Original article

Distribution of ageing changes of the goat aortic tunica media

Ogeng'o, JA.*, Malek, AKA. and Kiama, SG.

Department of Human Anatomy, University of Nairobi, P.O. Box 30197-00100, Nairobi, Kenya
*E-mail: jogengo@uonbi.ac.ke

Abstract

Ageing changes in the tunica media of the aorta may be influenced by the prevailing haemodynamic stress. These changes are associated with higher cardiovascular morbidity and mortality among the aged. Although the goat is a suitable model for vascular studies, little attention has been paid to aging changes in its aorta. This study investigated the histomorphological ageing changes in the goat aortic tunica media by light and transmission electron microscopy. Sixteen male domestic goats of age range 60-120 months were euthanised with sodium pentobarbital 20 mg.mL^{-1} and fixed with 3% phosphate buffered glutaraldehyde solution. Samples from various aortic regions were processed routinely for paraffin embedding and sectioning for light microscopy. 7 µ sections were stained with Mason's trichrome and Weigert resorcin fuchsin/Van Gieson. The specimens for transmission electron microscopy were post-fixed in osmium tetroxide, and ultrathin sections stained with uranyl acetate, counter stained with lead citrate and examined by EM 201 Phillips© microscope. It is observed that ageing is characterized by fragmentation of elastic lamellae, increased amounts and tangling of collagen, and disorganization smooth muscle cells. These aging changes are more pronounced in luminal than in adventitial zone, and in proximal than in distal parts of the aorta. This distribution of structural ageing changes in the goat aorta suggests that they are influenced by mural strain, and the amount of smooth muscle. Control of blood pressure in human beings constitutes a useful approach to reduction of age related vascular disruption.

Keywords: ageing, goat aorta, regional variation.

1 Introduction

Ageing structural changes in the mammalian aortic tunica media may be influenced by mural strain (NAKAMURA and OHTSUBO, 1992; GUO and KASSAB, 2004). These changes are thought to result in aortic stiffness, which is associated with higher cardiovascular morbidity and mortality (LESAUSKAITE, TANGANELI, BIANCIARDI et al., 1999; BENETOS, WAEBER, IZZO et al., 2002; REDDY, LI, PHAM et al., 2003). Although the goat is a suitable animal model for vascular studies due to similarity of vessel structure and disease alterations (LEMSON, DAEMEN, KITSHAAR et al., 1999), little attention has been paid to the ageing structural changes in the tunica media of its aorta. The present study aimed at describing histomorphological aging changes in the tunica media of the goat aorta.

2 Experimental animals and methods

The present study was done on 16 male healthy domestic goats *(Capra hircus)* aged between 60 and 120 months and weighing 10-60 kg, purchased from private livestock farmers in the outskirts of Nairobi. Eight animals were used for light, and eight for electron microscopic studies. The animals were weighed then euthanised with an overdose of sodium pentobarbitone 20 mg.mL^{-1} injected intravenously. After attaining complete euthanasia, the thorax and abdomen were opened, blood cleared by normal saline and the aorta fixed by gravimetric perfusion using 3% phosphate buffered glutaraldehyde solution introduced through the left ventricle. The pericardium was opened to expose the ascending aorta, and lungs and abdominal viscera retracted to expose the arch, descending thoracic and abdominal regions of the aorta.

For light microscopy, specimens were taken from each of the following areas: the ascending aorta; aortic arch, at each vertebral level down to T13 and abdominal aorta from pre-renal, renal and post renal segments. The specimens were further fixed by immersion in 3% phosphate buffered glutaraldehyde solution. The sections were trimmed and processed routinely for paraffin embedding. Seven micrometer sections were stained with the Weigert resorcin – fuchsin stain and counterstained with Van Gieson stain for demonstration of elastic fibres; and with Mason's trichrome stain for demonstration of other mural components.

Specimens for electron microscopy were taken from the ascending aorta, aortic arch, proximal thoracic aorta (T6); middle thoracic aorta (T9) and distal thoracic aorta (T12), and abdominal aorta, immersed in 3% phosphate buffered glutaraldehyde solution for further fixation, and subsequently post fixed with 1% phosphate buffered osmium tetroxide solution. The sections were then dehydrated in increasing grades of ethanol, cleared in propylene oxide, and embedded in 100% durcupan with catalyst, and polymerized in an oven at 60 °C, for 48 hours. Ultrathin sections were made with Reichert ultramicrotome©, collected on 200 mesh copper grids, stained with uranyl acetate, counterstained with lead citrate, and examined by EM 201 Phillips© electron microscope.

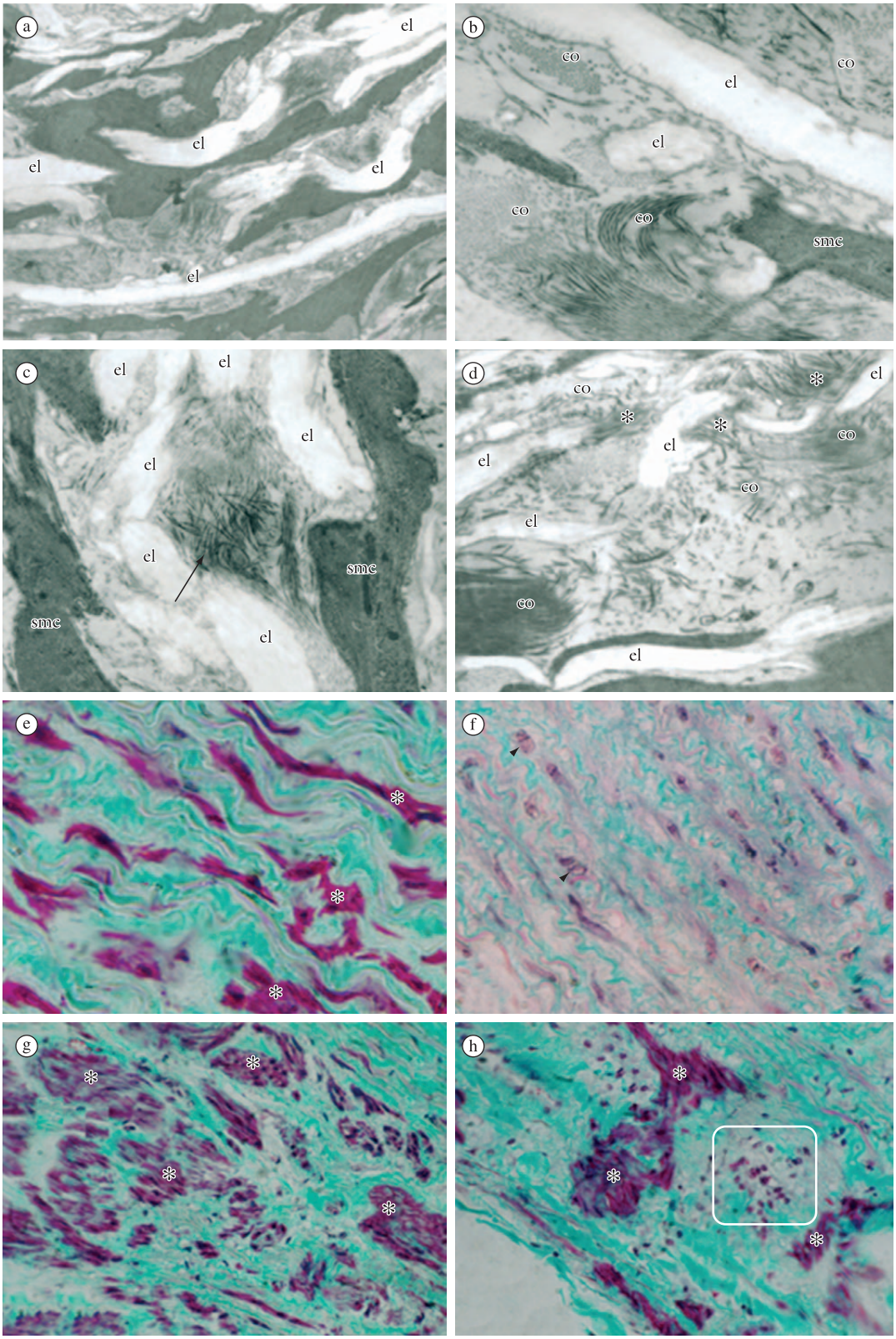

Figure 1. a-h) Micrographs showing Aging changes in the tunica media of the goat aorta. a) Fragmentation of elastic lamellae (el) ×8,760; b) increased amount of collagen fibres (co) running in various directions, between elastic lamellae (el) and smooth muscle cell (smc) ×8,760; c) tangling and knotting of collagen fibres (arrow) between fragments of elastic lamellae (el) close to smooth muscle cell (smc) ×27,800; d) collagen (co) joining the fragments (star) of elastic lamella (el) ×8,760; e) disoriented smooth muscle cells (star). Mason's trichrome stain ×400; f) binucleated cells (arrowheads). Masons trichrome stain ×250; g) distortion of smooth muscle islands (star) in the adventitial zone. Mason's Trichrome stain ×400; h) inflammatory-like cells (square) between smooth muscle cells (star). Mason's Trichrome ×400.

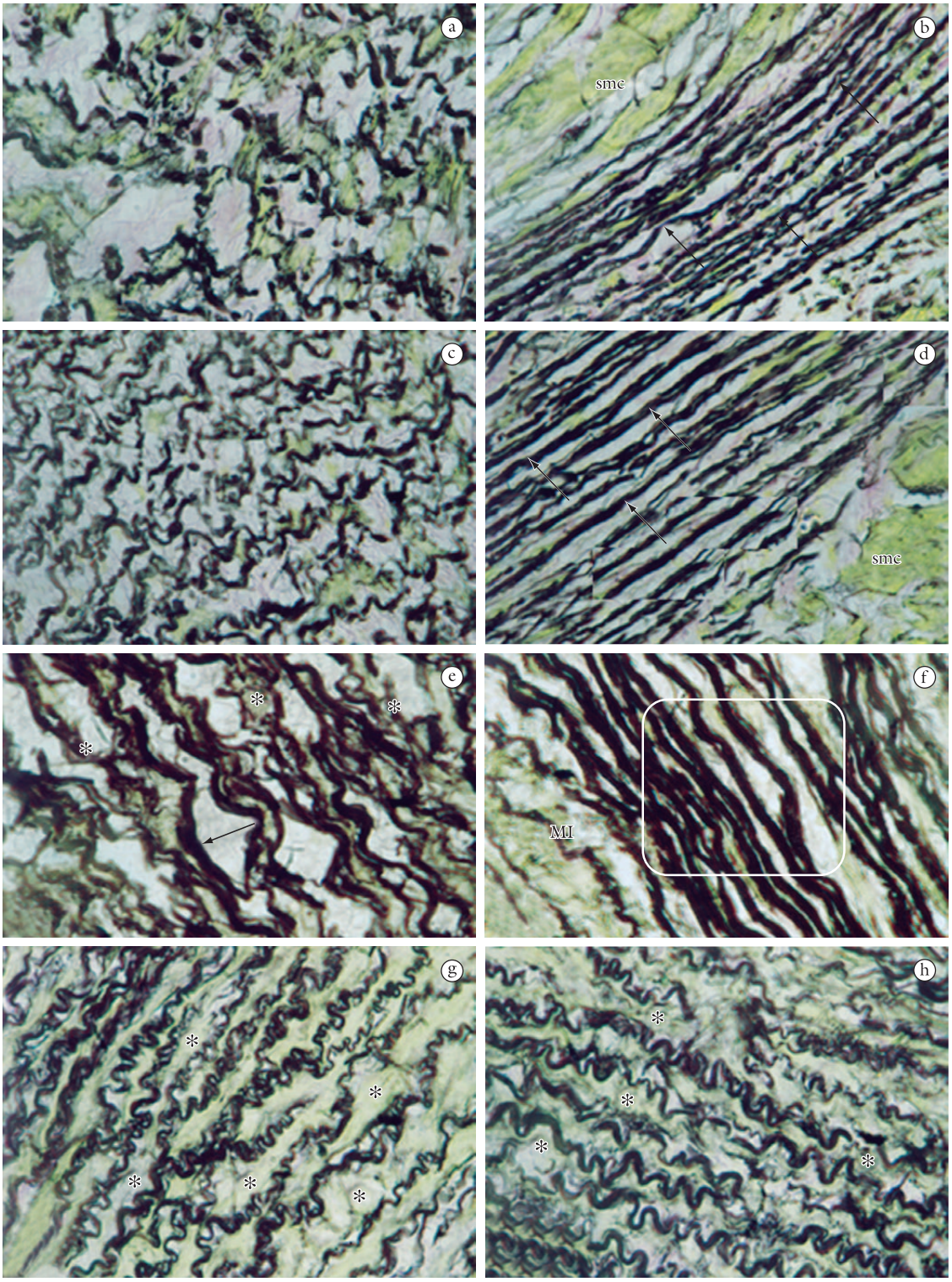

Figure 2. a-h) Micrographs showing Zonal and regional variations of ageing changes in the tunica media of the goat aorta. a) Fragmentation of elastic lamellae (stained black) in the luminal zone of the ascending aorta. Weigert elastic stain ×400; b) continuous elastic lamellae (arrows) next to bundles of smooth muscle (smc) in the adventitial zone of ascending aorta. Weigert elastic stain ×400; c) the fragmentation of elastic lamellae (stained black) in the luminal zone of proximal thoracic aorta. Weigert elastic stain ×400; d) continuous elastic lamellae (arrows) next to smooth muscle bundles (smc) in proximal thoracic aorta. Weigert elastic stain ×400; e) areas of elastic lamella fragmentation (star) among continuous elastic lamellae (arrows) in the luminal zone of distal thoracic aorta. Weigert elastic stain ×400; f) thick continuous elastic lamellae (square), next to a muscle island (MI) in the adventitial zone of distal thoracic aorta. Weigert elastic stain ×400; g) continuous wavy elastic lamellae (stained black) separated by wide interlamellar spaces (stars) in luminal zone of abdominal aorta. Weigert elastic stain ×400; h) continuous wavy elastic lamellae (stained black) separated by wide interlamellar spaces (stars) in the adventitial zone of abdominal aorta. Weigert elastic stain ×400.

3 Results

The tunica media of the goat aorta is made of regular concentric elastic lamellae between which are smooth muscle cells, collagen and elastic fibres. In the proximal segments down to T9, some outer lamellae are interrupted by muscle islands. Ageing changes affect the smooth muscle cells, elastic lamellae, collagen and elastic fibres. In the luminal zone of the ascending aorta and aortic arch, the elastic lamellae are discontinuous and fragmented (Figure 1a). Between the pieces of the fragmented lamellae there are collagen fibres running in various directions (Figure 1b). Some of the collagen is tangled (Figure 1c) and in some instances joins the elastic fibre fragments (Figure 1d). Concomitant with these connective tissue fibre changes, is cellular degeneration, whereby smooth muscle cells become randomly oriented and may assume bizarre shapes (Figure 1e), with some becoming binucleated (Figure 1f). The smooth muscle cells in the adventitial zone are also disorganized, (Figure 1g) and there is infiltration of small cells resembling inflammatory ones (Figure 1h).

These aging changes display regional and zonal variations. In the ascending aorta and aortic arch, the elastic lamellae of the luminal zone are thin, discontinuous and fragmented (Figure 2a, c). In the adventitial zone, on the other hand, the elastic lamellae between the muscle islands are thin but continuous with relatively less fragmentation (Figure 2b, d). These aging changes show a progressive craniocaudal diminution such that in the region around T10, the elastic lamellae are only mildly fragmented (Figure 2e) with minimal change in the adventitial zone (Figure 2f). In the most distal thoracic aorta extending between T11-T13, and the abdominal aorta, the tunica media comprises continuous fairly well preserved wavy elastic lamellae, in both zones (Figure 2g, h).

4 Discussion

Observations of the present study have revealed that goat aortic ageing is characterized by fragmentation of elastic lamellae in the tunica media similar to that described in literature (ZHU, UENO, MATTSUSHITA et al., 2001; CONNAT, BUSSEUIL, GAMBERT et al., 2002). Besides elastic lamella fragmentation, ageing is associated with deposition of more collagen in the interlamellar spaces, and also between the fragments of elastic lamellae, where it appears to join them. Increased secretion of collagen in the aorta with age has also been reported in other mammals (NICHOLS and O'ROURKE, 1998; ZHU, UENO, MATTSUSHITA et al., 2001; CONNAT, BUSSEUIL, GAMBERT et al., 2002). The increase in collagen may synergise the fragmentation of elastic fibres in causing aortic stiffness reported in the aged aorta (BENETOS, WAEBER, IZZO et al., 2002). The increased cardiovascular morbidity and mortality consequent to this increase in collagen may parallel that due to elastic fibre degradation.

The smooth muscle cells in both the luminal and adventitial zones of the tunica media in the proximal segments of the aged aortae appear distorted in shape and orientation. In some areas, the smooth muscle cells appear binucleated. These findings support reports that in aged aortae, there is irregularity in shape (TODA, TSUDA, NISHIMORI et al., 1980); cell necrosis (KOJIMAHARA, 1985) degradation of cells (OOYAMA and SAKAMATO, 1995 a,b); increased proliferation (MOON, CHA, KIM et al., 2003) and polypoidy (JONES and RAVID, 2004). It is probable that these changes represent both the degradation and attempted repair. The demonstration of "inflammatory" cells in old goat aortic tunica media is probably due to age related cellular activation similar to what occurs in atherosclerosis (HANSON, 2005; GALKINA, KADL, SANDERS et al., 2006). These inflammatory cells have been implicated in the production of matrix metalloproteinases (PALOMBO, MAIONE, CIFIELLO et al., 1999) some of which may be involved in elastic fibre degradation.

A notable observation of the present study is that, the elastic lamellae degradation and smooth muscle degeneration all decrease gradually in a cranio-caudal manner such that the abdominal aorta, is almost entirely spared. This is commensurate with reports that age changes are usually more pronounced where tension is highest (REDDY, LI, PHAM et al., 2003). It is therefore probable that shear stress and wall tension are the critical factors which influence the pattern of ageing change. An alternative explanation of this regional variation is the craniocaudal increase in cellularity of the tunica media. It is possible that short-term alterations in smooth muscle tone (KAWASAKI, SASAYAMA, YAGI et al., 1987; GREENWALD, 2007) off-load haemodynamic strain from the elastic fibres, thus protecting them from fatigue fracture.

Further, the present study has revealed that these aging changes display a transmedial zonation such that they are most noticeable in the luminal zone. This also appears to support previous reports that in both human and animal models, with ageing, the elastic lamellae of the tunica media begin to straighten and fragment from the luminal side (NAKAMURA and OHTSUBO, 1992). This pattern of degradation appears to be commensurate with the transmural gradient of wall tension in which there is higher strain on the luminal side (GUO and KASSAB, 2004). The observation that the elastic lamellae joining the muscle islands in the adventitial zone appear better preserved suggests that the smooth muscle islands in the adventitial zone of the thoracic aorta offload tension from the elastic fibres, thus protecting them from age change. Previous studies have suggested that by myogenic response, vascular smooth muscle uniformly distributes strain to maintain vascular haemeostasis (MATSUMOTO, TSUCHIDA and SATO, 1996).

In conclusion, the zonal and regional variation, and extent of the aging changes suggests that aortic wall tension and the amount of smooth muscle may be a relevant factors in their development. Control of blood pressure constitutes a useful approach to reduction of age related vascular disruption in human beings.

References

BENETOS, A., WAEBER, B., IZZO, J., MITCHELL, G., RESNICK, L., ASMAT, R. and SAFAR, M. Influence of age, risk factors, and cardiovascular and renal diseases on arterial stiffness: clinical applications. *American Journal of Hypertension*, 2002, vol. 15, no. 12, p. 1101-1108.

CONNAT, JL., BUSSEUIL, D., GAMBERT, S., ODY, M., TEBALDINI, M., GAMBONI, S., FAIVRE, B., QUIQUEREZ, AL., MILLET, M., MICHAUT, P. and ROCHETTE, L. Modification of the rat aortic wall during aging; possible relation with decrease of peptidergic innervation. *Anatomy and Embryology*, 2001, vol. 204, no. 6, p. 455-468.

GALKINA, E., KADL, A., SANDERS, J., KARUGHESE, D., SAREMBOK, IJ. and LEY, K. Lymphocyte recruitment into the aortic wall before and during development of atherosclerosis is partially L-selectin dependent. *The Journal of Experimental Medicine*, 2006, vol. 203, no. 5, p. 1273-1282.

GREENWALD, SE. Ageing of the conduit arteries. *Journal of Pathology*, 2007, vol. 211, no. 2, p. 157-172.

GUO, X. and KASSAB, GS. Distribution of stress and strain along the porcine aorta and coronary arterial tree. *American Journal of Physiology*, 2004, vol. 286, p. H2361-H2368.

HANSON, GK. Inflamation, atherosclerosis and coronary artery disease. *New England Journal of Medicine*, 2005, vol. 352, no. 16, p. 1685-1695.

JONES, MR. and RAVID, K. Vasuclar smooth muscle polyploidization as a biomarker for aging and its impact on differential gene expression. *Journal of Biology and Chemistry*, 2003, vol. 279, no. 7, p. 5306-5313.

KAWASAKI, T., SASAYAMA, S., YAGI, SI., ASAKAWA, T. and HIRAI, T. Non-invasive assessment of age related changes in stiffness of the human arteries. *Cardiovascular Research*, 1987, vol. 21, no. 9, p. 678-687.

KOJIMAHARA, M. Age induced changes in aortas of rats. *Experimental Pathology*, 1985, vol. 28, no. 4, p. 191-195.

LEMSON, MS., DAEMEN, MJ., KITSHAAR, PJ. and TORDOIR, JH. A new animal model to study intimal Hyperplasia in Av fistula. *Journal of Surgical Research*, 1999, vol. 85, no. 1, p. 51-58.

LESAUSKAITE, V., TANGANELLI, P., BIANCIARDI, G., SIMOES, C., TOTI, P. and WEBER, G. World Health Organization (WHO) and the world Heart Federation (WHF) pathobiological Determinants of Atherosclerosis in Youth (PBDAY) study. Histomorphometric investigation of the aorta and coronary arteries in young people from different geographical locations. *Nutrition Metabolism and Cardiovascular Disease*, 1999, vol. 9, no. 6, p. 266-267.

MATSUMOTO, T., TSUCHIDA, M. and SATO, M. Change in intramural strain distribution in rat aorta due to smooth muscle contraction and relaxation. *American Journal of Physiology*, 1996, vol. 271, no. 4, p. H1711-1716.

MOON, SK., CHA, YB. and KIM, CH. *In vitro* cellular aging is associated with enhanced proliferative capacity, GI cell cycle modulation, and matrix metalloproteinase-9 regulation in mouse aortic smooth muscle cells. *Archives of Biochemistry Biophysics*, 2003, vol. 418, no. 8, p. 39-48.

NAKAMURA, H. and OHTSUBO, K. Ultrastructural appearance of atherosclerosis in human and experimentally induced animal models. *Electron Microscopy Reviews*, 1992, vol. 5, no. 1, p. 129-170.

NICHOLS, WW. and O'ROURKE, MF. Aging changes in mouse aorta. In: NICHOLS, WW. and O'ROURKE, MF. *McDonald's blood flow in arteries*: theoretical experimental, and clinical principles. London: Arnold, 1998. p. 215-216.

OOYAMA, T. and SAKAMOTO, H. Arterial aging of aorta and atherosclerosis with special reference to elastin. *Nippon Ronen Igakkai Zasshi*, 1995a, vol. 32, no. 5, p. 326-331.

OOYAMA, T. and SAKAMOTO, H. Elastase in the prevention of arterial aging and the treatment of atherosclerosis. *Ciba Foundation Symposium*, 1995b, no. 192, p. 307-320.

PALOMBO, D., MAIONE, M., CIFIELLO, BI., UDINI, M., MAGGIO, D. and LUPO, M. Matrix metalloproteinases: Their role in degenerative chronic diseases of abdominal aorta. *Journal of Cardiovascular Surgery*, 1999, vol. 40, no. 2, p. 257-260.

REDDY, AK., LI, Y., PHAM, TT., OCHOA, LN., TREVINO, MT., HARTLEY, CJ., MICHAEL, LH., ENTMAN, ML. and TEFFER, GE. Measurement of aortic input impedence in mice: effects of age on aortic stiffness. *American Journal of Physiology*, 2003, vol. 285, no. 4, p. H1464-1470.

TODA, T., TSUDA, N., NISHIMORI, I., LESZCZYNSKI, DE. and KUMMEROW, FA. Morphometrical analysis of the aging process in human arteries and aorta. *Acta Anatomica* (Basel), 1980, vol. 106, n. 1, p. 35-44.

ZHU, BH., UENO, M., MATTSUSHITA, T., FUJIYAWA, H., SERIA, N., NISHIKAWA, T., NISHIMURA, Y. and HOSOKAWA, M. Effects of ageing and blood pressure on the structure of the thoracic aorta in SAM mice: a model for age-associated degenerative vascular changes. *Experimental Gerontology*, 2001, vol. 36, no. 1, p. 111-124.

Received April 20, 2010
Accepted February 17, 2011