUSSION

Cardiac ventricular weight is a variable of particular interest because it has been shown in human epidemiological studies that cardiac ventricular mass is one of the most important independent predictors of cardiovascular mortality and morbidity (Levy *et al.*, 1990; De Simone *et al.*, 2002). Mean diameters and volumes of nuclei of cardiomyocytes of atria and ventricles were not different with respect to age and sex (Table 1). The volumes of cardiomyocyte nuclei

**Table 1:** Mean±SEM of histometrical parameters of heart in both sexes and three different age groups of Teddy goat (*Capra hircus*)

Cardiac chamber	Parameters	Overall Mean	Male	Female	Kids (6-9 M)	Adults (13-21 M)	Old (22-24 M)
Right atrium	1	6.59±0.1	6.57±0.2	6.61±0.21	6.75±0.23	6.5±0.2	6.52±0.3
	2	167.89±10.7	161.94±11.9	173.83±18.1	172.72±17.8	158.16±12.3	72.79±24.9
Left atrium	1	7.02±0.14	7.05±0.19	6.99±0.2	7.2±0.28	6.85±0.2	7.01±0.3
	2	210.18±13.6	216.89±17.9	203.46±20.9	225.93±25.9	193.5±17.55	211.09±27.3
Right ventricle	1	6.8±0.12	6.73±0.1	6.9±0.21	6.75±0.2	6.99±0.3	6.7±0.2
	2	186.85±9.97	178.2±10.77	195.49±16.8	172.49±13.99	206.31±21.3	53.89±15.56
Left ventricle	1	5.92±0.1	5.78±0.23	5.96±0.2	5.77±0.3	5.84±0.3	6.01±0.2
	2	127.93±8.5	127.62±11.5	128.24±12.8	133.51±17.1	122.67±16.2	127.61±11.2
Right atrium	3	6.06±0.2	6.03±0.3	6.09±0.4	6.08±0.2	6.24±0.5	5.85±0.5
Left atrium	3	6.38±0.3	6.4±0.4	6.36±0.4	6.48±0.4	6.57±0.5	6.09±0.5
Right ventricle	3	6.21±0.3	6.44±0.4	5.96±0.4	5.92±0.5	6.75±0.5	5.94±0.6
Left ventricle	3	6.03±0.2	6.1±0.2	6.09±0.4	5.9±0.4	6.17±0.4	6.03±0.3

1=Mean diameter of cardiomyocyte nuclei (µm); 2=Mean volume of cardiomyocyte nuclei (µm<sup>3</sup>); 3=Intramural Connective Tissue (%).

**Table 2:** Mean±SEM of histometrical parameters of right and left kidneys in both sexes and three different age groups of Teddy goat (*Capra hircus*)

Parameters	Side	Overall mean	Male	Female	Kids (6-9 M)	Adults (13-21 M)	Old (22-24 M)
MSG diameter (µm)	Rt	91.0±1.8	88.3±2.1	93.2±2.9	86.1±3.1 <sup>a</sup>	91.8±3.0 <sup>ab</sup>	96.7±2.7 <sup>b</sup>
MSG surface area (µm <sup>2</sup> ×10 <sup>3</sup> )		7.72±0.4	7.25±0.5	8.19±0.5	5.67±0.5 <sup>c</sup>	8.01±0.52 <sup>b</sup>	9.48±0.4 <sup>a</sup>
MSG volume (µm <sup>3</sup> ×10 <sup>3</sup> )		387.2±24.75	352.61±31.61	421.8±37.2	269.49±29.5 <sup>c</sup>	390.96±39.8 <sup>b</sup>	501.15±30.8 <sup>a</sup>
MSG diameter (µm)	Lt	95.56±2.1	94.35±3.4	96.98±2.3	89.79±2.6 <sup>b</sup>	94.37±2.9 <sup>b</sup>	104.06±3.7 <sup>a</sup>
MSG surface area (µm <sup>2</sup> ×10 <sup>3</sup> )		8.44±0.5	8.28±0.8	8.6±0.4	6.97±0.6 <sup>b</sup>	7.45±0.3 <sup>b</sup>	10.9±0.8 <sup>a</sup>
MSG volume (µm <sup>3</sup> ×10 <sup>3</sup> )		439.95±35.6	421.9±66.4	457.1±27.5	344.9±21.4 <sup>b</sup>	357.4±41.7 <sup>b</sup>	617.53±74.6 <sup>b</sup>
MJG diameter (µm)	Rt	100.37±2.3	97.93±3.3	102.8±3	94.68±3 <sup>a</sup>	101.92±3.08 <sup>ab</sup>	105.6±4.2 <sup>b</sup>
MJG surface area (µm <sup>2</sup> ×10 <sup>3</sup> )		9.72±0.5	9.38±0.8	10.06±0.7	7.62±0.7 <sup>b</sup>	10.08±0.8 <sup>b</sup>	11.46±0.8 <sup>a</sup>
MJG volume (µm <sup>3</sup> ×10 <sup>3</sup> )		537.8±39.9	497.1±63.7	578.54±47.9	433.18±48.2 <sup>b</sup>	477.79±65.6 <sup>b</sup>	702.5±68.9 <sup>a</sup>
MJG diameter of (µm)	Lt	102.33±2.2	99.33±3.8	105.27±2.2	94.69±2.7 <sup>a</sup>	100.18±3 <sup>ab</sup>	111.82±3.6 <sup>b</sup>
MJG surface area (µm <sup>2</sup> ×10 <sup>3</sup> )		9.72±0.5	9.24±0.9	10.2±0.5	8.17±0.8 <sup>a</sup>	8.41±0.5 <sup>ab</sup>	12.57±0.9 <sup>b</sup>
MJG volume (µm <sup>3</sup> ×10 <sup>3</sup> )		571.89±42.1	523.23±75.3	618.55±37.2	430.47±38.7 <sup>a</sup>	504.26±52.5 <sup>ab</sup>	780.93±80.6 <sup>b</sup>

MSG=Mean subcapsular glomerular; MJ= Mean juxtamedullary glomerular; Rt= Right; Lt=Left.

**Table 3:** Mean±SEM of histometrical parameters of adrenal glands in both sexes and three different age groups of Teddy goat (*Capra hircus*)

Parameter	Site	Side	Overall mean	Male	Female	Kids (6-9 M)	Adults (13-21M)	Old (22-24M)
T	<i>Zona glomerulosa</i>	Rt	161.8±7.3	159.5±9.2	164.1±11.6	191.7±14.8	136.3±9.8	157.8±7.3
T	<i>Zona fasciculata</i>		756.53±24.1	757.29±37.1	757.77±32	627.18±22.2 <sup>b</sup>	789.73±36.7 <sup>a</sup>	852.69±35.5 <sup>a</sup>
T	<i>Zona reticularis</i>		355.27±21.9	379.11±28.2	331.43±33.4	259.43±22.81 <sup>c</sup>	346.51±37.1 <sup>b</sup>	459.86±29.1 <sup>a</sup>
T	<i>Zona glomerulosa</i>	Lt	166.76±31.7	171.4±8.9	162.11±5.7	188.3±8.4 <sup>a</sup>	142.47±7.6 <sup>c</sup>	169.5±6.4 <sup>b</sup>
T	<i>Zona fasciculata</i>		734.3±27.7	691.28±31.6	777.32±44.1	673.39±42.4 <sup>b</sup>	732.64±60.8 <sup>b</sup>	796.27±34.0 <sup>a</sup>
T	<i>Zona reticularis</i>		353.38±19.4	378.23±32.9	328.52±19.7	269.84±12.9 <sup>b</sup>	333.56±28.1 <sup>b</sup>	456.72±31.4 <sup>a</sup>
D	<i>Zona glomerulosa</i>	Rt and Lt	4.67±0.3	4.59±0.3	4.73±0.3	4.53±0.4 <sup>b</sup>	4.76±0.3 <sup>b</sup>	5.46±0.4 <sup>a</sup>
V	<i>Zona glomerulosa</i>	Rt and Lt	86.5±14.9	85.49±15.3	88.67±13.8	78.31±12.9 <sup>a</sup>	86.13±14.7 <sup>b</sup>	90.21±15.8 <sup>a</sup>

T= Thickness (µm); D= Diameter of nuclei (µm); V=Volume of nuclei (µm<sup>3</sup>); Rt= Right; Lt=Left.

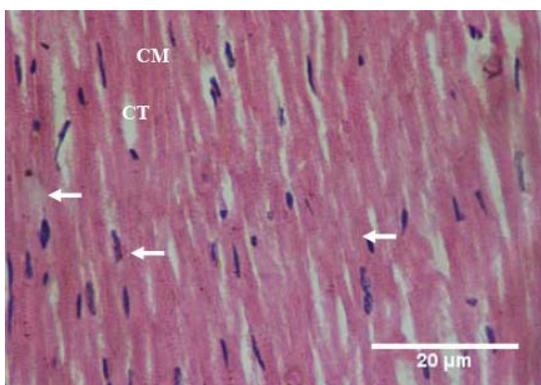
of various chambers of heart in the cattle heifers reported earlier (Tiwari and Swarup, 1977; Spieler, 1995) were lower than in the present findings. This suggests a species difference in the size of cardiomyocytic nuclei. No literature could be found regarding this parameter in goats.

Mean intramural connective tissue percentage of atria and ventricles in teddy goats are in general conformation with earlier findings (Spieler, 1995), in different age groups including kids, adults and old groups of teddy goats. Age induced a statistically significant (P<0.05) rise in subcapsular and juxtamedullary glomerular diameter, surface area and volume in the right and left kidneys. However, relatively higher values of subcapsular glomerular diameter (127 µm) and juxtamedullary glomeruli (126 µm) were reported in goats (Zolnai and Palkovits, 1965). The lower values of glomerular diameter can be attributed to the small body size of the teddy goat. A mean subcapsular glomerular diameter of 162 µm and a juxtamedullary glomerular diameter of 142 µm were reported earlier in adult buffaloes (Tiwari and Swarup, 1977). Mean subcapsular and juxtamedullary glomerular diameters in two species of cattle were reported as 197 and 175 µm in *Bos indicus* and 194 and 201 µm in *Bos taurus*, respectively (Mbassa, 1988). Present

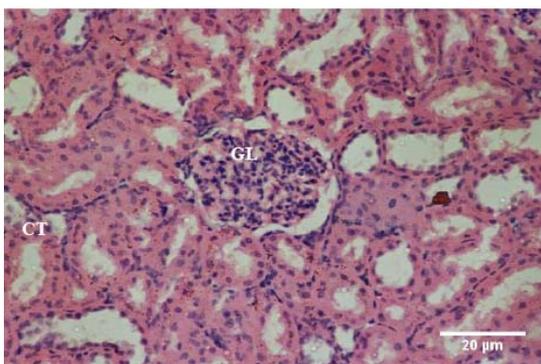
findings were significantly (P<0.05) lower than the values of subcapsular glomerular diameter in the right and left kidneys of young one-humped camels (114.01±2.35 and 117.4±2.33µm, respectively) (Rehan and Qureshi, 2007). Likewise, higher mean volumes of glomeruli of kidneys in young and adult buffaloes were documented earlier (Hussain and Qureshi, 2007) as compared to the present study. Significantly (P<0.05) higher values of subcapsular glomerular volume (1020.78±65.9 µm<sup>3</sup> × 10<sup>3</sup> and 1635.14±94.3 µm<sup>3</sup> × 10<sup>3</sup>) were reported earlier in the right and left kidneys of young one humped camels (Rehan and Qureshi, 2007).

Significantly higher values of subcapsular and juxtamedullary glomerular surface area (11.86±0.48 and 7.64±0.28µm<sup>2</sup> × 10<sup>3</sup>) were reported formerly in camel calves (Rehan and Qureshi, 2007) as compared to teddy goats. The reduced size of glomeruli in teddy goats may be due to their smaller size than other domestic animals.

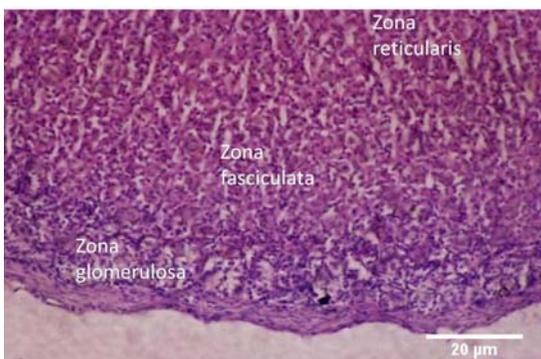
The diameter, volume and surface area of the subcapsular and juxtamedullary glomeruli increased significantly (P<0.05) with advancing age, while minimal influence of the sex was observed upon these parameters.



**Fig. 1:** Photomicrograph of atrial muscles of teddy goat showing the cardiomyocytes (CM), cardiomyocytic nuclei (arrows) and connective tissue contents (CT); Stain H&E. × 200



**Fig. 2:** Photomicrograph of kidney of teddy goat showing the juxtamedullary glomerulus (GL) and convoluted tubules (CT); Stain H&E. × 100



**Fig. 3:** Photomicrograph of adrenal cortex of teddy goat showing the different zones; Stain H&E. × 100

These findings are in accordance with earlier studies in teddy goats (Shah *et al.*, 2010), rats (Deschepper *et al.*, 2004), cattle heifers (Mischke, 1997) and roe-deers (Genschow, 1997). According to Deschepper *et al.* (2004) differences observed in kidney cortices were due to an increase of the mesangial compartment (with increased mesangial cellularity and mesangial matrix), or enlarged juxtglomerular apparatus. This increased mass of the kidney tissue may account for better filtration in the adult goat.

Overall mean thickness of *zona glomerulosa* ( $161.83 \pm 7.3$ ; 12.7%), *zona fasciculata* ( $756.53 \pm 24.14$ ;

$59.40\%$ ) and *zona reticularis* ( $355.27 \pm 21.89$ ; 27.90%) of adrenal glands were recorded on the left and right sides. Sex had no significant effect on these parameters whereas there was a consistent increase in percentage with age. The increase in thickness of zones with age contributes to the increased weight and dimensions of adrenal glands as reported earlier (Shah *et al.*, 2010). These results are in line with the previous findings regarding the *zona glomerulosa* and *zona fasciculata* in sheep (Tiwari and Swarup, 1977; Deschepper, *et al.*, 2004). According to Akana *et al.* (1983) the trophic effect ACTH on the adrenal fasciculate might be the strongest determinant of changes in adrenal weight.

Mean values of diameters and volumes of nuclei of *zona glomerulosa* cells of right and left adrenal glands of teddy goat are depicted in Table 3. Higher values of mean diameter ( $7.3 \pm 0.08$  and  $7.17 \pm 0.1$  μm) and volume ( $93.83 \pm 23.24$  and  $88.68 \pm 28.01$  μm<sup>3</sup>) of nuclei of *zona glomerulosa* cells of right and left adrenal glands were reported earlier in camel calves (Rehan and Qureshi, 2007). Sex had no significant effect on these two parameters. However, age induced a consistent rise in these parameters. A comparable trend with advancing age (Tiwari and Swarup, 1977) was recorded in sheep and buffaloes (Hussain and Qureshi, 2007).

It is conceivable from these findings that development of heart, kidneys and adrenals, morphologically as well as histologically, showed an increase parallel to advancing age to adjust with the increasing blood pressure and filtration rate due to physiological development processes.

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