

Faculty of Veterinary Medicine
Dept. of Anatomy & Histology
Head of the Dept.: Prof. Dr. Aziza Abdel Aziz

**SCANNING ELECTRON MICROSCOPICAL STUDY
OF THE VASCULAR ARCHITECTURE OF THE
ADRENAL GLANDS IN DONKEY
(With 4 Figures)**

By
**M.M. ABD-ELNAEIM; A.E. ZAYED
and A.O. SALEM**

(Received at 29/12/1998)

دراسة بالماسح الالكترونى على التركيب الوعائى للغدة الكظرية فى الحمار

محمود محمد عبد النعيم ، أحمد الزهرى زايد
أحمد عمر سالم

أجرى هذا البحث بغرض دراسة التركيب الوعائى للغدة الكظرية فى الحمار وذلك باستخدام الماسح الإلكترونى. أجريت الدراسة على عدد عشرة حيوانات من كلا الجنسين مع الإشارة إلى مورفولوجية هذه الغدد. أظهرت الدراسة أن المدد الدموى الشريانى للغدة الكظرية يأتى من الشريان الكظرى القحفى والأوسط والذنبى التى تتوزع على أسطح الغدة وهذه تتفرع بدورها إلى شريانات عديدة فى محفظة الغدة (الطبقة المفككة). ولقد أظهرت الدراسة أيضا أن بعض هذه الشريانات يكون شبكة متسعة من الشعيرات الدموية (الضفيره المحفظية). أما الضفيره التحت محفظية فتتكون من باقى الشريانات بالإضافة إلى شريانات صغيرة أخرى من الضفيره المحفظية حيث تظهر فى صورة فصيصات محدبة مكونة من شبكة من الشعيرات الدموية تغطى المنطقة المقوسة. أما المنطقة الحزمية فتتغذى بشعيرات طويلة مستقيمة متوازية متجهة إلى اسفل مع حدوث زيادة بسيطة فى أقطارها مكونة جيبان شعيرية فى المنطقة الشبكية التى تتصل بدورها فى النهاية بالشبكة الشعيرية لنخاع الغدة. أوضحت الدراسة أيضا أن المدد الدموى لنخاع الغدة يأتى من الاتصال المباشر للجيبان الشعيرية للمنطقة الشبكية بالإضافة إلى الشريانات القشرية الطويلة التى تعطى بدورها بعض الأفرع الجانبية لتشارك أيضا فى تغذية القشرة. وقد وجد أن الشعيرات الدموية تكون شبكة كثيفة لها صورة عينية فى منتصف منطقة النخاع وشبكة متسعة فى المنطقة المجاورة النخاعية. وقد تم مقارنة ومناقشة نتائج هذا البحث مع ما تم الحصول عليه من مراجع سابقة.

SUMMARY

The present study was carried out on the adrenal glands of ten healthy adult donkeys of both sexes. The corrosion casts were prepared from eight adrenals by injection of a mixture of 4:1 Mercocox and Methylmethacrylate. The adrenal glands of the donkey received their arterial blood supply through three main sources, the cranial, middle and caudal adrenal arteries. These arteries were distributed all over the gland surfaces and gave rise to several arteriolar branches in the loose layer of the capsule. Some of them formed the widely meshed capillary network (capsular plexus). The subcapsular plexus was formed from the rest of arterioles in addition to those of the capsular plexus. This plexus appeared in the form of convex lobules of densely meshed capillary network covering the arcs of the Zona arcuata. The Zona fasciculata was supplied by long straight parallelly oriented capillaries that continued downward with a little increase in the diameter forming the sinusoidal capillaries of the Zona reticularis and lastly join the capillary network of the adrenal medulla. The adrenal medulla received its blood from the direct continuation of the sinusoidal capillaries of the Zona reticularis as well as from the long cortical arterioles that gave rise to small collaterals to the cortex. In the medulla, the capillaries constituted a densely meshed network having an acinar form in the center and a widely meshed network at the juxtamedullary region.

Key words: Vascular architecture, corrosion casting, SEM, adrenal gland, donkey.

INTRODUCTION

Since Eustachius (1563) had firstly described the adrenal glands in man, there is an increasing interest for studying their structural components, the steroid secreting cortex and the catecholamines secreting medulla, by progressive series of physiological, histological and histochemical investigations in relation to functions of other organs. The morphology of the adrenal glands of the domesticated animals was fully investigated by El-Hagri (1967), Venzke (1975), Bareedy (1980), Jamader and Ema (1982), Hullinger (1993) and Dyce, Sack and Wensing (1996). The adrenal glands are the most important endocrine organ of

the body. This is due to the role played by the adrenocortical hormones that may affect many physiological processes in the body.

The adrenal glands have the highest rate of blood flow in the body (Banks, 1993; Fawcett, 1994). The arterial blood supply of the adrenal glands of the domesticated animals is fully described in textbooks of veterinary anatomy. The adrenal arteries are derived from three main sources viz., caudal phrenic, abdominal aorta and renal arteries which detach cranial, middle and caudal adrenal arteries respectively (Ljubomudrov, 1939; El-Hagri, 1967; Venzke, 1975; Wilkens, 1981). In man each gland receives its major blood supply from the inferior phrenic artery via the superior suprarenal arteries, the middle suprarenal arteries from the aorta and the inferior suprarenal arteries from the renal arteries (Fawcett, 1994). The microvasculature of the adrenal gland was studied in rat (Kikuto and Murakomi, 1982) and in man (Pitynski, Litwin, Nowogrodzka-Zagorska and Miodonski, 1996). However, nothing has been mentioned on the microvascular architecture of the donkey adrenal gland in the available literature so far. This study aimed to give some information on the general morphology of the donkey adrenal gland with special attention to its microvascular architecture using Mercox-methylemethacrylate microcorrosion casts.

MATERIAL and METHODS

Twenty adrenal glands from ten healthy adult donkeys (5 males and 5 females) were used in this study. Dimensions of the glands including the length, width and thickness as well as the weight were determined in all specimens. Four latex injected animals were dissected and examined grossly. Pieces of both right and left glands, from another two animals, were fixed in Bouin's fixative, processed for paraffin sectioning and stained with Hx & E (Bancroft, Stevens and Turner, 1996).

For preparation of the microvascular casts eight adrenals (from four animals) were injected by a mixture of 4:1 *Mercox* CL2-2B (Vilene, Tokyo, Japan) and *Methylmethacrylate* (Fluka, Neu-Ulm, Germany) through the middle adrenal artery. The mixture was freshly prepared and instilled at low constant hand pressure until the venous outflow consisted of pure plastic, then all vessels of outflow were ligated and the artery was clamped to allow complete polymerization of the plastic for 30-60 min. Once hardened, the injected adrenal was stored at 60°C overnight. Corrosion was conducted by alternating immersion in 20 % KOH and

running tap water several times. The casts were embedded in 20 % gelatin for cracking in liquid nitrogen. Gelatin was removed by second corrosion, then the casts were dried at 37°C. Suitable specimens were mounted on aluminum stubs, sputter-coated with gold and examined with JEOL 5400 LV scanning electron microscope.

RESULTS

Morphology of the adrenal glands:

The donkey adrenal glands were elongated flattened brown organs having two extremities (cranial and caudal), two borders (medial and lateral) and two surfaces (dorsal and ventral). The cranial extremity was broader in the right gland. The two adrenals were related to the cranial poles of the corresponding kidney craniomedially and ventrally, the right adrenal was slightly cranial in position. Both glands were related cranially to the pancreas and caudally to the renal hilus. The caudal vena cava crossed the ventral aspect of the right adrenal gland, which was related medially to the psoas muscles and crura of the diaphragm. The left adrenal gland was related medially to the cranial mesentric artery and to the left celiacomesentric ganglia and aorta dorsally. Ventrally, the left gland was related to the root of the mesentery (Fig. 1a & b). The average length, width and thickness of the right adrenal were about 6.2, 3.5 and 0.4 cm, respectively, while the left one measured about 5.5, 3.6 and 0.5 cm respectively. Concerning the weight, the right adrenal was slightly heavier, weighing 8.26 gm while the left one weighed 7.16 gm.

Both right and left adrenal glands received their arterial blood supply from three sources (Fig. 1a & b). The first source (cranial adrenal artery) was represented by very fine branch coming from the caudal phrenic artery. The second contribution (middle adrenal artery) arose from the ventral aspect of the abdominal aorta and seemed to be the main source. This artery passed laterally towards the medial border of the gland and divided into 3-4 branches distributed on the dorsal and ventral surfaces of the gland. The third source (caudal adrenal artery) originated from the renal arteries, directed to the caudal extremity of the gland and split into 4-5 fine branches. The adrenal veins appeared on the ventral aspect of both right and left glands. The right adrenal vein joined the caudal vena cava while the left one drained into the left renal vein.

The donkey adrenal gland consisted of cortex and medulla, encapsulated by a thick connective tissue capsule. The capsule consisted

of two layers, an outer loose layer that contained small and large arterioles and an inner dense layer. The adrenal cortex was the thickest region of the gland consisting of three zones, Zona arcuata, Zona fasciculata and Zona reticularis. The adrenal medulla occupied mostly the central position of the gland (Fig. 2a).

Microvascular architecture:

The adrenal arteries split into smaller branches that attained a highly tortuous course over the surface of the glands (Fig. 2b, 3a & 3d). From these branches, some arterioles originated in the loose layer of the capsule to form at first a widely meshed capillary network (capsular plexus). This network consisted of smooth capillaries having very small but nearly uniform diameters (Fig. 3b). The rest of arterioles in addition to those of the capsular plexus constituted the subcapsular plexus, which appeared in the form of convex lobules of densely meshed capillary networks covering the arcs of the Zona arcuata (Fig. 3c). From the subcapsular plexus short-arcuate arteries, arterioles and capillaries originated to vascularize first, the Zona arcuata (Fig. 3d). Straight or parallel oriented capillaries extended thereafter distalwards to vascularise the Zona fasciculata. These capillaries were relatively large and have nearly uniform diameter (Fig. 3e). They continued downward with a little increase in the diameter forming sinusoidal capillaries that vascularized the Zona reticularis and lastly join the capillary network of the medulla.

Long cortical arterioles originated also from the subcapsular plexus, passing through the cortex directly to the medulla, but they shared also in the vascularization of the cortex through collateral communications (Fig. 3e).

The vascular pattern of the medulla was completely different from that of the cortex. It received blood from two main sources, the first one appeared as a direct continuation of the sinusoidal capillaries of the Zona reticularis, while the second was coming from the long cortical arterioles. The medullary capillary network was more densely meshed and consisted of capillaries which had a smaller diameter and convoluted appearance (Fig. 4a). This capillary network constituted more or less an acinar form in the center of the medulla (Fig. 4b). However, at the juxtamedullary position the capillaries appeared slightly larger in diameter and formed a widely meshed network around the medullary epithelial cords. The venous drainage of adrenal gland started at the corticomedullary junction through the post-capillary venules

which continued as small and large veins (Fig. 4a, b) that joined the large central vein in the medulla (Fig. 2a). The latter emerged from the hilus of the gland on its ventral surface.

DISCUSSION

Although the adrenal gland has the highest rate of blood flow per gram tissue (Banks, 1993; Fawcett, 1994) little has been published on the microvascular architecture of this organ. The present study revealed that the adrenal glands of the donkey received their arterial blood supply from three main sources; the cranial, the middle and the caudal adrenal arteries. These results simulate that mentioned by Junqueira and Carneiro (1983) and Fawcett (1994) in man and rat (Kikuta & Murakami, 1982). Among these sources the middle adrenal artery (originating from the abdominal aorta) seems to be the main source. However, Wilkens (1981) mentioned that the adrenal glands of the horse receive their blood only from the caudal adrenal artery, which arises from the renal artery. He added also that those of goat, sheep and cow are supplied with the cranial and the caudal adrenal arteries, while the adrenal glands of carnivores and pig received, in addition, the middle adrenal artery. El-Hagri (1967) and Venzke (1975) stated that, in the domestic animals, the main blood supply of the adrenal glands comes from the renal artery and the abdominal aorta or from the aorta directly or through its branches (Frandsen, 1981). On the other hand, Dyce *et al.* (1987) stated that the vascular branches of the adrenal gland come from four sources namely, renal, lumbar, phrenicoabdominal and cranial mesenteric arteries.

The arteries supplying the donkey adrenal glands rapidly ramified into small ones over the capsule, which had a highly tortuous or convoluted appearance, that has not been described either in man or animals, giving the donkey's adrenal arteries unusual pattern of distribution. In addition, the small arteries and arterioles, that constituted the convex lobules of the subcapsular plexus, represents another point of specification to the donkey adrenal microvasculature in contrast to the smooth subcapsular plexus of man and rat adrenals (Fawcett, 1994 and Kikuta & Murakami, 1982). The vascular pattern of cortex of the donkey adrenal gland resembles that described in man (Fawcett, 1994) and rat (Kikuta & Murakami, 1982). It consisted of extensive network of parallel oriented capillaries that occupied interstices among the arcs of parenchymal cells in the Zona arcuata and among cell columns of Zona

fasiculata. These capillaries passed down to form a rich plexus in the Zona reticularis. Similar to that described in the rat, cat and pig adrenal cortex (Motta, Muto and Fujita, 1979), the organization of the blood capillaries in the cortex of the donkey adrenal gland was in the form of parallel columns separated by the cortical cells producing a labyrinthic system.

In the present study, the blood capillaries in the deeper zone of the cortex (Zona reticularis) appeared enlarged and sinusoidal. At the corticomedullary junction, these sinusoidal capillaries joined wide collecting vessels (veins) in the medulla, that there is no venous system in the donkey adrenal cortex. This simulates that mentioned by Junqueira and Carneiro (1983), Liebich (1990), Dellmann (1993) and Fawcett (1994). On the contrary, Banks (1993) stated that, the cortex contains subcapsular veins.

In addition to the before mentioned sinusoidal capillaries, the medulla received also long cortical arterioles. Hence, its vascular bed appeared densely meshed that consisted of irregular capillaries. The latter constituted a typical acinar form for increasing the surface area of contact and exchange. The sinusoidal capillaries and the long cortical arterioles indicate a dual blood supply of the adrenal medulla. This pattern of vascularization has an important physiological consequence not only of transporting the adrenocortical hormones directly into the blood stream through the adrenal medulla but also to control the synthesis of catecholamines in the chromaffin cells (Liebich, 1990). This occurs through induction and maintenance of the enzyme phenylethanolamine N-methyl transferase which is necessary for the synthesis of epinephrine (Fawcett, 1994), or conversion of norepinephrine to epinephrine (Wurlman and Pohorecky, 1971).

The before mentioned long cortical arterioles were described as long non-branched cortical arteries in rat and man adrenal gland (Kikuta & Murakami, 1982 and Fawcette, 1994) respectively, as well as in cat and pig adrenal glands (Motta *et al.*, 1979). In donkey, they shared also in the vascularisation of the adrenal cortex through small side branches. However, Liebich (1990) and Dellmann (1993) stated that these arterioles course through the cortex without branching.

In the current work, the blood collected via small venules from the cortico-medullary junction to join the medullary veins that open in a large central vein. In this concern, Griffith and Cameron (1975) mentioned that the cortico-medullary veins of the human adrenal gland have longitudinal muscle bundles that regulate the blood flow through

the cortico-medullary vascular area and hence may provide a system by which uptake of adrenocortical hormones is controlled by the various cells.

No evidence of portal circulation was detected in the adrenal gland of the donkey. This simulates that mentioned, in human adrenal gland, by Sasano, Takezawa, Sato and Horikawa (1971). This finding disagrees with that reported by Rufeng and Bailian (1987) who ascertained the existence of a portal circulation in the human fetal adrenal glands as well as in adults (Coupland and Mac Dougall, 1966; Dobbie and Symington, 1966; Henderson and Daniel, 1978). They based this opinion on their observation of small veins near the central region of the gland that broke up into capillaries that converged again into venules and finally drain into branches of the central vein. However, Dempster (1974) who attributed the suggestion of the previous authors into misinterpretation or injection artifacts has rejected the existence of the portal circulation in the adrenal gland.

REFERENCES

- Bancroft, D. J.; Stevens, A. and Turner, D. R. (1996):* Theory and practice of histological techniques. 4th edition, Churchill Livingstone, Hong Kong.
- Banks, W. J. (1993):* Applied veterinary histology. 3rd Ed. Mosby. Year. Book. St. Louis. Baltimore. Boston. Chicago. London. Philadelphia. Sydney. Toronto.
- Bareedy, M.H. (1980):* Histological, histochemical and volumetric post-natal changes of the adrenal gland of one-humped male camel. (*Camelus dromedarius*). Ph.D. Thesis. Faculty Vet. Med., Zagazig University.
- Coupland, R.E. and McDougall, J.D.P. (1966):* Adrenaline formation in noradrenaline-storing chromaffin cells in vitro induced by corticosterone. *J. Endocrinol.*, 36: 317-324.
- Dellmann, H.-D. (1993):* Textbook of veterinary histology. 4th Ed. Lea & Febiger. Philadelphia.
- Dempster, W.J. (1974):* The nature of the venous system in the adrenal gland. *Tohoku J. Exp. Med.*, 112: 63-77.
- Dobbie, J.W. and Symington, T. (1966):* The human adrenal gland with special reference to the vasculature. *J. Endocrinol.*, 34: 479-489.

- Dyce, K.M.; Sack, W.O. and Wensing, C.J.G. (1996):* Textbook of veterinary anatomy. 2nd ed. Saunders Co. Philadelphia, London, Montreal, Sydney, Tokyo.
- El-Hagri, m.A.A. (1967):* Splanchnology of the domestic animals. 1st Ed. The public organization for books and scientific applicants. Cairo University Press.
- Eustacius, B. (1563):* Opuscula Anatomica. Libellus de Renibus, Venet. Cited by Venzke, W.G. (1975).
- Frandsen, R. D. (1981):* Anatomy and physiology of the farm animals. 3rd Ed. Lea & Febiger. Philadelphia.
- Fawcett, D. W. (1994):* A textbook of histology. In Bloom and Fawcett. 12th Ed. W.B. Saunders Co. Philadelphia.
- Griffith, K. and Cameron, E.H.D. (1975):* The adrenal cortex. In: F. Beck, J.B.L. Ioyd (eds), The cell in the Medical Science, Vol. 3, p. 155. Academic press, London.
- Henderson, J.R. and Daniel, P.M. (1978):* Portal circulations and their relation to countercurrent systems. Quart. J. Exp. Physiol., 63: 355-369.
- Hullinger, R.L. (1993):* The endocrine system. In: Evans, H.E.: Miller's anatomy of the dog, 3rd Ed. W.B. Saunders, Philadelphia, London, Toronto, Montreal, Sydney, Tokyo.
- Jamdar, M.N. and Ema, A.N. (1982):* Relationship of cortex and medulla in the adrenal gland of the donkey. Res.Vet. Sci., 32: 261-264.
- Junqueira, I. C. and Carneiro, J. (1983):* Basic histology. 4th. Ed. Librairie de liban. Beirut. Lebanon. Lange medical publications. Los Altos. California.
- Kikuto, A. and Murakami, T. (1982):* Microcirculation of the rat adrenal gland: a scanning electron microscope study of vascular casts. Am. J. Anat., 164: 19-28.
- Liebich, H.-G. (1990):* Functionell Histologie. Farbatlas und kurzlehrbuch der mikroskopischen Anatomie der Haussaeugetiere. Schattauer. Stuttgart. New York.
- Ljubomudrov, A.P. (1939):* The blood supply of the suprarenal glands in the dog. Arkhiv. Anat. Gistol. Embriol., 20: 220-224.
- Motta, P.; Muto, M. and Fujita, T. (1979):* Three dimensional organization of mammalian adrenal cortex, a scanning electron microscopic study. Cell Tissue Res., 196: 23-38.

- Pitynski, K.; Litwin, J.A.; Nowogrodzka, Zagorska, M. and Miodonski, A.J. (1996):* Vascular architecture of the human fetal adrenal gland: A SEM study of corrosion casts. *Ann. Anat.*, 178: 215-222.
- Rufeng, J. and Bailian, D. (1987):* The vascular architecture of the adrenal gland in the fetuses and newborns. *Acta Anat.*, 18: 59-64.
- Sasano, N.; Takezawa, Y.; Sato, H. and Horikawa, N. (1971):* Microangiography of normal and pathologic human adrenals in prenatal and aging course. *Tohoku J. Exp. Med.*, 104: 129-141.
- Venzke, W.G. (1975):* General endocrinology. In: Sisson and Grossman. *The anatomy of the domestic animals.* Rev. by Getty, R. 5th Ed. W.B. Saunders Co., Philadelphia, London, Toronto.
- Wilkens, H. (1981):* Arteries. In: Schummer, Wilknes, Vollmerhaus and Habermehl. *The circulatory system, the skin and the cutaneous organs of the domestic mammals.* Vol. III. Verlag Paul Parey. Berlin and Hamburg.
- Wurlman, R. J. and Pohorecky, L. A. (1971):* Adrenocortical control of epinephrine synthesis in health and disease. *Adv. Metab.*, 5: 53-54.

LEGENDS

- Figs. 1a & b:** Micrographs showing the topography of the donkey adrenal glands (ventral views) and their blood supply after removal of the caudal vena cava. Right adrenal (Ra), left adrenal (La); cranial adrenal artery (arrowhead); middle adrenal artery (arrow); caudal adrenal artery (double arrows); abdominal aorta (aa); renal artery (ra); cranial mesenteric artery (cm); right kidney (Rk); left kidney (Lk); ureter (U); pancreas (P).
- Fig. 2a:** Light micrograph of the donkey adrenal gland showing the capsule (C), Zona arcuata (A), Zona fasciculata (F), Zona reticularis (R) and the medulla (M). Small arteries and arterioles in the outer loose layer of the capsule (arrows), part of the central vein (arrowhead). H & E. X 50.
- Fig. 2b:** Scanning electron micrograph of a frozen-cut microvascular cast of the donkey adrenal gland showing an overview of the vascular architecture of the capsule (C), Zona arcuata (A), Zona fasciculata (F), Zona reticularis (R) and the medulla (M). Bar 1mm.

- Fig. 3a:** Scanning electron micrograph of the donkey adrenal microvascular cast showing small arteries (arrows) having a highly tortuous course over the surface of the gland, ramifying into arteriolar branches (arrowheads) that form the subcapsular plexus (stars). Bar 500 μm .
- Fig. 3b:** Scanning electron micrograph of the donkey adrenal microvascular cast showing a widely meshed capillary network of the loose layer of the capsule. Bar 50 μm .
- Fig. 3c:** Scanning electron micrograph of the donkey adrenal microvascular cast showing the convex lobules of the subcapsular plexus of the Zona arcuata. Bar 500 μm .
- Fig. 3d:** Scanning electron micrograph (top and side view) showing the vascularization of the Zona arcuata. Corrugated artery (arrow); arcuate arterioles (arrowheads). Bar 100 μm .
- Fig. 3e:** Scanning electron micrograph showing the radial arrangement of the capillaries of the Zona fasciculata. Long cortical arterioles (arrows) with slightly larger diameters than the capillaries and collaterals (arrowhead) are seen. Bar 100 μm .
- Figs. 4a & b:** Scanning electron micrographs of a microvascular cast of the donkey adrenal medulla illustrating the branching pattern of capillaries and the venous drainage. Post-capillary venules (arrowheads); small and large veins (arrows). Notice the acinar formation of the capillaries (asterisks). Bar 100 μm .









