International Journal of Innovation and Applied Studies
ISSN 2028-9324 Vol. 7 No. 3 Aug. 2014, pp. 920-928
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http://www.ijias.issr-journals.org/

# SEX-DEPENDENT KIDNEY MORPHOLOGY OF GUINEA PIG – LIGHT MICROSCOPY AND IMMUNOHISTOCHEMICAL STUDY

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**ABSTRACT:** The objective of this study was to investigate the sex-dependent kidney morphology of male and female guinea pigs using light microscope and different immunohistochemistry techniques. Hamatoxyline and Eosin (H&E) and Periodic Acid Schiff (PAS) staining techniques were performed for the general histological observations. Immunohistrochemistry was performed to detect renin positive site, expression of Cyclo-oxygenase -2 (COX-2) and Nitric Oxide Syntheses (nNOS), Number of renin, COX-2, and nNOS positive sites were counted and tabulated. Both Light microscopic examination and immuno-histochemical study reveals distinct differences between the male and female kidneys. Parietal layer of Bowman's capsule was consisted of a single layer of simple squamus epithelium in female, but in male, it was composed of a single layer of simple cuboidal epithelium. PAS-positive brush border and vacuoles of different shapes and sizes were appeared in the proximal straight tubules (PST) and collecting ducts in female guinea pig. Whereas in male, vacuoles were not detected in the PST epithelium and collecting ducts. Strong staining intensity for PAS-positive brush border in the PST and collecting ducts was observed in the outer medulla of female guinea pig kidney, but the reaction was observed poor in male. Neural Nitric Oxide Synthase (nNOS)-positive reaction was observed in PST epithelium and collecting ducts in female kidney, but male kidney revealed nNOS-negative reaction for PST and collecting ducts. Renin, COX-2 and nNOS-positive sites were detected in the juxtaglomerular cells (JGCs) cells and macula densa cells of both male and female kidneys. When counted, total numbers of glomeruli, renin, COX-2, and nNOS positive-sites were higher in female, when compared to that of the male. However, number of glomeruli, areas of renin, COX-2 and nNOS-positive sites reveals no significant differences between male and female species ($P<0.05$).

**KEYWORDS:** Sex-dependent, Kidney morphology, Guinea pig.

## INTRODUCTION

It is now well understood that Kidney morphology changes based on the functional status and condition of the organ. A thorough understanding of the complex structure of the mammalian kidney provides a basis for comprehending the multitude of functional characteristics of this organ in both healthy and disease states. Renal structural alterations are closely related to their functional alterations and renal histopathological analysis is indispensable to investigate kidney related diseases as well as kidney failure. To make a correct interpretation of histopathological observations in laboratory experiments, a clear understanding of normal histological features of laboratory animals is very important. In a very recent times mouse has been used frequently and extensively in laboratory experiments, and there have been many reports regarding sexual dimorphism of the mouse kidneys [1, 2, 14]. Morphological feature of kidney differs based on their strain differences also [4]. However, sex-and strain-dependent structural features have not been fully elucidated with the exception of very few reports described previously. The present work has been undertaken to clarify the sex differences of kidney morphology of guenia pigs together with the differences of their expression of three important enzymes, such as renin,

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secreted from the juxtaglomerular cells (JGCs), Cyclooxygenase-2 (COX-2), and Neuronal nitric oxide synthase (nNOS) secreted from the macula densa cells. Renin is secreted from JGCs, is a key enzyme of the renin-angiotensin (RA) system that control blood pressure. Furthermore, renin secretion from JGCs is also regulated by the expression of COX-2 and nNOS from macula densa cells. Any alterations and / or deviations of the functions of these three important enzymes will be the great concern for renal functions. Therefore, the aim of the present study using guinea pig kidney was to assess the sex differences of kidney morphology together with the expression of these 3 (three) important proteins using immunohistochemistry.

## MATERIAL AND METHODS

A total of sixteen (16) guinea pigs of 2 month of age were used in this experiment. They were purchased from Charles River Laboratories, Japan (Crj). The body weight of the animals was ranged from 280-300g. They were acclimatized in our animal laboratory facilities for another 1 month prior to use. The guinea pigs were housed three per one plastic cage, maintained on a 12h light/dark cycle at a constant temperature (70°F ± 2°F) and humidity (35% to 70%), and provided water and rodent Pellets (Oriental Yeast, Japan) ad libitum. The handling of all animals was maintained in accordance with the National Institute of Health (NIH) Guideline for the Care and Use of Laboratory Animals. The Use of animals for this experiment was approved by the Institutional Animal Care and Use Committee (IACUC) of the University of Tokyo, Japan. All the experimental animals anesthetized with sodium pentobarbital (Nembutal; 50mg/kg body weight), and subsequently sacrificed by decapitation. Ventral abdomens was cleaned and disinfected with ethanol. Both right and left kidneys were surgically removed. Central slices including hilum of the kidney were cut perpendicular to the long axis of the organ and preserved in Zamboni solution. The specimens were kept at 4°C for overnight. After several wash with Phosphate buffer solutions (0.1MPB, PH 7.4) the specimens were dehydrated with a graded series of ethanol & embedded in paraffin. The paraffin blocks were cut at 5 µm in thickness, dried at 40°C for overnight. The tissue sections were then stained with Meyer's Hamatoxyline and Eosin (H&E) and Periodic Acid-Schiff (PAS)-Hamatoxyline staining for general morphological studies of the kidney. Immunohistochemical study was conducted for the detection of renin, Cyclooxygenase -2 (COX-2), and Neuronal Nitric Oxide Synthase (nNOS). The materials and methods for immunohistochemical study were followed based on the protocol supplied by the company and according to the report published previously by Yabuki *et al* [5]. A total count of glomeruli for both male & female species was counted by using a light microscope connected with a digital camera (Nikon ECLIPSE E 600) and a computerized monitor (BUFFALO, Japan). Similarly, a total count of localization of renin, Cyclooxygenase -2 (COX-2), and Neuronal Nitric Oxide Synthase (nNOS) - positive site was counted and tabulated. Areas of renin, COX-2 and nNOS positive sites were counted and analyzed statistically by using student's *t* test (Stat View). Separate graphs were prepared for the number of glomeruli, renin, COX-2 and nNOS-positive sites.

## OBJECTIVE OF THE STUDY

- Sex-dependent kidney morphology of guinea pig.
- Using of light Microscopy and immunohistochemical methods.

## RESEARCH METHODS

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## RESULTS

### Light Microscopy

Histological features of glomerular capsule in guinea pig kidney were found different among the male and female species. The parietal layer of the glomerular capsule in male guinea pig kidney consisted of a single layer of simple cuboidal epithelium, whereas it was single layer of simple squamous epithelium in female. Vacuoles of different size and shapes were appeared in the proximal convoluted tubules (PCT) in male, but this histological feature was not observed in female kidney. PAS-positive brush border in proximal straight tubules (PST) and collecting ducts in female reveals stronger staining intensity when compared with that of the male species. PAS-positive granules (small and giant granules) were observed in PST epithelium and collecting ducts in female kidney, but this feature was absent in male. Strong nNOS positive reaction for PST epithelium and collecting ducts was observed in female, but this character was absent in male kidney. A total number of glomeruli, renin, COX-2, nNOS positive sites were comparatively higher in female kidneys then that in male. However, statistical analysis reveals no significantly differences of the areas of renin, nNOS and COX-2 positive sites between the male and female kidneys ($P<0.05$)

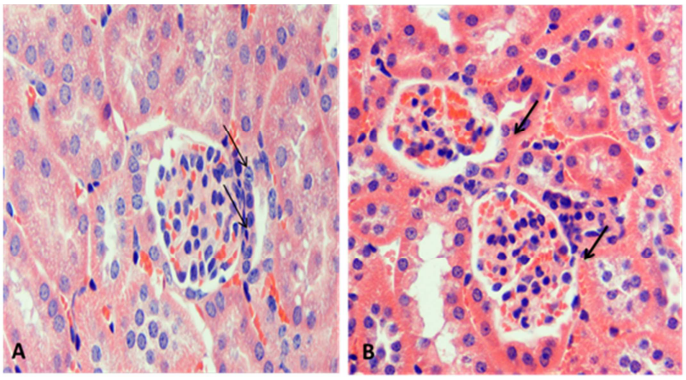



Fig.1 Parietal layer of the glomerular capsule consisted of a single layer of simple cuboidal epithelium in male guinea pig (**Fig.1A,** small arrow), whereas in female guinea pig it was a single layer of simple squamous epithelium (**Fig.1B**,Big arrow). H &E Stain, 20 X.

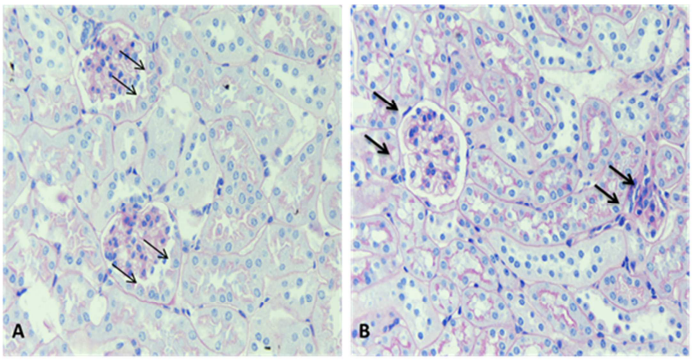



Fig.2 Parietal layer of the glomerular capsule consisted of a single layer of simple cuboidal epithelium in male guinea pig (**Fig. 2A,** small arrow), whereas in female guinea pig it was a single layer of simple squamous epithelium ( **Fig.2B,** big arrow) PAS Stain, 20 X.

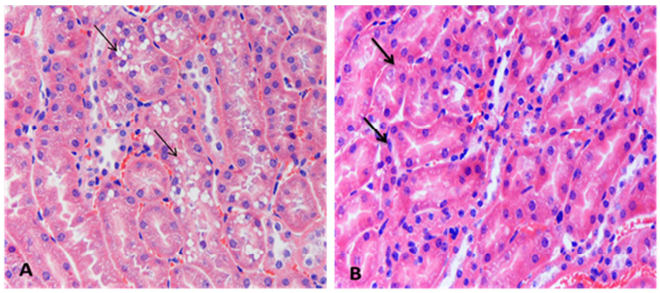



Fig.3 Vacuoles of different Shapes and Sizes appear in the PCT epithelium in male guinea pig kidney (**Fig. 3A,** Small arrow), but this histological feature was absent in females (**Fig. 3B,** big arrow), H&E stain, 20 X.

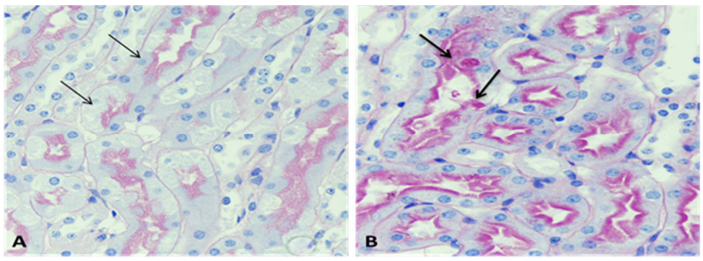



Fig. 5 PAS positive brush border appears in the PST epithelium in male (**Fig.5A,** small arrow). Strong reaction of for-positive brush border & small and giant granules were observed in the PST epithelium in female Guinea pig kidney (**Fig.5B,** big arrow). PAS stain, 20X.

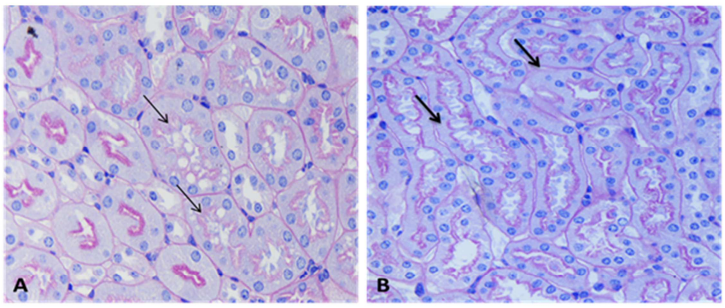



Fig.4 Vacuoles of different shapes and sizes appear in the PCT epithelium in male guinea pig kidney (**Fig. 4A**, Small arrow), but this histological feature was absent in females (**Fig. 4B,** big arrow). PAS Stain, 20 X.

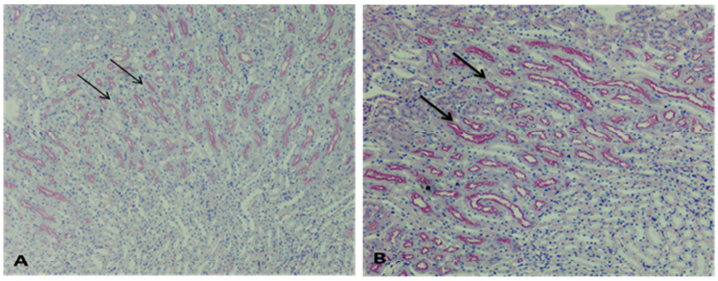



Fig.6 PAS positive brush border of PST epithelium in the outer medulla was observed in male (**Fig.6A** small arrows). Stronger staining reaction for PAS positive brush border of PST epithelium was prominent in the outer medulla of female guinea pig kidney (**Fig 6B,** big arrows). PAS stain; 20 X.

**Immunohistochemistry**

Male guinea pig kidney revealed strong staining intensity for renin-positive site in the juxtaglomerular area. Although female kidney showed renini-positive site in the juxtaglomerular area, the reaction was comparatively weak. In male guinea pig kidney, nNOS-positive reaction for juxtaglumerular area was distinctly observed, but female kidney revealed comparatively weak reaction. Female guinea pig kidney showed strong reaction for nNOS–positive site in the PST epithelium and cell lining in the collecting duct, but male guinea pig kidney showed nNOS –negative reaction. Although both male and female guinea pig kidney shows COX-2-positive reaction in macula densa area but in female guinea pig kidney, this reaction was observed comparatively weak.

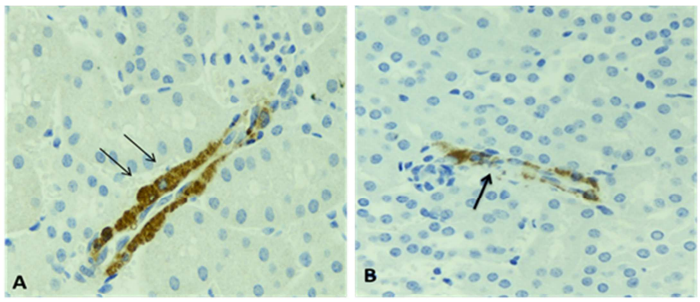



Fig7. Strong reaction for renin-positive site in the juxtaglomerular area was observed in Male guinea pig kidney ( **Fig. 7A** Small arrow). In female guinea pig kidney, reaction for renin-positive site was not prominent (**Fig. 7B**, Big arrow) .

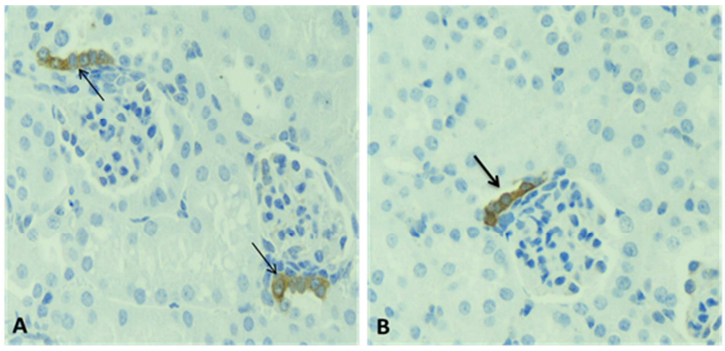



Fig 8. In male guinea pig kidney, nNOS - positive reaction was distinctly observed (**Fig. 8A** small arrow) where as in female guinea pig kidney, nNOS-positive reaction was not distinct (**Fig. 8B** big arrow).

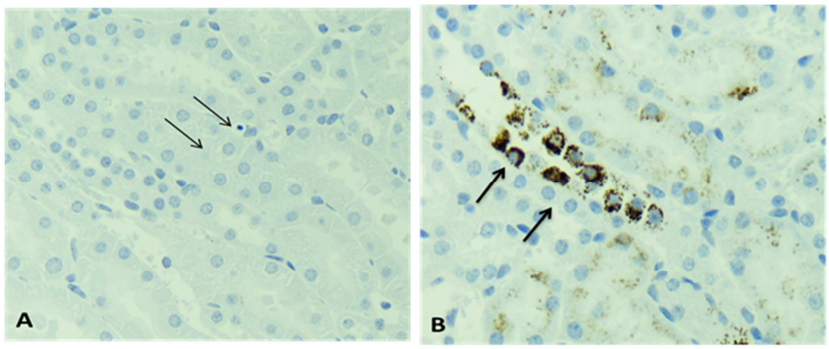
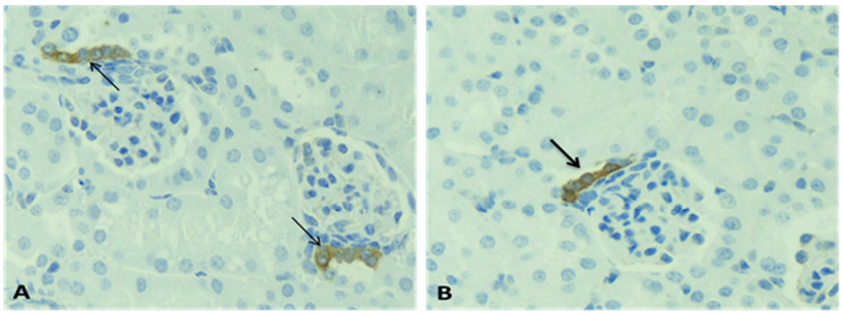



Fig. 9 In male guinea pig kidney shows no reaction for nNOS–positive site in PST epithelium and collecting ducts (**Fig. 9A** small arrow) but female guinea pig kidney shows strong reaction for PST epithelium and collecting ducts (**Fig. 9B** big Arrow).

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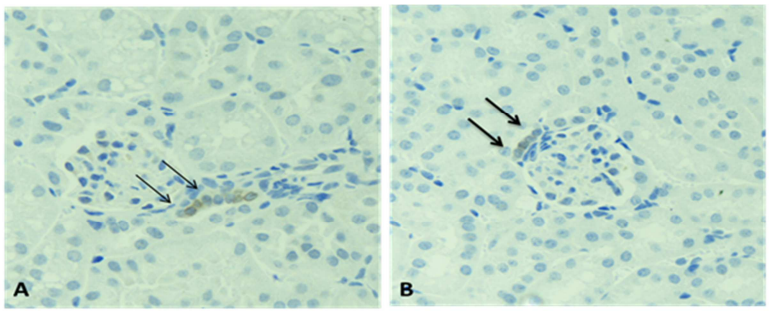


Fig. 10 Both male and female guinea pig kidney shows the COX-2 –positive reaction in mecula densa area **( Fig 10A & 10B**). In male, COX-2 positive reaction in mecula densa area was observed prominent (**Fig, 10A**, small arrow) but in female, Cox-2 positive reaction was poorly detected ( **Fig. 10B** big arrow).

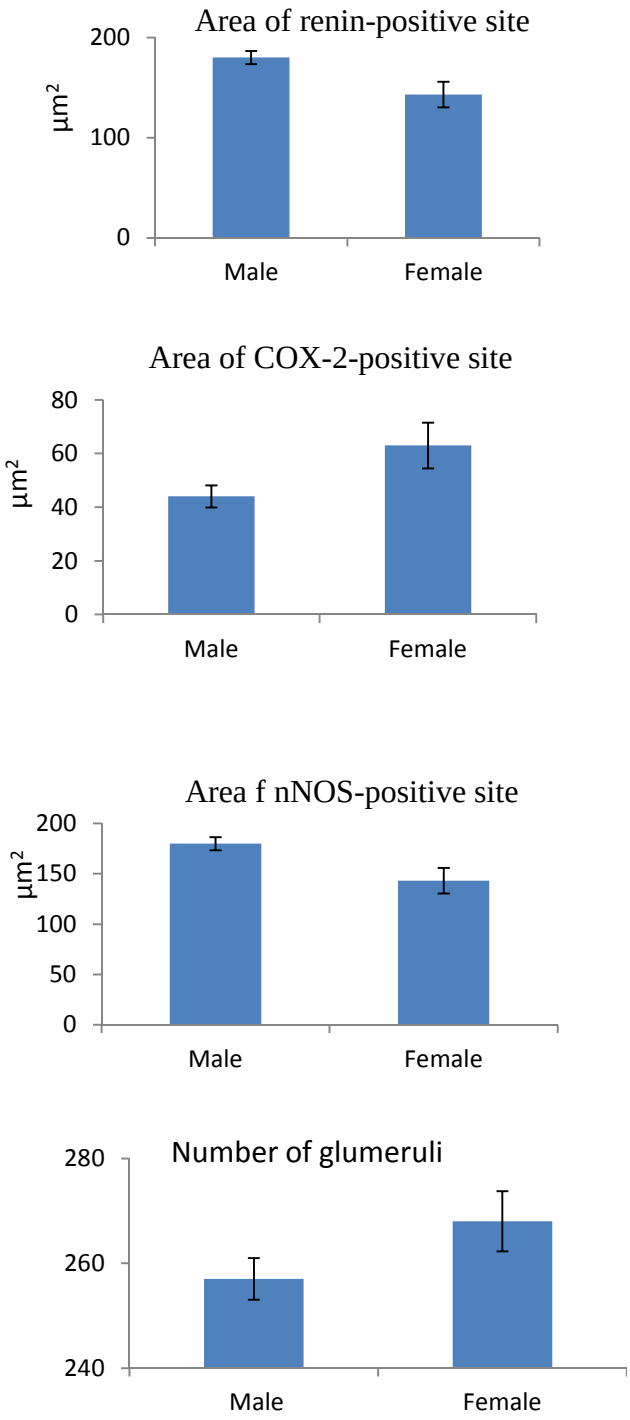


***Fig.11. Expression of Renin, COX-2, and nNOS-positive sites, Number of glomeruli was observed during light microscopy and immunohistochemical studies, but when the data was analyzed statistically the differences were not significant between male and female guinea pig kidneys (P > 0.05, Fig.11).***

## DISCUSSION

Sex- and strain-dependent differences in the morphology of glomerular capsule of have been described in different laboratory animals **[1, 2, 3, 4].** The male and female guinea pig kidney also revealed sex-dependent kidney morphology in the present investigation. Parietal layer of the glomerular capsule in male guinea pigs consisted of a single layer of simple cuboidal epithelium, whereas it was single layer of simple squamous epithelium in female guinea pig kidney. Sex - and strain-dependent morphology of the glomerular capsule was observed in DBA/2Cr mouse **[7]** and was consistent in our present study using guinea pig kidney. Vacuoles of different sizes and shapes were appeared in the proximal convoluted tubules (PCT) in male, but were not observed in female species. These vacuoles were not appeared with PAS or HE stains in our observation and were very much similar with the observation described by Yabuki *et al.* [3]. They have reported that were light microscopic morphological characters of the male DBA/2Cr mouse kidney. They also mentioned that these structures were appeared as electron-dense multilamellar bodies and have been cytochemically identified as lysosomes. Lysosome has been demonstrated in the PCT of ICR, BALB/c, C57BL/6, C3H/Hen, DBA/2 mice, and Wistar rat, kidney **[ 8, 1, 9, 6, 2, 10, 4].** They also mentioned that these vacuolar structures were especially remarkable in male DBA/2 mice and in consistent with our present observation in male guinea pigs kidney. Recent reports indicated that the renal proximal tubule plays an important role in the catabolism of low-density lipoprotein (LDL) and LDL receptor family is distributed throughout the renal proximal tubules [**11, 12, 13, 14].** It is assumed that, detection of vacuolar structures in PCT epithelium of male kidney which was previously confirmed ultra-structurally as lysosomes [15] is directly related with the LDL metabolism. Renal structures resembling vacuolar structures of DBA/2 mice were reported in cat [16] and mastomys kidneys [17]. When stained with Periodic Acid Schiff Reagent (PAS), the brush border of the proximal straight tubules, and collecting ducts reveals stronger staining intensity than that of male, and this histological characters are very much similar with the findings of Yabuki *et al*.(2003) in DBA/2Cr mouse kidney [**7].** In our present study, female guinea pig kidney reveals PAS-positive granules of different sizes (small and giant granules) in PST epithelium and collecting ducts, but this cytological feature was found absent in male. These PAS-positive granules in the PST of female mouse kidneys were first reported in the ICR strain and these granules were observed prominent in female [10] . Moreover, the number and size of these granules were subsequently found to differ based on the strains [4]. Strong Nitric Oxide Synthase (nNOS) Positive reaction for PST epithelium and collecting ducts was observed in female guinea pigs, but this character was absent in male and was consistent with the observation reported by Yabuki *et al*.2003 in DBA/2 [7] and DBA/1 mouse kidney. An increased number of glomeruli, renin, COX-2, nNOS positive sites were observed in DBA/2CrSl female guinea pigs when compared with that of the male, but when analyzed statistically the differences were not significant ($P<0.05$). Sex-and – strains dependent expression of rennin, COX-2, and nNOS was reported by many authors using many of laboratory animals **[8, 1, 9, 6, 2, 10, 4]** and their findings were very much similar to our present study.

## CONCLUSION

Sex-dependent histological features of the guinea pigs kidney have been observed. The reasons for nNOS positive reactions for the PST epithelium and collective ducts have not yet been clearly understood. A further study is needed to elucidate the reasons for nNOS positive PST and collecting ducts for female species. Guinea pigs revealed renin, COX-2, nNOS positive reactions in the present study & suggesting that this species can be experimentally used in the laboratory for explaining kidney functions and its related pathological studies.

## ACKNOWLEDGEMENTS

The skillful and cordial cooperation of Dr. Akira Yabuki, laboratory of Veterinary Anatomy, Kagoshima University, Japan has gratefully acknowledged. This work was supported in part by Grants-in Aid from the Ministry of Education, Science, Sports and culture in Japan.

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