Closure of the ductus arteriosus of indigenous South African goats at high altitude

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ABSTRACT

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The closure of the ductus arteriosus (DA) of 31 indigenous South African goats, whose ages ranged from 30 days prenatal to 60 days postnatal, were studied at an altitude of 1 514 m above sea level by vascular injection as well as histologically and ultrastructurally. The vascular injection results showed that functional occlusion started from the pulmonary end of the DA in kids 6 days old and progressed to the aortic end in kids 8 days old. Histologically, anatomical obliteration was observed in kids from 35 days of age. The functional closure was preceded by enlargement of the subendothelial region, progressive intimal thickening, presence of subendothelial vacuolization and endothelial detachment. There was radial orientation of the subintimal smooth muscle cells and subsequent migration towards the intima. The inner tunica media contained mast cells and areas of cytolysis. Following functional closure, the subendothelial region showed migrating subintimal smooth muscle cells with extensive cytoplasmic processes and, ultrastructurally a fragmented internal elastic lamina. In 15-day-old kids there were prominent, progressively enlarged cisternae of the rough endoplasmic reticulum and numerous free, dispersed ribosomes.

In kids 19 and 25 days old, there was, additionally, rarefaction of the cell cytoplasm and appearance of intracellular myofibrils and extra cellular collagen in the surrounding amorphous matrix, which culminated in the complete anatomical closure of the DA in 35-day-old kids.

Keywords: Ductus arteriosus, goats

INTRODUCTION

The precise physiology of foetal circulation and the interactions of the various factors during the transition from intrauterine to postnatal conditions have generated considerable research interest. Postnatal changes in the foramen ovale and ductus arteriosus (DA) are usually adequate to effect the final postnatal realignment of the circulation of blood with minimum circulatory disturbances. However, their prompt closure may not be realized due to genetic, chemical, environmental and other numerous pathophysiological factors, leading to circulatory disorders (Heymann & Rudolph 1975; Horton, Crea & Perry 1989). Moreover, studies on the morphological sequence of events culminating in the closure of the DA have shown considerable resemblance to that which has been described for arteriosclerosis (Ross 1986; Chervu & Moore 1990). This sequence of events in the DA has therefore served as a model for the study of differentiation and dedifferentiation, and for phenotypic expressions of smooth muscle cells during development and in disease (Schwartz, Campbell & Campbell 1986; Slomp, Gittenberger-De Groot, Glukhova, Van Munsteren, Kockx, Schwartz & Koteliansky 1997). The DA plays a very important role during the embryo-foetal period, and at varying times

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after birth it undergoes functional and anatomical occlusion due to modified circulatory conditions. Observations at high altitude and at sea level reveal that, in humans, persistent patency or delayed closure of the DA is more prevalent among persons living at high altitude than those at sea level (Born, Dawes, Mott & Rennock 1956; Alizamora-Castro, Battilana, Abugattas & Sialar 1960; Penaloza, Arias-Stella, Sime, Recavarren & Martecorena 1964). In addition, marked species differences have been observed in the onset and timing of the closure process. In the human, intimal cushion formation starts in the second trimester of pregnancy, while in the dog it starts just before birth (Gittenberger-De Groot, Strengers, Mentink, Poelmann & Patterson 1985). Strengers, Poelmann, Patterson & Gittenberger-De Groot (1986) have ascribed the onset in the dog to genetic factors while in the human it is thought to be due to more variable factors (Gittenberger-De Groot, Moulaert & Hitchcock 1980).

In the present study, consideration has been given to the altitude of Pretoria, South Africa which is 1 514 m above sea level and the possibility of delayed closure being a factor in the high mortality rate recorded in neonate kids at the Animal Production Unit of the Medical University of Southern Africa. The object of this study was to determine the functional and anatomical closure times of the DA in indigenous South African kid goats at high altitude and to evaluate the histological sequence of events involved in this phenomenon.

MATERIALS AND METHODS

The animals (Table 1) consisted of 28 indigenous South African goat kids varying in age from 1–60 days, and three foetuses, one of which was four, and the other two, 5 months of gestation age, respectively. All animals used in the study were born and maintained at the Animal Production Unit of the Medical University of Southern Africa, which is situated at an elevation of 1 514 m above sea level. The foetuses were obtained by caesarean section of three specially bred does using standard surgical procedures. Each foetus was euthanased with pentobarbitone sodium (1 g/9 kg body mass) and perfused through the umbilical vein with buffered 3 % glutaraldehyde. The DA was excised and routinely processed for light and electron microscopy.

Prior to each investigation, each neonate kid was physically examined, and its respiratory and heart rates monitored. All kids with signs of respiratory distress were replaced with others of the same age. Each animal was deeply anaesthetised with pentobarbitone sodium (5 mg/2 kg body mass) and intramuscularly heparinized (150 units/kg body mass). The common carotid artery was unilaterally dissected out for exsanguination and perfusion with normal saline. Following cardiopulmonary arrest, a thoracotomy was performed and the DA exposed. The brachiocephalic trunk, pulmonary arteries and descending aorta were ligated as previously described (Ohale 1993), in two kids from each age group (1, 3, 6 and 8 days). In each of these, the ascending aorta was catheterised with appropriately sized polyethylene tubing and methylene blue (0,1 % in normal saline) was injected. Each DA was dissected out, cut into aortic and pulmonary halves (Fig. 1) and placed in 10 % formol saline. The extent of any effusion of the dye from both ends of each half was observed as an indication of lumen patency. The DA from the remaining kids were perfused with a fixative consisting of 3 % glutaraldehyde containing 0.067 M of cacodylate as buffer, using a 5 mℓ syringe. The DA was then excised and further fixed in the same fixative for 24 h, after which it was transversely sliced into sections 2 mm thick, most of which were stored in the fixative. Some sections were transferred to 10 % formalin solution for light microscopy. The sections were then routinely processed for light and electron microscopy.

RESULTS

The dye injection experiments revealed that in 0–3-day-old kids the entire DA was perfused with the methylene blue, indicating patency. In the 6-day-old kids the dye effusion was observed at the aortic end

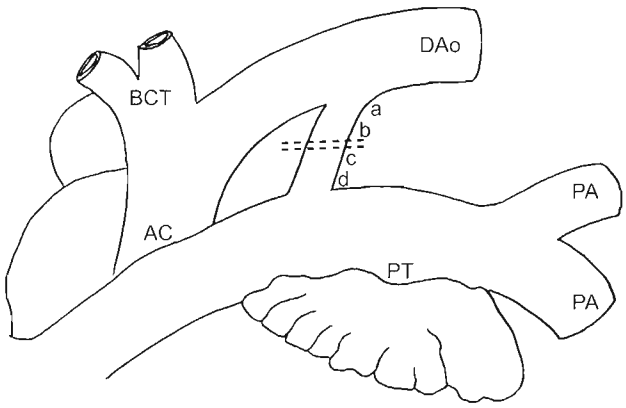
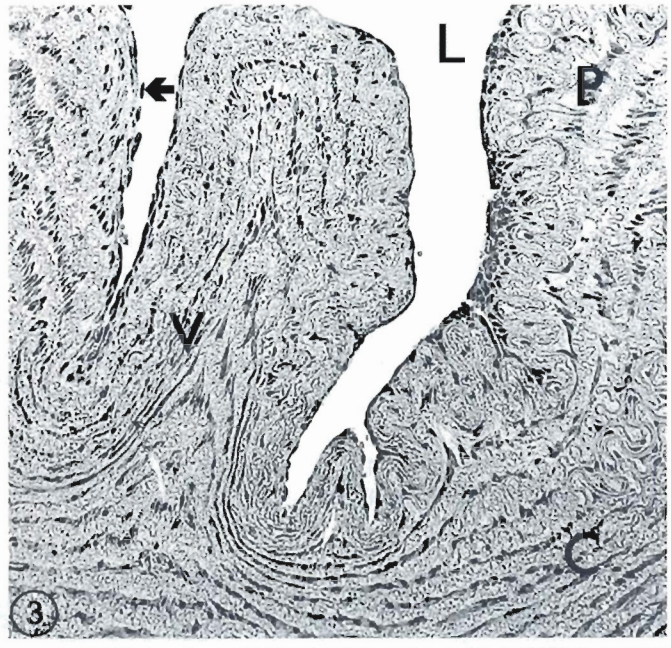
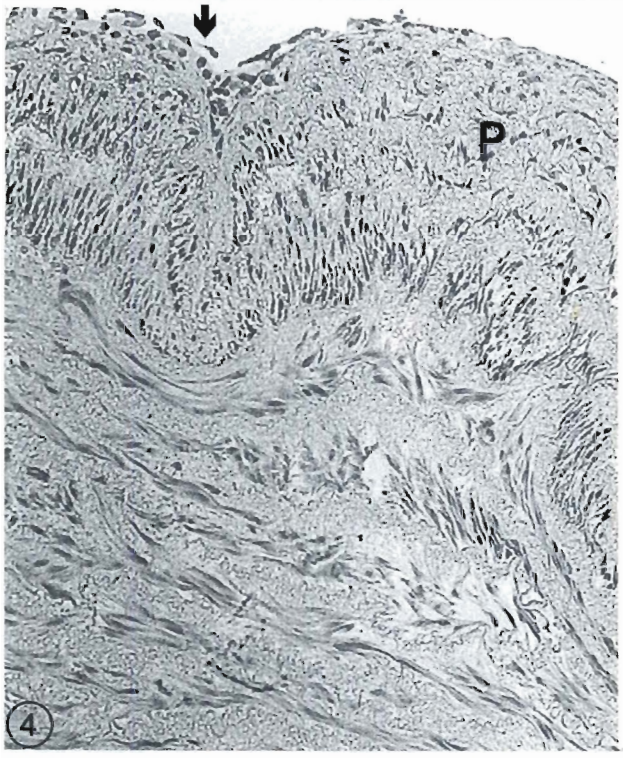


FIG. 1 Schematic line diagram of the DA between the pulmonary trunk and the descending aorta

BCT = brachiocephalic trunk
AC = ascending aorta
DAo = descending aorta
PA = pulmonary artery
PT = pulmonary trunk
ab = aortic half of the DA
a = aortic end
b = pulmonary end of the aortic half
cd = pulmonary half
c = aortic end of the pulmonary half
d = pulmonary end





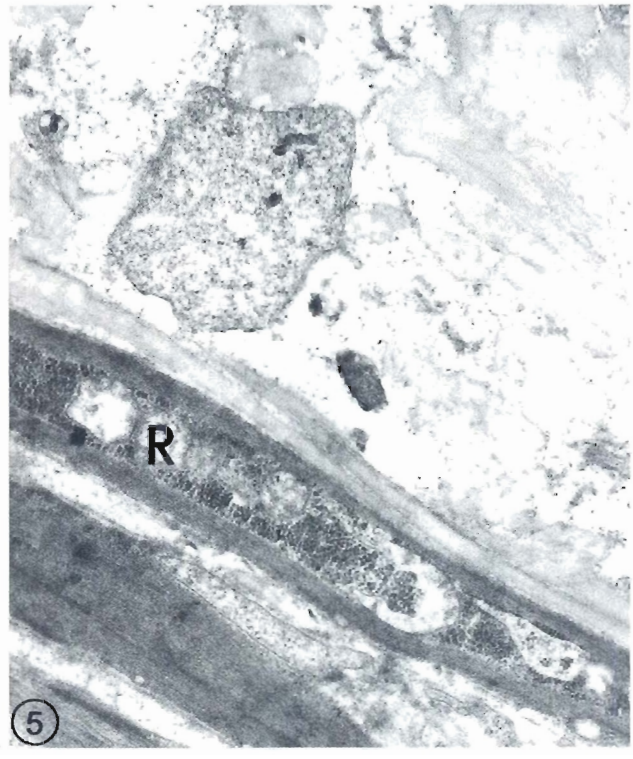


FIG. 2 A light micrograph of the transverse section of the DA of a five-month-old goat foetus. Arrows show circularly arranged subintimal smooth muscle cells X 125

FIG. 3 A light micrograph of the ductus wall of a 3-day-old kid showing irregular intimal evagination

P = early intimal cushion enlargement
L = lumen
C = circularly orientated smooth muscle cells
V = vertically arranged smooth muscle cells
Arrow head = protruding or detached endothelial cells X 200

FIG. 4 A light micrograph of the transverse section of the ductus wall of a 6-day-old kid showing a progressive increase in the intimal thickening, (P); and detached endothelial cells (arrow) X 200

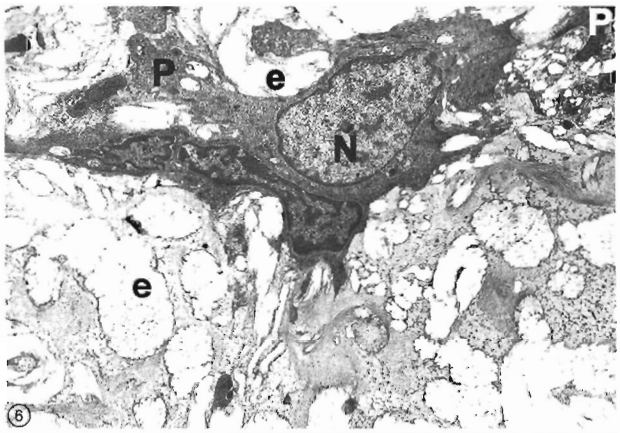
FIG. 5 An electron micrograph of the middle part of the tunica media showing a smooth muscle cell with a fragmented nucleus (R) X 15 000

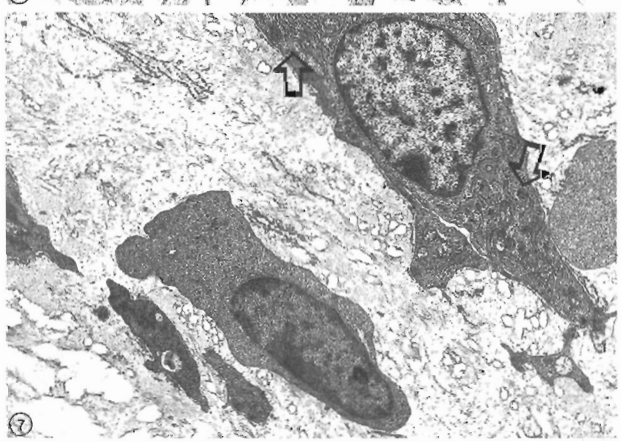
of the pulmonary half and also from both ends of the aortic half, but not at the pulmonary end of the pulmonary half, indicating functional closure from the pulmonary end. In the 8-day-old kids, there was no dye in the DA, rather, there was a prominent bulge due to the accumulation of dye at the aortic end indicating a complete functional closure of the DA.

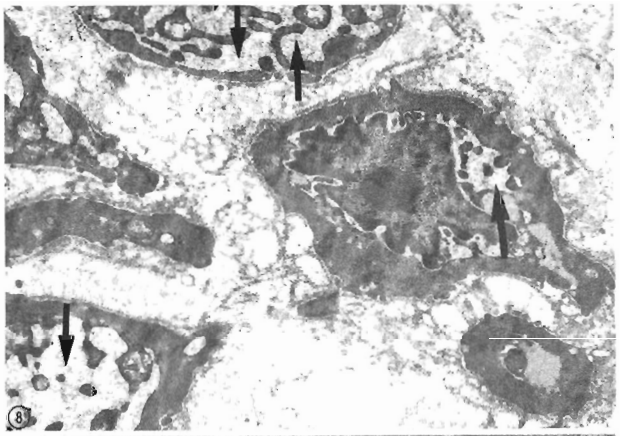
Histologically, the DA of the foetal and one day old kids, showed endothelial cells firmly attached to the basal lamina of an intact internal elastic membrane and there was no enlargement of the subendothelial region. The tunica media contained closely packed layers of circularly orientated smooth muscle cells (Fig. 2).

In the 3-day-old kids, the DA showed stages of early intimal cushion formation. The most prominent histological stages were separation of endothelial cells from the internal elastic lamina, increase or enlargement of the subendothelial region and the appearance of vertically orientated smooth muscle cells in the subintimal region of the tunica media (Fig. 3). In the 6-day-old kids, the DA showed progression in the enlargement of the subendothelial region and intimal thickening with varying degrees of endothelial cell detachment (Fig. 4) culminating in the functional closure of the DA in the 8-day-old kids.

Ultrastructurally, the middle part of the tunica media showed areas of cytolysis with some smooth muscle cells displaying fragmented nuclei, characteristic of apoptosis (Fig. 5). The migrating smooth muscle cells in the subendothelial region showed extensive cytoplasmic processes, which penetrated and fragmented the internal elastic lamina (Fig. 6).







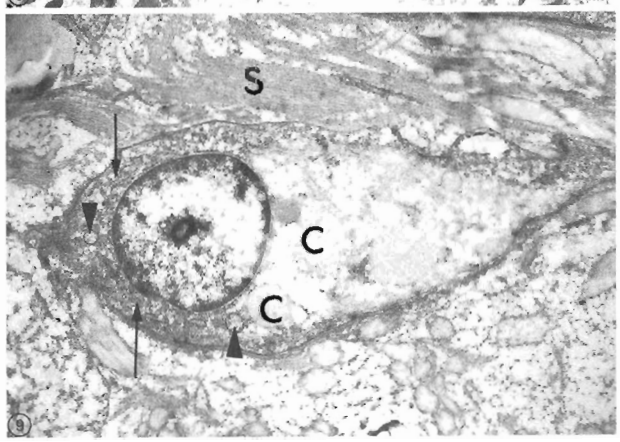


FIG. 6 An electron micrograph of the enlarged subendothelial region of the ductus wall showing migrating smooth muscle cells

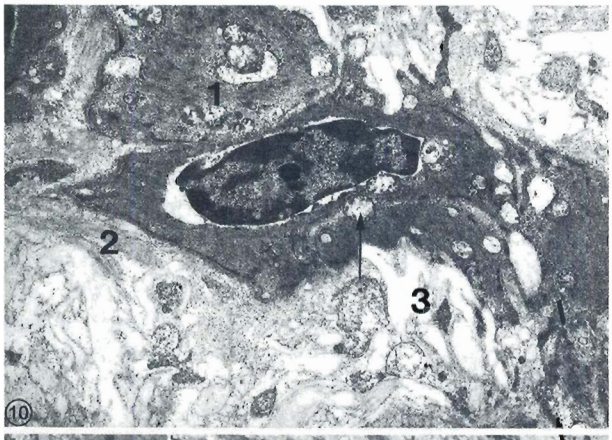
N = nucleus
e = fragmented internal elastic membrane
P = cytoplasmic processes of the cells X 15 000

FIG. 7 An electron micrograph of smooth muscle cells (synthetic phenotype) in the subendothelial region of the ductus wall of a 15-day-old kid showing prominent rough endoplasmic reticulum with dispersed ribosomes (open arrows) X 8 000

FIG. 8 A synthetic phenotypic smooth muscle cell in the subendothelial region of the ductus wall of a 19-day-old kid. Arrows show very enlarged cisternae of the rough endoplasmic reticulum X 10 000

FIG. 9 Rarefaction of the cytoplasm of a smooth muscle cell in the subendothelial region of the ductus wall of a 33-day-old goat

C = fat droplets
S = collagen fibres
Arrows = degenerating cytoplasmic organelles
Arrow head = possible lysosomes X 10 000



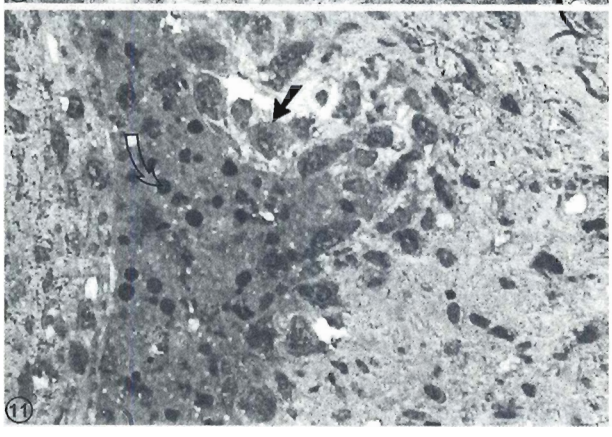


FIG. 10 An electron micrograph of the subendothelial region of the ductus wall showing:

1 = smooth muscle cells with intracellular myofibrils
2 = extracellular collagen fibres
3 = elastic fibres
Arrow = degenerating mitochondria X 15 000

FIG. 11 A light micrograph of an occluded DA lumen, showing cells with clumped nuclei (open arrow) surrounded by more vesicular cells (dark arrow) X 125

TABLE 1 Number of foetal and neonate goats and their respective ages

Kids	Ages	No. used
A. Foetal	(Gestational age—months)	
	4	1
	5	2
B. Neonates	(Days) 1	4
	3	4
	6	4
	8	4
	10	2
	15	2
	19	2
	33	2
	35	2
	60	2

In the 15 and 19-day-old kids, the migrating smooth muscle cells displayed a very prominent rough endoplasmic reticulum (RER) with dispersed ribosomes (Fig. 7). Some of these cells possessed very enlarged cisternae of the RER (Fig. 8).

The intimal region of the 19 and 33-day-old kids exhibited smooth muscle cells with varying degrees of rarefaction of the cytoplasm (Fig. 9). This process of rarefaction coincided with the appearance of intracellular myofibrils as well as extracellular collagen fibres in the surrounding amorphous matrix (Fig. 10). At the ages of 35 and 60 days, the lumen of the DA contained numerous cells with clumped and fragmented nuclei in a collagenous matrix, which is indicative of the final anatomical closure (Fig. 11).

DISCUSSION

The dye injection and histological results reveal that the DA was haemodynamically patent in the kids 0–5 days old, with functional occlusion starting from the pulmonary end in the 6-day-old kids and completed in the 8-day-old kids. The anatomical occlusion was observed in the 35 and 60-day-old kids. This observation is in contrast to that at sea level in West African dwarf goats where the DA was patent in kids 0–3 days old, with functional occlusion starting at the pulmonary end in 4-days-old kids and completed in 6-days-old kids (Ohale 1993). Anatomical obliteration was observed in kids 28–33 days old (Ohale 1993). The morphological sequence of events leading to the closure of the DA of kids at both sea level and at the high altitude of 1 514 m above sea level appear similar except for the marked difference in the onset and timing of the closure process. At sea level in West African dwarf goats, intimal cushion formation was first observed in the foetal DA just before term (Ohale 1993), in contrast to those in the present study in which the first signs of intimal cushion were observed in 3-day-old kids.

Intimal thickening is considered to be a specific process which can be influenced by various endogenic and exogenic factors (Ross 1986). In the dog, the morphological changes during intimal thickening have been described as a physiological process preceding normal closure, and in the hereditary form of persistent DA, there is an impairment of the normal process of intimal cushion formation (Gittenberger-De Groot *et al.* 1985). Although the trigger for the onset of intimal thickening remains unclear, De-Reeder, Girard, Poelmann, Van Munsteren, Patterson & Gittenberger-De Groot (1988) hypothesized that the activated endothelial cells of the normal DA both produce hyaluronic acid and trigger the smooth muscle cells to produce hyaluronic acid. The result is the swelling of the subendothelial region, detachment and invagination of the endothelium and migration of the subintimal smooth muscle cells. In dogs

with a genetically determined persistent DA, the endothelial cells lack the ability to initiate intimal thickening probably due to failure to synthesize a certain specific factor (De-Reeder *et al.* 1988).

Most reports concur that the formation of intimal thickening is a normal developmental process that begins in the foetal DA before birth (Broccoli & Carinci 1973; Gittinberger-De Groot *et al.* 1985). This observation is also true for foetal DA of the kids studied at sea level (Ohale 1993), but contrasts with that in the present study at 1 514m above sea level in which the initial closure process was delayed. Penaloza *et al.* (1964) claimed that the maturation process and physiological changes occurring in the heart and pulmonary arterial tree takes place more rapidly at sea level. Comparing their findings in children of the same age at sea level with those at high altitude, there was evident delay in the anatomical and physiological changes in the heart and pulmonary circulation of the children living at high altitude. They attributed this delay to an environment in which there is a lower oxygen content in the air.

Fay (1971) demonstrated in guinea pigs that cytochrome a_3 oxidation preceded an increase in a force-related rise in oxygen tension; in reverse, a reduction in cytochrome a_3 oxidation was preceded by a decrease in force, associated with a fall in oxygen tension. He observed that an increase of oxygen in the environment increased the availability of oxygen to the terminal cytochrome chain leading to an increase in oxidative phosphorylation and an eventual increase in the synthesis of high-energy compounds which trigger off the DA smooth muscle constriction. It is therefore probable that the sustained lower levels of oxygen and pulmonary hypertension which remain after birth are factors related to the delayed onset and closure of the DA which takes place at high altitude compared to that at sea level. This assumption is confirmed by the reports of a higher incidence of persistent ductus arteriosus among people living at high altitudes than those at sea level (Alizamora-Castro *et al.* 1960).

The observation of myofibroblast-like cells, with well-developed RER in the intima appears to justify the remodelling theory of Mosse, Campbell, Wang & Chamley-Campbell (1985) and Mosse, Campbell & Chamley-Campbell (1986), which states that smooth muscle cells in atherosclerotic lesions are modified structurally, with decrease in myofilaments and an increase in the size of RER and Golgi complex. Chamley-Campbell & Champbell (1981) also suggested that the change of smooth muscle cells from the contractile to the synthetic phenotype is a prerequisite for cellular proliferation and vascular remodelling, a process evidenced by the conversion of the DA to ligamentum arteriosum. This shift in the differentiated properties of the smooth muscle cells has been demonstrated by Slomp *et al.* (1997) as an expression of cytoskeletal and other proteins of the smooth muscle cells during intimal thickening of the ductus wall.

In conclusion, irrespective of possible genetic differences between the two breeds of goat, the apparent delay in the onset and timing processes of the closure of the DA of the goats in this study, when compared to those at sea level (Ohale 1993), may be attributed to the reduced oxygen content of the air at high altitude. The histogenesis of cells in the DA showed smooth muscle cells in different stages of differentiation and dedifferentiation in both the intima and inner media, a process indicating vascular remodelling. This apparent delay in the closure process may cause respiratory stress to kids predisposing them to early infections.

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