

## Research Article

# Histochemical and Immunohistochemical Study of Peripolar Cells in Sheep

Passos Joana,<sup>1</sup> Prada Justina,<sup>2</sup> Bento Lígia,<sup>2</sup> Rodrigues Paula,<sup>2</sup> and Pires Isabel<sup>2</sup>

<sup>1</sup> Escola Superior de Tecnologia da Saúde do Porto, Rua da Linha 224, 4900-739 Viana do Castelo, Portugal

<sup>2</sup> Escola de Ciências Agrárias e Veterinárias, Universidade de Trás-os-Montes e Alto Douro, Quinta de Prados, Apartado 1013, 5001-801 Vila Real, Portugal

Correspondence should be addressed to Passos Joana; [rigormortum@gmail.com](mailto:rigormortum@gmail.com)

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Peripolar cells are granulated cells located in the vascular pole of the renal corpuscle. Even though these cells have already been described, there are still many unknown histological and physiological characteristics. We carried out histochemical and immunohistochemical analyses of peripolar cells in sheep and compared their number in both normal and injured kidneys, discriminating according to the age of the animal. We tested HE, Toluidine Blue, PAS, and Masson's Trichrome stains to select the best stain for identification and quantification. Masson Trichrome yielded the best results and was selected for this purpose. We identified the cells by the presence of cytoplasmic granules and by their position in the vascular pole. We found no statistically significant association between the number of peripolar cells and the age of the animal or the occurrence of lesions. In the immunohistochemical analysis, we found that the cells were positive to  $\alpha$ -smooth muscle actin and less consistently positive to NSE and S100 protein. Chromogranin A, cyclooxygenase-2, AE1/AE3, and Wide Spectrum Cytokeratin and desmin yielded negative results. We conclude that although there was evidence of a contractile function, there was no evidence to support that peripolar cells have either a neuroendocrine or an epithelial nature.

## 1. Introduction

The kidneys are the main urinary system organ, being responsible for maintaining the body hydroelectrolytic balance. Besides these vital homeostatic functions, they are also responsible for the excretion of metabolic degradation by-products, arterial tension regulation, and hormone production (mainly erythropoietin and renin) [1].

In 1979, Ryan et al. described a new kind of cells found in the vascular pole of renal corpuscle in sheep [2]. These were designated by peripolar cells (PPCs) due to their ring-like distribution around the vascular pole [3]. They are located near the inflexion point between the parietal and visceral layers [4]. They were initially classified as epithelial cells due to their location above the basal membrane of Bowman's corpuscle, and by an abundance of junctions between the basal membrane and the PPCs [2, 5].

PPCs' main identifying characteristic is the presence of cytoplasmic granules. These granules have been shown

before to be stainable for optical microscopy by PAS, Toluidine Blue, Methylene Blue, and Trichrome methods [2, 5–7].

PPCs are also present in many other animals including humans [4–10]. The percentage of PPCs in an animal varies with species and age: in humans, they were found in 6.5% of the renal corpuscles, 45% in laboratory mice, and 85% in sheep [11]. This study was performed in sheep since sheep's PPCs are bigger and spread among more renal corpuscles [2, 3]. It has also been shown by Alcorn et al. that newborn sheep have an increased number of PPCs when compared to adults or fetuses [12].

A significant association has been suggested between an increase in the number of granular PPCs and the occurrence of some diseases, such as pathologic sodium depletion in sheep [13] and chicken [8], and in cases of malignant hypertension in humans [14].

By electron microscopy, it was shown that peripolar cells have membrane-bound electron-dense cytoplasmic granules, as well as a preeminent endoplasmic reticulum

and Golgi apparatus [15]. These ultrastructural characteristics suggest that these cells may have some role in protein synthesis [12] and/or secretion [2]. It has been hypothesized that peripolar cells are related to the renin-angiotensin-aldosterone system and the kallikrein-kinin system [5, 7], but their function remains unknown.

Peripolar cells' location, in the vascular pole of the renal corpuscle, suggests the possibility of an anatomical relationship with the juxtaglomerular apparatus [2]. The apparatus' major function is to regulate blood pressure via the renin-angiotensin-aldosterone system and the control the concentration of sodium ions by the macula. In response to reduced blood pressure, the juxtaglomerular cells (derived from smooth muscle cells) secrete renin in order to restore potassium and sodium levels in the blood [16].

Studies by Gibson and his colleagues [11] suggested that, despite an anatomical proximity between peripolar cells and juxtaglomerular cells, there is no correlation between the numbers of peripolar cells and renin-secreting cells. This excludes the hypothesis of PPCs belonging to the juxtaglomerular apparatus. Moreover, studies by Gardiner and Lindop [6] showed that human peripolar cells do not contain immunostainable renin, as it was described in sheep [7]. Therefore, the nature of the relationship between peripolar cells and juxtaglomerular apparatus remains unclear.

Due to the anatomical proximity to juxtaglomerular cells, it was proposed that peripolar cells may have a contractile function [17]. Therefore, a variation of the arteriolar diameter could cause an alteration in peripolar cells, increasing their secretory activity. This view is reinforced by the presence of microfilaments and microtubules in peripolar cells in sheep. However, microtubules were not seen in human peripolar cells [4].

Previous studies found that peripolar cells were positive to albumin, immunoglobulins, IgG [17], and transthyretin (TTR) [15], and 10% of the cells were positive to neuron-specific enolase (NSE) [18]. The NSE immunoreactivity is suggestive of a putative neuroendocrine role [19]. The presence of immunoreactive immunoglobulins and albumin can be explained by a glomerular leakage of plasma proteins [18]. This protein leakage is present in kidneys of newborn rats, due to the immaturity of the glomerular filtration barrier [20]. It has been suggested that there may be such a leakage (of IgG and TTR), in the developing kidneys of newborn sheep, that can be absorbed by peripolar cells [15]. Nevertheless, this filtration mechanism is not yet well understood.

As far as we know, this is the first study where a battery of antibodies was used to characterize peripolar cells in sheep.

The aim of our study was to perform histochemical and immunohistochemical analyses of peripolar cells in sheep, comparing their number according to the age of the animal, in both normal and lesioned kidneys. In the histochemical analysis, the samples were stained by HE, Toluidine Blue, PAS, and Masson's Trichrome. In the immunohistochemical analysis, we used  $\alpha$ -smooth muscle Actin, NSE, Chromogranin A, S100 Protein, desmin, Cox-2, AE1/AE3, and a Wide Spectrum Cytokeratin.

## 2. Materials and Methods

Our samples were composed of ten kidneys from male lambs (identified as R1 to R10) and ten kidneys from adult male sheep (R11 to R20), without visible macroscopic lesions. These were collected at a slaughterhouse and fixed in 10% neutral buffered formalin, embedded in paraffin, and sectioned at 3  $\mu$ m for the histochemical and immunohistochemical study.

We also used five kidneys with a previous diagnosis of lesions for the histochemical study. These were selected from the Archive of the Histology and Pathological Anatomy Laboratory of the Trás-os-Montes e Alto Douro University and had been diagnosed with the following lesions: congestion, glomerular capillary thrombosis and haemorrhage, chronic interstitial nephritis, embolic nephritis, intercortical and medullary haemorrhage, and congestion and haemorrhage. None of the selected kidneys had glomerulonephritis to avoid difficulties in peripolar cells identification.

**2.1. Histochemical Study.** Consecutive sections from all samples were stained with Hematoxylin and Eosin (HE), Toluidine Blue, Periodic acid-Schiff (PAS), and Masson's Trichrome [21] for conventional light microscopical observation. These allowed the determination of the best stain to identify peripolar cells.

Masson's Trichrome yielded the best results and was used for posterior cell quantification.

**2.2. Quantification of Peripolar Cells and Statistical Analysis.** For each kidney, a minimum of 49 renal corpuscles with evident vascular pole were photographed. Each picture was analysed by three different observers in order to ensure a correct identification of the peripolar cells.

The peripolar cells were identified as granular cells located in the glomerular capsule, near the inflexion point between the parietal and visceral layers. For the peripolar cells' quantification in normal and injured kidneys, the following index was used [6]:

Peripolar Cell Index (PPI):

PPI (%)

$$= \frac{\text{number of vascular poles with at least one peripolar cell}}{\text{total number of vascular poles}} \times 100. \quad (1)$$

We determined the number of vascular poles (VP), the number of vascular poles with peripolar cells (VPPPC), and the total number of PPCs (PPCt). We also determined the average number of PPCs in a renal corpuscle (PPCa) for each vascular pole with PPCs.

**2.2.1. Statistical Analysis.** We performed a one-way ANOVA test, in SPSS 12.0, to analyse differences in the PPI between kidneys of young sheep and old sheep, with or without lesions. Results with  $P < 0.05$  were considered statistically significant.

TABLE 1: Primary antibodies used and informative data.

Antibody	Supplier	Dilution	Antigen retrieval	Primary antibody—incubation time
$\alpha$ -Smooth muscle actin	Novocastra ( $\alpha$ sm-1)	1:50	Microwave—5 minutes	3 h RT
NSE	Zymed (Lote 20771997)	1:50	Microwave—5 minutes	2 h RT
Chromogranin A	Dako	1:50	Microwave—5 minutes (2x)	2 h RT
S100 protein	Novocastra	1:200	Microwave—5 minutes	1 h RT
Desmin	Dako (D33)	1:100	Microwave—5 minutes	2 h RT
Cox-2	Transduction Laboratories, Kentucky (Clone SP-21)	1:50	Microwave—5 minutes (4x)	ON at 4.°C
AE1/AE3	Dako (AE1/AE3)	1:50	Microwave—5 minutes	2 h RT
Wide Spectrum Cytokeratin	Dako	1:1000	Microwave—5 minutes (2x)	2 h RT

RT: room temperature; ON: overnight.

**2.3. Immunohistochemical Study.** We cut consecutive 3- $\mu$ m sections which were mounted on silane-coated slides. The most representative sections (with a higher PPI) were examined by the streptavidin-biotin-peroxidase complex method with a commercial detection system (Ultra Vision Detection System; Lab Vision Corporation, Fremont, CA, USA) following the manufacturer's instructions. Antibodies to alpha smooth muscle actin ( $\alpha$ sm-1, Novocastra), NSE (Zymed), Chromogranin A (Dako), S100 Protein (Novocastra), desmin (Dako, D33), Cox-2 (SP-21, Transduction Laboratories, KY, USA), AE1/AE3 (Dako) and Wide Spectrum Cytokeratin (Dako) were applied as described in Table 1.

Following dewaxing in xylene and washing in PBS, we carried out an antigen retrieval procedure. We performed a differently timed treatment (Table 1) in the microwave for each antigen, at 750 W and in a 0.01 M solution of citrate buffer with a pH of 6.2. After the microwave treatment, the sections were allowed to cool down for 20 min at room temperature (RT). After the antigen retrieval procedure, the endogenous peroxidases were inactivated using 3% hydrogen peroxide (Panreac Quimica, SA). Following this inactivation, the sections were washed twice in PBS and were incubated at RT with Ultra V Block to block unspecific background staining. Then, the tissues were incubated with the primary antibody. The dilution and incubation time used for each antibody is described in Table 1.

After incubation with Streptavidin Peroxidase Complex, the immunoreaction was made visible by incubation with 3,3'-diaminobenzidine tetrahydrochloride (DAB) at 0.05% with 0.01% H<sub>2</sub>O<sub>2</sub> as the final substrate, for 5 minutes. After a final washing in distilled water, the sections were counterstained with haematoxylin, dehydrated, cleared, and mounted.

Negative controls were prepared by omitting the primary antibodies and replacing them with PBS. Tissue sections known to express the corresponding antigens served as positive controls.

### 3. Results

**3.1. Histochemical Study.** Peripolar cells were identified by their position, close to the vascular pole, and by the presence

of cytoplasmic granules (Figure 1). For the identification of PPCs, different stains and histochemical methods were used: HE, PAS, Toluidine Blue, and Masson's Trichrome.

The HE stain, despite allowing a good visualization of the different histological components of the kidney, was not considered ideal for the visualization of the PPC. PPCs stained with HE were identified with a small, oval and elongated nuclei, with slightly acidophilic granules in the cytoplasm (Figure 1(a)).

With PAS, PPCs were identified by their characteristic position, and their cytoplasmic granules were stained magenta (Figure 1(b)).

Using Toluidine Blue, peripolar cells' granules were not clearly visible (Figure 1(c)). Using Masson's Trichrome, the peripolar cells were easily identified in the previously mentioned location. The PPCs were identified due to their bulky cytoplasm and the presence of cytoplasmic granules stained in red (Figure 1(d)). Due to a good evidentiatio of the granules, the Masson's Trichrome method was the one elected for the identification and quantification of the PPCs.

Using this stain, we analysed the differences between PPCs morphology and number. The cells' morphology was diverse: rounded or elongated, flat, or with a bulky cytoplasm. We also identified the presence of two or more individualized cells that were contiguous to each other.

During the histochemical analysis, we found, in average, a higher number of peripolar cells in the vascular poles from young animals. These cells, however, were smaller. In the kidneys of adult sheep, the PPCs were bigger but less numerous by renal corpuscle.

The PPCs were more easily identified in the samples of kidneys previously diagnosed with renal lesions where the Bowman space was dilated (e.g., those with a diagnosis of congestion and haemorrhage) (Figure 2(a)). On the other hand, for kidneys with microthrombi (with a diagnosis of thrombosis of the glomerular capillary), the identification was harder (Figure 2(b)).

**3.2. Quantification of PPCs and Statistical Analysis.** The quantification results for the peripolar cells are shown in Table 2.

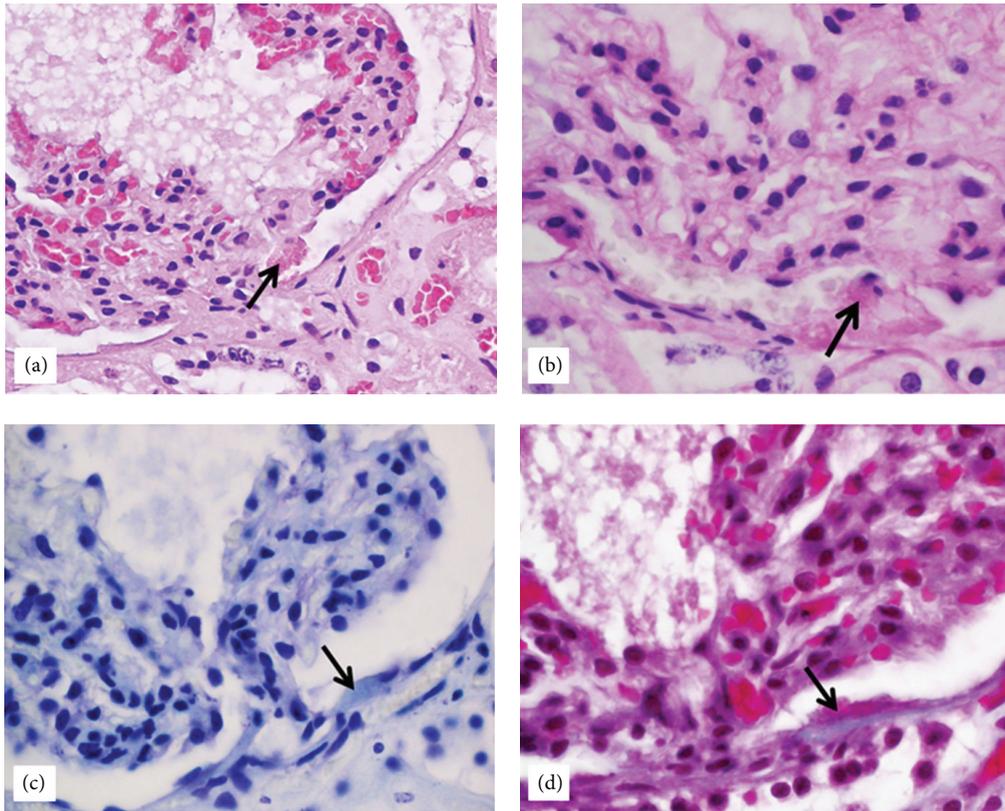


FIGURE 1: Peripolar cells (arrow) near the vascular pole of the renal corpuscle. (a) Hematoxylin and Eosin, 1000x. (b) PAS, 1000x. (c) Toluidine Blue, 1000x. (d) Masson's Trichrome, 1000x.

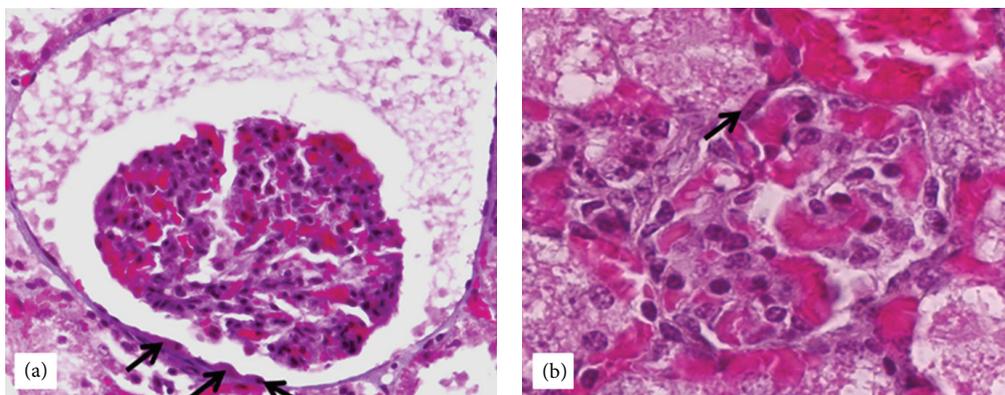


FIGURE 2: Peripolar cells (arrows) near the vascular pole of the renal corpuscle. In the kidneys in which the Bowman space was dilated ((a), 400x), PPCs were easily identified. In the kidneys with diagnosis of thrombosis of the glomerular capillary ((b), 600x), PPCs (arrow) were less visible. Masson's Trichrome method.

In kidneys without visible macroscopic lesions, the PPI varied between 29.4% (R16) and 72.5% (R13). In the kidneys from young animals, the PPI average was 56.39%, while in the kidneys from adult animals it was 59.57%.

In kidneys with lesions, the PPI varied between 27.5% and 68%, with the PPI average of 48%.

The newborn sheep' PPI was lower when compared to that of adult sheep, although we found a possible nonsignificant increase in the average number of peripolar cells

per vascular pole in newborn sheep ( $1.483 \pm 0.150$ ) when compared to adult animals ( $1.402 \pm 0.153$ ).

The differences in the number of peripolar cells between kidneys from different ages ( $P = 0.53$ ) and between kidneys with and without lesions ( $P = 0.06$ ) were not statistically significant.

**3.3. Immunohistochemical Study.** In our samples, peripolar cells were positive to alpha smooth muscle actin (Figure 3). The staining was cytoplasmatic, with a granular pattern.

TABLE 2: Quantification of peripolar cells.

Kidney	Number of corpuscles studied	VP	VPPPC	PPt	PPI (%)	PPCa
Young kidneys, without visible macroscopic lesions						
R1	50	41	22	36	54	1.6
R2	50	47	20	24	42.6	1.2
R3	50	48	24	33	50	1.4
R4	53	49	31	40	63.3	1.3
R5	51	51	29	45	56.9	1.6
R6	51	51	32	47	62.7	1.5
R7	50	50	23	36	46	1.6
R8	51	51	32	52	62.7	1.6
R9	50	50	34	55	68	1.6
R10	52	52	30	45	57.7	1.5
Adult kidneys, without visible macroscopic lesions						
R11	50	50	34	46	68	1.35
R12	51	51	27	42	52.9	1.6
R13	51	51	37	58	72.5	1.6
R14	51	51	24	28	47.1	1.2
R15	52	48	32	42	66.7	1.3
R16	51	51	15	19	29.4	1.3
R17	51	50	34	50	68	1.5
R18	50	49	35	55	71.4	1.6
R19	53	53	31	45	58.5	1.5
R20	51	49	30	36	61.2	1.2
Kidneys with lesion						
Congestion, glomerular capillary thrombosis, and haemorrhage	49	49	19	21	38.8	1.1
Chronic interstitial nephritis	50	50	21	29	42	1.4
Embolic nephritis	52	41	22	28	53.7	1.3
Intercortical and medullary haemorrhage	51	51	14	17	27.5	1.2
Congestion and haemorrhage	50	50	34	52	68	1.5

VP: number of vascular poles; VPPPC: number of vascular poles with PPCs; PPt: total number of peripolar cells; IPP: PPCs Index; PPCa: average number of PPCs in a renal corpuscle.

Only 25% of the peripolar cells were also positive to NSE (Figure 4). The macula densa and the collector tubules were also positive to NSE.

The S100 protein antibody gave positive results in 10% of the PPCs (Figure 5). The staining was cytoplasmatic, granular and easily identified in the renal corpuscle.

Peripolar cells did not react to anti-Chromogranin A (Figure 6), antidesmin, and anti-Cox-2 (Figure 7). Cox-2 immunoreacted with the macula densa and the Loop of Henle.

For AE1/AE3 antibody, the PPCs were also negative (Figure 8). A visible staining was identified in the renal tubes, in the urothelium, and, inconsistently, in the parietal cells of Bowman's.

Finally, using the Wide Spectrum Cytokeratin, the peripolar cells were also not stained (Figure 9). An immunoreaction was identified with the distal convoluted tubules,

the urothelium, and with some parietal cells of the Bowman's capsule.

#### 4. Discussion

Peripolar cells are granulated cells located near the inflexion point between the parietal and visceral layers of the renal corpuscle. Although PPCs are present in a large number of species, they are more numerous in sheep and goats and present in the majority of the renal corpuscles.

The high incidence of PPCs in these species can be justified by the fact that renal corpuscles from sheep and goat are also more cellular when compared to other species [1]. The present study was conducted in sheep, given the previous fact and since sheep's PPCs are better described in the current scientific literature. This species is also a suitable comparative model for studying human peripolar cells.

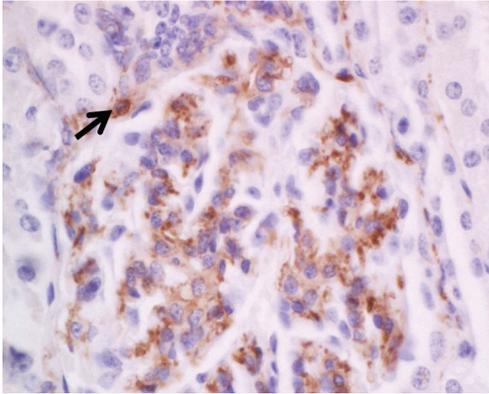


FIGURE 3: Peripolar cell (arrow) positive to  $\alpha$ -smooth muscle actin. Streptavidin-Biotin-Peroxidase Method, 600x.

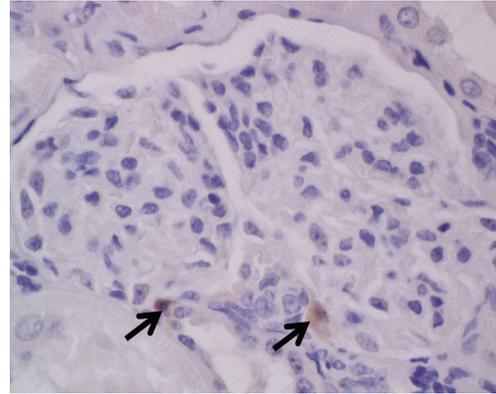


FIGURE 5: Peripolar cell (arrows) positive to S100 Protein. Streptavidin-Biotin-Peroxidase Method, 600x.

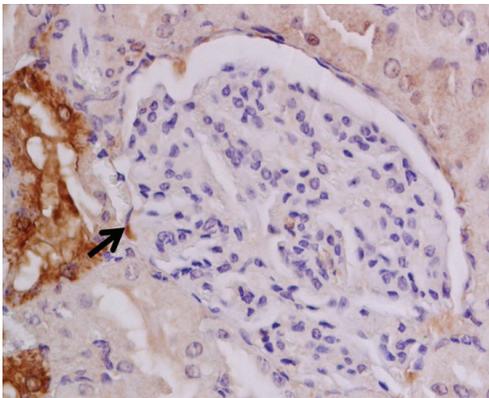


FIGURE 4: Peripolar cell (arrow) positive to Neuron-Specific Enolase (NSE). Streptavidin-Biotin-Peroxidase Method, 400x.

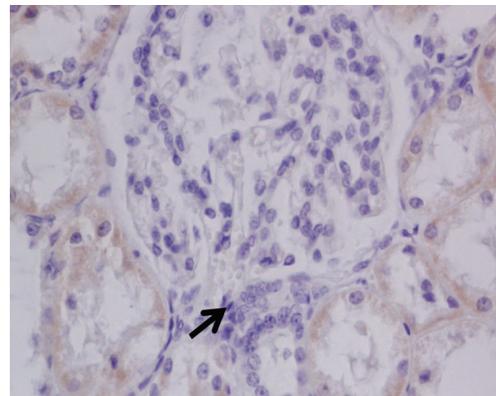


FIGURE 6: Peripolar cell (arrow) negative to Chromogranin A. Streptavidin-Biotin-Peroxidase Method, 600x.

We used different histochemical stains to identify peripolar cells. The cells were PAS-positive, and their cytoplasmic granules were stained blue with Toluidine Blue and red with Masson's Trichrome, according to previous studies [5, 7]. The Masson's Trichrome method yielded the best results in the identification of peripolar cells. The PPCs' granulosity was not clearly visible with PAS, Toluidine Blue, and Hematoxylin-Eosin.

The trichromic method applied (Masson's Trichrome, Bradbury and Gordon [21]) allowed for clearer and more accurate identification of PPCs since the cytoplasm, muscle, and erythrocytes were stained in red with the acid fuchsin and the collagen fibres were stained in green with the light green dye. Thus, the different structures were stained in different colours, which allows for a better observation of PPCs. Previous studies also used another trichromic method—Martius Scarlet Blue (MSB) [22]. Using this method, the nuclei are stained dark-brown, collagen blue, erythrocytes yellow and muscle and fibrin fibres are stained in red. Since in both trichromic methods PPCs' granules are stained in red, we decided to use a method more routinely used in a histology laboratory.

The ease of identification of PPCs in normal kidneys and in kidneys with lesion was slightly different. In the kidneys with a diagnosis of congestion and haemorrhage, the PPCs were more easily identified. However, in kidneys with microtrombi (with a diagnosis of thrombosis of the glomerular capillary), the identification was harder. Thus, the maintenance of normal histological features of Bowman's space and the absence of intraglomerular lesions are favourable to the identification of PPCs.

After the quantification of PPCs, the PPI (Peripolar Cell Index) in the kidneys without visible macroscopic lesions varied between 29.4% and 72.5%. These results are consistent with previous studies, in which PPCs varied between 20% and 100% [3]. In the lesioned kidneys, the PPI varied between 27.5% and 68%.

In our study, PPI of young sheep was lower (56.39%) when compared to the adult sheep (59.57%). Our data do not support a study performed by Alcorn et al. [12], where the PPI in kidneys from newborn lambs was higher than that of the adult sheep.

According to Alcorn et al., the high number of PPCs in newborn lambs (from 12 h to 9 days) is due to peripolar cell hypertrophy [12]. This hypertrophy may reflect a functional adaptation of the kidney to immediate postnatal life, when

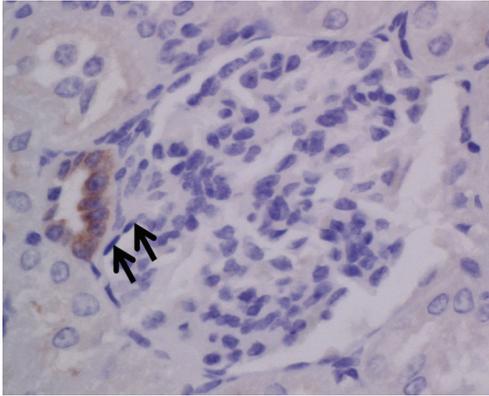


FIGURE 7: Peripolar cell (arrows) negative to Cyclooxygenase-2 (Cox-2). Streptavidin-Biotin-Peroxidase Method, 600x.

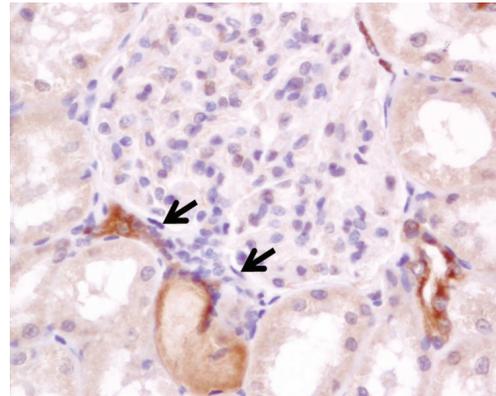


FIGURE 9: Peripolar cell (arrows) negative to Wide Spectrum Cytokeratin. Streptavidin-Biotin-Peroxidase Method, 400x.

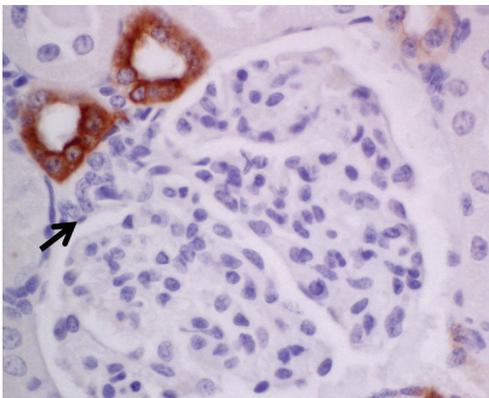


FIGURE 8: Peripolar cell (arrow) negative to AE1/AE3. Streptavidin-Biotin-Peroxidase Method, 600x.

the kidneys assume the water and electrolyte homeostasis for the first time. Similar results were found by Gall et al. [7] on mice and newborn sheep.

In our study, young sheep had a slightly higher number of PPCs per vascular pole ( $1.483 \pm 0.150$ ) when compared with adult sheep ( $1.402 \pm 0.153$ ). However, PPCs from adult sheep were bigger in size. This observation can be explained by the different cellular secretion pathways.

There are two different cellular secretion pathways described [23]: constitutive and regulated. In the constitutive secretion pathway, proteins are continuously secreted for cell maintenance, regardless of environmental factors. These secreted proteins are released and transported in the secretion vesicles to their final destination. On the other hand, in regulated secretion, protein's production and transport are only active when there is a release of specific secretion signals. Therefore, proteins are synthesized, stored in secretory vesicles, and only transported to their final destination in response to a specific signal. This regulated secretory pathway is characteristic of some nerve and endocrine cells.

The size of PPCs from adult kidneys can be a result of a regulated secretion, while PPCs from younger kidneys can be a result of a constitutive secretion. Different results were found by Thumwood et al. [9], in which PPCs from

newborn lambs had more granules, which could be suggestive of a regulated secretion, while PPCs from adult kidneys were smaller and with less granules, which can be related to a constitutive secretion.

The difference in the number of PPCs can be justified by the possible absence of mitosis observed in these cells. Thus, the loss of adult peripolar cells is not compensated by the formation of new others. Consequently, the kidneys from young sheep had higher number of peripolar cells per vascular pole, when compared to kidneys from adult sheep.

Concerning the number of peripolar cells in kidneys with lesion, previous studies have shown a significant increase of PPC's, namely, in sodium depletion in sheep [13] and in chickens [8], and in human malignant hypertension [14]. In this study, the number of peripolar cells in normal kidneys and kidneys with lesion was not statistically significant ( $P = 0.06$ ).

The composition of the cytoplasmatic granules that characterize peripolar cells is still not known. In 1988, Trahair and Ryan [18] showed the presence of plasmatic proteins in PPCs. Peripolar cells were also shown to be immunoreactive to transthyretin by Hollyweel et al. [15] and also to NSE by Trahair et al. [19]. However, until this date, it was not shown whether these proteins are a product of PPCs' synthesis or if they are a reabsorbed product from the glomerular ultrafiltrate [24].

The main goal of our study was to understand the role of PPCs by testing for the presence of the following proteins:  $\alpha$ -smooth muscle Actin, NSE, Chromogranin A, S100 Protein, desmin, Cox-2, AE1/AE3, and a Wide Spectrum Cytokeratin. As far as we know, there are no other studies about the positivity of these antibodies in PPCs (with the exception of NSE). Even though these antibodies were raised against human epitopes, we believe that the risk of cross-reactions is minimal since the proteins under study are highly conserved in the phylogenetic scale.

We obtained positive results to  $\alpha$ -smooth muscle actin that support our initial hypothesis that peripolar cells have a contractile function [17]. This protein plays a role in cell motility, structure, and integrity [25] and is a major constituent of the contractile apparatus. However, desmin,

an intermediate filament of muscle cells [16], appears to be absent in these cells.

PPCs have ultrastructural characteristics similar to neuroendocrine cells that are characterized by the presence of dense granules (neuroendocrine granules) associated with the cell membrane [26, 27]. We tested for the presence of NSE to explore the possibility of a neuroendocrinal origin of PPCs since NSE is an isoenzyme found in nerve and neuroendocrine cells [28]. We found that 25% of PPCs were positive to NSE, which is in line with previous studies. Trahair et al. [19] identified that a portion of PPCs exhibited NSE immunoreactivity, which provides some evidence of a nerve or neuroendocrine origin. However, since not all cells were immunoreactive, it is difficult to draw any conclusions.

S100 proteins comprise a family of 21 low molecular weight (9–13 kDa) proteins [29] that are implicated in inflammatory response, growth, cellular differentiation, cytoskeleton dynamics, enzyme activity, and  $\text{Ca}^{2+}$  homeostasis [30–32]. Regardless of their poor specificity, S100 proteins are also widely used for the diagnosis of neuroendocrine tumours. In our study, PPCs were positive to S100 proteins, which may also indicate a neuronal or neuroendocrine origin of the cells.

Chromogranin A is a member of the granin family of neuroendocrine secretory proteins, being located in secretory vesicles of neurons and endocrine cells [33]. In this study, PPCs were not positive to Chromogranin A, which contradicts our previous results that suggested a neuroendocrine role of PPCs.

Our tests for a neuroendocrine role of PPCs yielded contradictory results. However, even for the diagnosis of neuroendocrine tumours, it is advisable to use a battery of antibodies to minimize the occurrence of common false positives and false negatives to Chromogranin A [34].

Given the negative results of PPCs to Cox-2, we found no evidence to support an association between PPCs and the juxtaglomerular apparatus. Cyclooxygenase-2 (COX-2) is an isoenzyme that converts arachidonic acid to prostaglandins. When renin levels are low, Cox-2 expression and the synthesis of activated prostaglandins increase. Consequently, the renin levels increase as well as the levels of angiotensin II and aldosterone. This conducts a boost of tubular reabsorption to restore homeostasis. Therefore, the presence of Cox-2 in peripolar cells would indicate a relation with the juxtaglomerular apparatus.

In a study by Gardiner and Lindop [14], it was shown that there were more PPCs and other granulated cells in the cases of human malignant hypertension. However, there was no correlation between the number of peripolar cells and the number of renin-containing cells. Similar results were obtained by Gibson et al. [11]. Gardiner and his colleagues [35] also verified that neither the sodium depletion in Addison's disease nor the reduction in renal perfusion pressure affected the number of peripolar cells. Our findings support the results of these previous studies, which suggest that peripolar cells are not related to the juxtaglomerular apparatus.

We also had negative results to both A1/AE3 and Wide Spectrum Cytokeratin, which suggest that PPCs may not have

an epithelial nature. Cytokeratins are proteins of keratin-containing intermediate filaments, found in the cytoskeleton of epithelial tissue [36].

Peripolar cells are granulated cells located near the inflexion point between the parietal and visceral layers of the renal corpuscle. By histochemistry, the granularity of PPCs with PAS, Toluidine Blue, and HE was not clearly visible. Masson's Trichrome was the method that yielded the best results in the identification of peripolar cells. After quantification, we found no statistically significant difference between the number of cells in either kidneys of old and newborn animals ( $P = 0.53$ ) or normal kidneys and kidneys with lesion ( $P = 0.06$ ). By immunohistochemistry, peripolar cells were positive to  $\alpha$ -smooth muscle actin and less consistently positive to NSE and S100 protein. Peripolar cells did not show positive reaction to chromogranin A, desmin, cyclooxygenase-2, AE1/AE3, and Wide Spectrum Cytokeratin. Therefore, we believe that although there is evidence of a contractile function, we found no evidence supporting neither a neuroendocrine nor an epithelial nature of peripolar cells.

Peripolar cells are still poorly studied in humans and other animals. Since these cells are attached to the glomerular basement membrane by junctions, we believe that it would be interesting to perform tests for the presence of the cell adhesion molecules (e.g., E-Cadherin, vascular cell adhesion protein 1 (VCAM-1), or selectin). We believe that we found solid evidence supporting a contractile function of peripolar cells and that further studies should be performed in order to better understand the form and function of this cell population.

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