

An Immunohistochemical Study of the Oviduct in the Domestic Fowl (*Gallus domesticus*)

M.-C. MADEKUROZWA

Department of Anatomy and Physiology, University of Pretoria, Private bag X04, Onderstepoort 0110, South Africa. Tel: +27 12 529 8417. e-mail: mary.madekurozwa@up.ac.za

Summary

This study describes the distribution of vimentin, desmin, smooth muscle actin (SMA) and laminin in the oviduct of the laying domestic fowl. Vimentin immunostaining was localized in the luminal epithelium of the infundibulum, magnum, magnum isthmus junction and isthmus. The luminal epithelium of the shell gland regions displayed weak vimentin immunostaining. Vimentin immunostaining was demonstrated in the glandular grooves of the tubular infundibular region. In contrast, gland cells in the magnum, isthmus and shell gland regions were vimentin immunonegative. Fibroblasts and vascular endothelial cells in the lamina propria of the oviductal regions studied exhibited vimentin immunostaining. Strong desmin and SMA immunostaining was present in the smooth muscle cells of the tunica muscularis and vascular tunica media. In this study, basement membranes underlying the luminal and glandular epithelia were immunopositive for laminin. In addition, basement membranes associated with smooth muscle cells exhibited laminin immunostaining. The results of the study indicate that the immunolocalization of desmin, SMA and laminin in the oviduct of the domestic fowl is similar to that in the mammalian uterus. The immunolocalization of vimentin in the domestic fowl varies depending on the oviductal region.

Key words: avian, oviduct, vimentin, desmin, smooth muscle actin, laminin.

Introduction

The oviduct in birds is composed of the infundibulum, magnum, isthmus, tubular shell gland, shell gland pouch and vagina (Solomon, 2002). Extensive histological and ultrastructural studies have been conducted on the various regions of the oviduct in several avian species (Wyburn et al., 1970; Draper et al., 1972; Fertuck and Newstead, 1970; Madekurozwa, 2005, 2007a).

Vimentin, desmin and smooth muscle actin (SMA) are cytoskeletal proteins, which are involved in structural support, cell migration, cellular differentiation and contractility (Amsterdam and Aharoni, 1994; Goldman et al., 1996; Galou et al., 1997; Fletcher and Mullins, 2010). Vimentin is localized in cells of mesenchymal origin, as well as in certain epithelial cells, while desmin and SMA are expressed by muscle cells (Lazarides, 1980; Kohnen et al., 2000; Korgun et al., 2007). Desmin is a marker for all muscle types, while SMA is specific for smooth muscle (Lazarides, 1980). In addition to desmin and SMA, smooth muscle cells are characterized by the presence of an enclosing basement membrane, which is immunopositive for the glycoprotein laminin (Abd-Elmaksoud, 2009). Laminin is also a component of the basement membrane underlying luminal and glandular epithelia (Tanaka et al., 2009). Studies have shown that the sequences of vimentin, desmin, SMA and laminin have been conserved during evolution (Nelson and Traub, 1982; Kabsch and Vandekerckhove, 1992; Galkin et al., 2010). The cross reactivity of mammalian cytoskeletal and basement membrane antibodies to avian tissue has been demonstrated in several studies (Madekurozwa, 2007b; Aire and Ozegbe, 2007, 2008; Rodler and Sinowatz, 2011).

In mammals, changes in the expression of cytoskeletal proteins and laminin have provided an insight into the effect of toxins on the cytoskeleton and basement

membranes of uterine tissue (Mehasseb et al., 2009). Thus, cytoskeletal proteins and laminin have been extensively used in the diagnosis of uterine tumours (Faber et al., 1986; Perez-Martinez et al., 2001; McCluggage 2002; Gil da Costa et al., 2009; Stewart and Little, 2010). There is currently a lack of information on the immunolocalization of cytoskeletal proteins and basement membrane markers in the avian oviduct. The present study was undertaken to provide baseline information on the distribution of the intermediate filaments, vimentin and desmin, as well as the microfilament SMA in the oviduct of the laying domestic fowl. In addition, basement membranes in the oviductal regions were studied using an antibody against laminin.

Materials and Methods

A total of 10 actively laying white Leghorn hens (*Gallus domesticus*) were kept in individual cages under a light regime of 16 h light: 8 h dark. Feed and water were provided *ad libitum*. The birds were euthanized with an overdose of sodium pentobarbital (Sagatal, May and Baker, Port Elizabeth, South Africa). All the procedures used in this study were approved by the Animal Use and Care Committee of the University of Pretoria.

The thoraco-abdominal cavity was cut open, and the oviduct was removed. The position and state of the forming egg in the oviduct was noted. Tissue samples were collected from the infundibulum (funnel and tubular regions), middle region of the magnum, magnum-isthmus junction (MIJ), the proximal and middle regions of the isthmus and the tubular and pouch regions of the shell gland.

The tissue samples were immersion-fixed in 4% phosphate buffered formaldehyde for 48 h. After fixation, the tissues were processed routinely for histology and embedded in paraffin wax. The immunostaining technique was

performed on 5 µm thick sections using a Universal LSAB-plus kit, Peroxidase (DakoCytomation, Glostrup, Denmark).

The sections were deparaffinized and endogenous peroxidase activity was blocked, using a 3% (v/v) hydrogen peroxide solution in water for 5 min. The slides were then rinsed in a 0.01 M phosphate buffered saline solution (PBS, pH 7.4) for 5 min. Thereafter, the sections for vimentin, desmin and SMA immunohistochemistry were microwaved at 750 W for three cycles of 7 min each. After being allowed to cool for 20 min the sections were rinsed with PBS. Antigen retrieval on sections for laminin immunohistochemistry was performed by incubating the sections with Proteinase K (Dakocytomation, Glostrup, Denmark) in a 0.05 mol/L Tris-HCl (pH 7.6) solution for 6 min.

The sections were incubated for 1 h at room temperature with monoclonal antibodies against desmin, vimentin and SMA at dilutions of 1:50, 1:25 and 1:50 respectively. In addition, a polyclonal laminin antibody at a dilution of 1:100 was used. The primary antisera were purchased from Dakocytomation, Glostrup, Denmark. After the incubation with primary antibodies the slides were rinsed with PBS and then incubated for 15 min with a biotinylated secondary antibody (LSAB-plus kit). Thereafter, the slides were rinsed in PBS and subsequently incubated for 15 min with the streptavidin peroxidase component of the LSAB-plus staining kit. Slides were then rinsed in PBS and bound antibody was visualized after the addition of a 3,3'-diaminobenzidine tetrachloride solution (LSAB-plus kit). The sections were counter-stained with Mayer's haematoxylin.

In the negative controls the monoclonal desmin, vimentin and SMA antibodies were replaced with mouse IgG1 (Dakocytomation, Glostrup, Denmark), while the polyclonal laminin antibody was substituted with rabbit immunoglobulin fraction

(Dakocytomation, Glostrup, Denmark). The negative control reagents were diluted to the same concentration as the primary antibodies. Smooth muscle was used as a positive control for desmin, SMA and laminin, while tonsillar tissue was used as a positive control for vimentin. Variations in the immunostaining of sections used in this study were minor. No background staining was detected in the negative control sections (Fig. 5c), while positive immunostaining for vimentin, desmin, SMA and laminin was observed in the control sections.

On the basis of visual examination, the relative intensities of vimentin, desmin and SMA immunostaining were designated as absent (-), weak (+), moderate (++) and strong (+++) as described previously (Mdekurozwa and Kimaro, 2006).

Table 1. Summary of the immunohistochemical localization of vimentin, desmin and smooth muscle actin (SMA) in the oviduct of the domestic fowl

	Vimentin	Desmin	SMA
<i>Luminal epithelium</i>			
Infundibulum	+++	-	-
Magnum	++/+++	-	-
MIJ	++/+++	-	-
Isthmus	+ / ++	-	-
Shell gland	- / +	-	-
Vagina	-	-	-
<i>Glandular epithelium</i>			
Infundibulum (tubular)	+++	-	-
Magnum	-	-	-
Isthmus	-	-	-
Shell gland	-	-	-
<i>Fibroblasts</i>			
	+ / ++ / +++	-	-
<i>Endothelial cells</i>			
	++ / +++	-	-
<i>Mesothelial cells</i>			
	+++	-	-
<i>Smooth muscle cells</i>			
	-	+++	+++
<i>Pericytes</i>			
	-	+++	++

Intensities of immunostaining: -, absent; +, weak; ++, moderate; +++, strong.

Results

The distribution and intensity of immunostaining were unaffected by the location of the forming egg. The immunostaining intensities of vimentin, desmin and SMA in various cells of the oviduct are summarized in Table 1.

Luminal epithelium

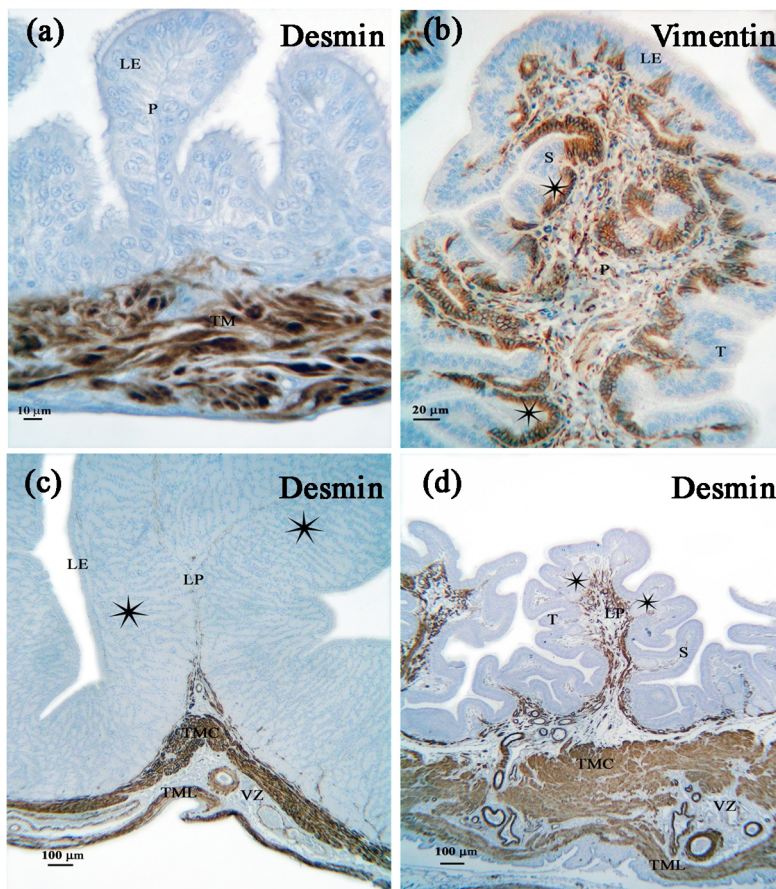


Figure 1. Survey photomicrographs of **(a)** infundibular funnel, **(b)** infundibular tubular region, **(c)** magnum, **(d)** isthmus. Primary (P), secondary (S) and tertiary (T) mucosal folds. TM: Tunica muscularis. TMC: inner circular layer. TML: outer longitudinal layer. VZ: vascular zone. LP: lamina propria core. Asterisks: tubular glands. LE: luminal epithelium.

The oviductal mucosa was arranged in folds of varying complexity (Fig. 1-3). The mucosal folds were lined with pseudostratified columnar epithelium consisting of

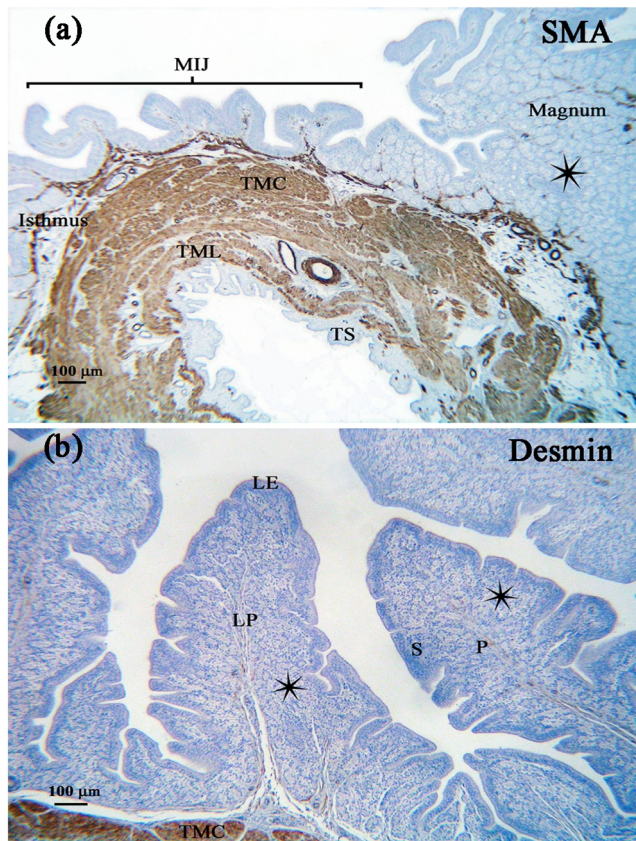


Figure 2. Survey photomicrographs of **(a)** MIJ, magnum, isthmus, **(b)** tubular shell gland. Primary (P) and secondary (S) mucosal folds. Tunica muscularis with inner circular (TMC) and outer longitudinal (TML) layers. LP: lamina propria core. Asterisks: tubular glands. LE: luminal epithelium. TS: tunica serosa.

ciliated and non-ciliated cells. Underlying the epithelium was a laminin immunopositive basement membrane (Fig. 3a).

In the funnel region of the infundibulum, the luminal epithelium (LE) lining the basal and lower lateral regions of the mucosal folds displayed strong vimentin immunostaining. Strong vimentin immunoreactivity in the tubular region of the infundibulum was observed in groups of cells lining the apical regions of primary folds (Fig. 4a). In both funnel and tubular regions vimentin immunostaining was observed throughout the cytoplasm of the epithelial cells.

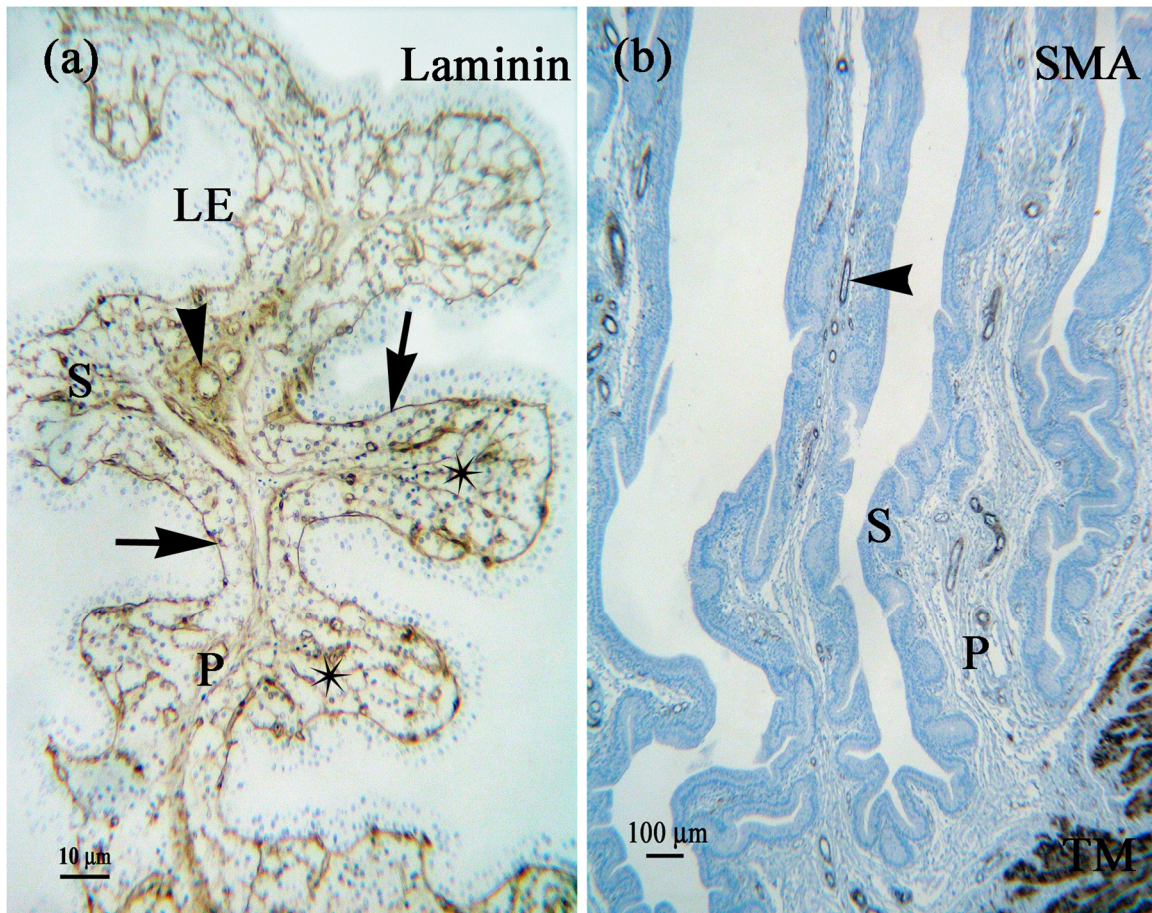


Figure 3. Survey photomicrographs of primary (P) and secondary (S) folds in **(a)** shell gland pouch and **(b)** vagina. Arrows: basement membrane. Asterisks: tubular glands. LE: luminal epithelium. Arrowheads: blood vessels. TM: tunica muscularis.

Vimentin immunostaining in the magnum was localized predominantly in the basal regions of non-ciliated cells lining the lateral and basal regions of the mucosal folds (Fig. 5a). Occasional non-ciliated cells displayed perinuclear vimentin immunostaining. At the magnum-isthmus junction (MIJ) the LE lining the basal regions of the mucosal folds displayed moderate to strong vimentin immunostaining. In the isthmus strong vimentin immunostaining was confined to epithelial cells lining the apical regions of secondary folds. Cells lining the basal and lateral regions of the secondary folds exhibited weak to moderate vimentin immunostaining.

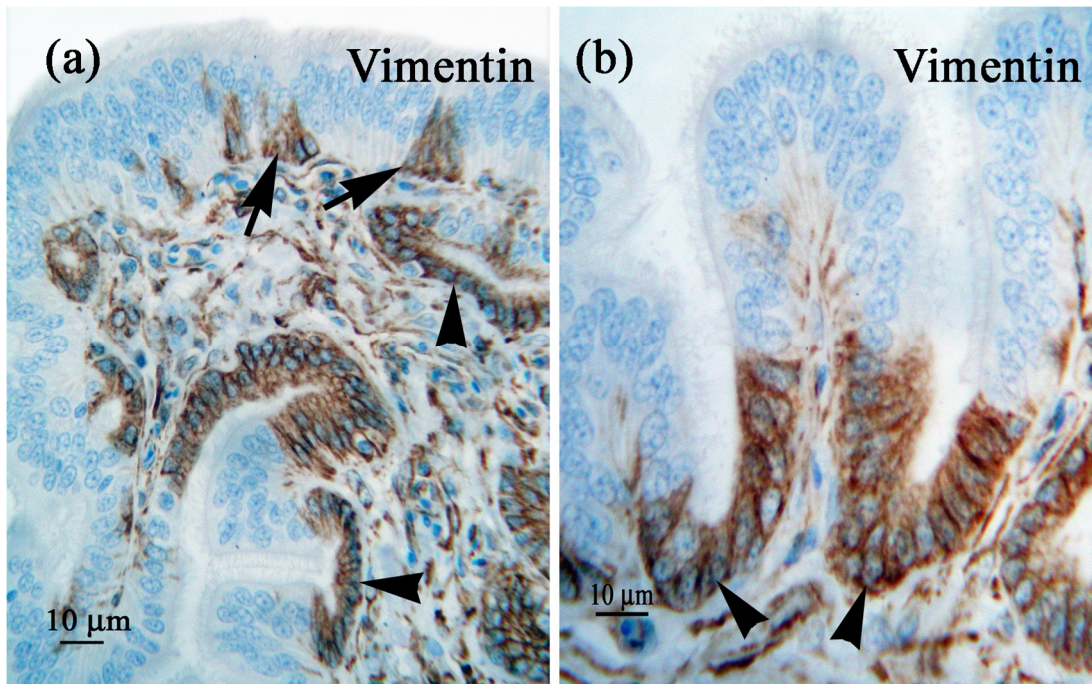


Figure 4. **(a) & (b)** Infundibulum tubular region. Strong immunostaining in apical epithelial regions (arrows) and in glandular grooves (arrowheads).

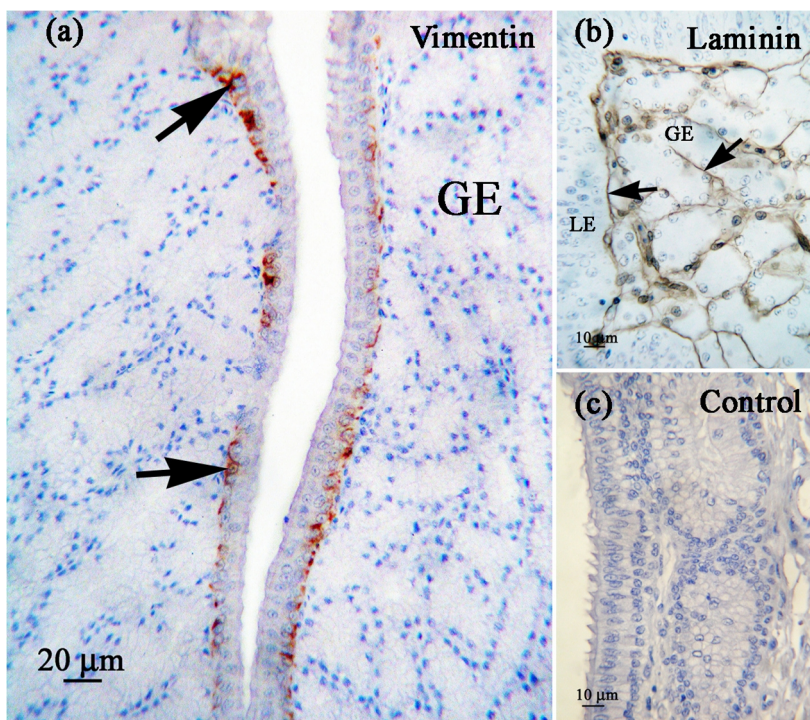


Figure 5. **(a)** Magnum. Immunopositive LE (arrows). GE is immunonegative. **(b)** Tubular shell gland. Immunopositive basement membranes (arrows) underlie GE and LE. **(c)** Negative control. Incubated with normal mouse IgG.

Vimentin immunostaining in the LE of the shell gland regions was weak or absent. The LE of the vagina was vimentin immunonegative.

Glandular epithelium

The mucosa of the MIJ was non-glandular (Fig. 2a). The glandular grooves in the tubular region of the infundibulum were lined by vimentin immunopositive non-ciliated cells (Fig. 4 a & b). In the magnum, isthmus and shell gland regions the glandular epithelium (GE) consisted of vimentin immunonegative cells (Fig. 5a). Underlying the GE was a laminin immunopositive basement membrane, which defined the branching profile of the tubular glands (Fig. 5b). Branched tubular glands were observed in the magnum, isthmus and shell gland regions. In the isthmus several tubular glands drained into a single duct, which opened onto the luminal surface.

Lamina propria

In the magnum, isthmus and shell gland regions, tubular glands filled the lamina propria between the LE and the centre of the primary mucosal folds (Fig. 1c; Fig. 2b). The lamina propria in the central areas of the primary mucosal folds contained fibroblasts, blood vessels and smooth muscle bundles (Fig. 1c & d). Vimentin immunostaining in the fibroblasts varied from weak to strong. The endothelial cells lining blood vessels displayed moderate to strong vimentin immunostaining (Fig. 6a). The tunica media surrounding the endothelial cells was immunopositive for SMA, desmin and laminin (Fig. 3b; Fig. 6b & c). Immunostaining for vimentin, desmin and SMA was cytoplasmic, while laminin was localized in basement membranes associated with smooth muscle cells.

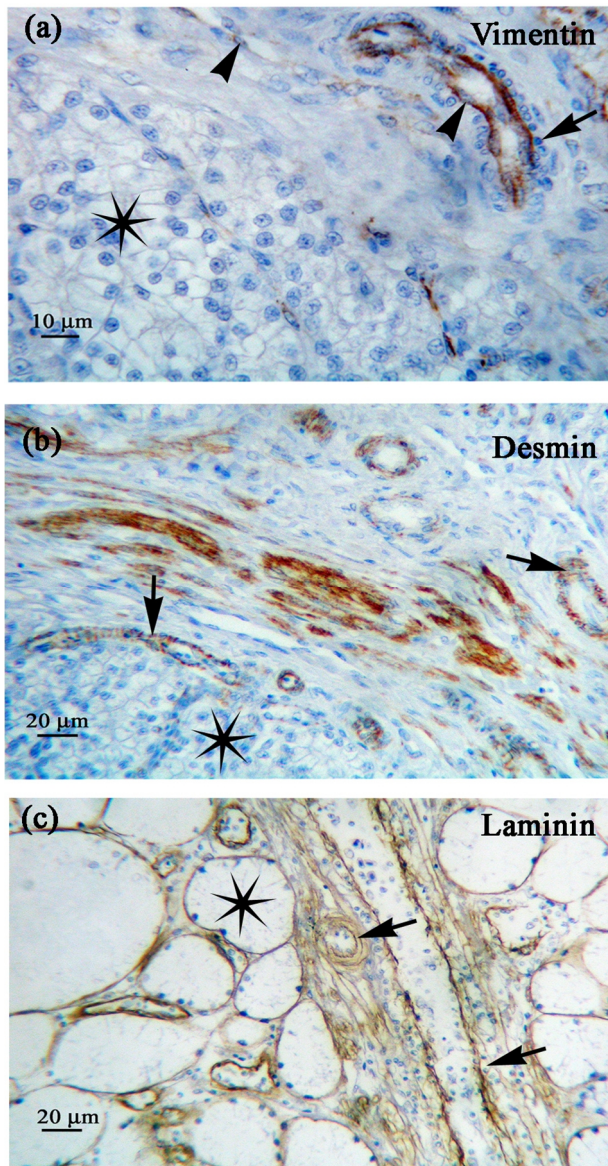


Figure 6. **(a) & (b)** Tubular shell gland. **(c)** Magnum. Arrowheads: endothelial cells. Arrows: tunica media. Asterisks: tubular glands.

Branches from blood vessels located in the central lamina propria core coursed between the tubular glands and eventually formed a capillary network below the LE. Endothelial cells of the sub-epithelial capillary networks were immunopositive for vimentin, but immunonegative for desmin and SMA (Fig. 7a). Desmin and SMA immunopositive pericytes encircled the endothelial cells (Fig. 7b). A laminin

immunopositive basement membrane separated the pericytes from the endothelial cells (Fig. 7c).

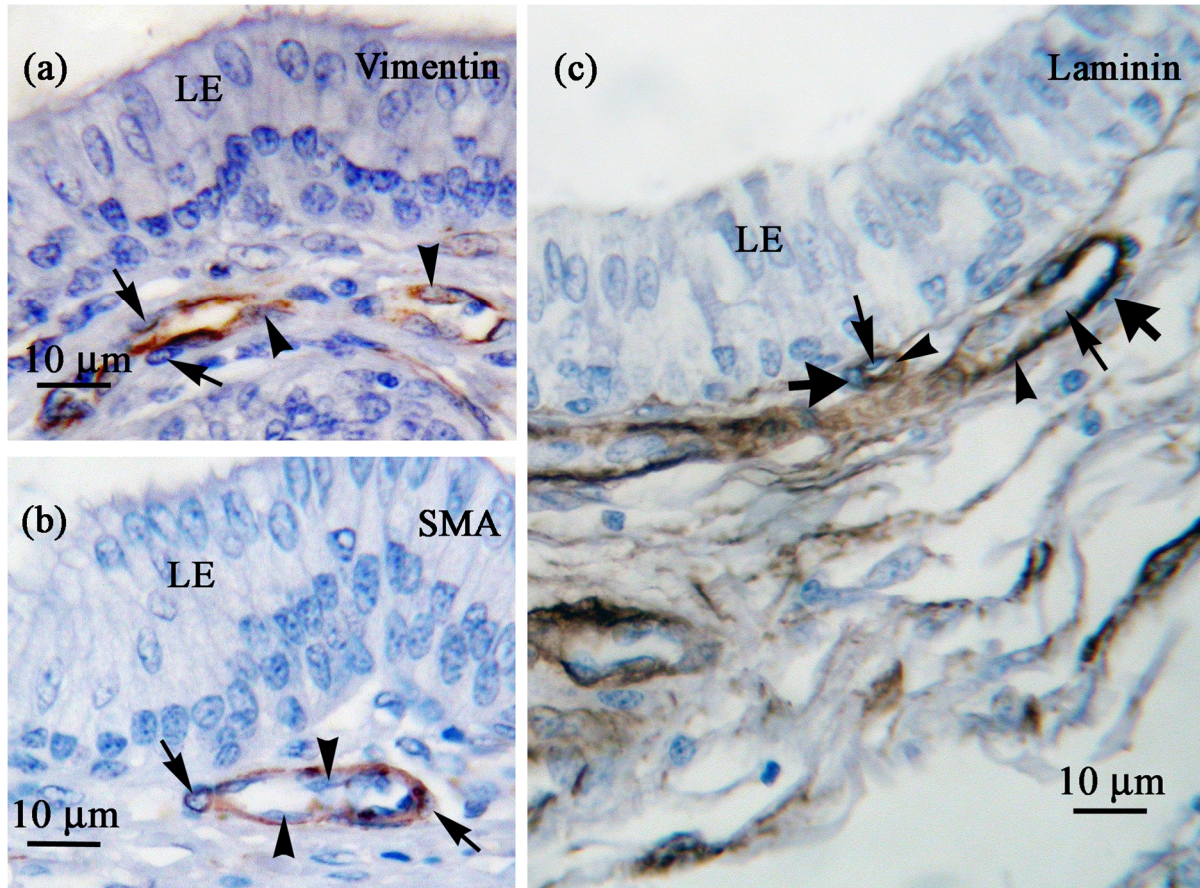


Figure 7. Vagina. LE: luminal epithelium. **(a)** Immunopositive endothelial cells (arrowheads) encircled by immunonegative pericytes (arrows). **(b)** SMA immunopositive pericytes (arrows). Arrowheads: endothelial cells. **(c)** Basement membrane (arrowheads) between pericytes (thick arrows) and endothelial cells (thin arrows).

Tunica muscularis

The tunica muscularis in the funnel and tubular regions of the infundibulum consisted of randomly orientated smooth muscle bundles (Fig. 1a). The tunica muscularis consisted of inner circular and outer longitudinal smooth muscle layers in the magnum, isthmus, shell gland and vagina (Fig. 1c & d; Fig. 2a). The muscle layers

were separated by vascular areas. Smooth muscle cells in the tunica muscularis expressed strong immunostaining for desmin, SMA and laminin (Fig. 1a; Fig. 2a).

Tunica serosa

The tunica serosa (Fig. 2a) underlying the tunica muscularis consisted of a thin, undulating layer of loose connective tissue, which was lined by vimentin immunopositive mesothelial cells.

Discussion

The present immunohistochemical study, which describes the oviductal distribution of vimentin, desmin, SMA and laminin, complements extensive morphological observations of the domestic fowl oviduct (Johnston et al., 1963; Breen and De Bruyn, 1969; Draper et al., 1972; Wyburn et al., 1970; Yu and Marquardt, 1973).

In the present study vimentin immunostaining was expressed in the LE lining the basal and lower lateral regions of the infundibulum, magnum, MIJ and isthmus. The reason for the basolateral immunolocalization of vimentin in the present study is unclear. Bailey et al. (2010) hypothesized that in the pig uterus the basolateral immunoexpression of vimentin was associated with the presence of cells undergoing remodeling. Studies have shown that the expression of vimentin increases in cells undergoing remodeling and proliferation (Wang and Stamenovic, 2000, 2002).

Underlying the LE in the present study was a capillary network. In mammals the sub-epithelial capillary network in the endometrium is vital during the establishment of the placenta (Dantzer and Leiser, 1994). In the present study the sub-epithelial capillary network was presumably important in the supply of nutrients to the secretory cells of the luminal epithelia. The results of the present study have

shown that the endothelial cells in the sub-epithelial capillary network are immunopositive for vimentin, but immunonegative for desmin. The presence of either vimentin, desmin or a co-localization of the two intermediate filaments has been reported in endothelial cells of capillary networks in various organs of the domestic fowl (Fujimoto and Singer, 1986). The reason for variations in the expression of vimentin and desmin by capillary endothelial cells in the domestic fowl is unclear.

In the current study pericytes enclosing endothelial cells in the sub-epithelial capillary network were immunopositive for both desmin and SMA, but immunonegative for vimentin. In contrast, desmin and vimentin have been co-localized in pericytes in the kidney, pancreas and cardiac muscle of the domestic fowl (Fujimoto and Singer, 1987). The expression of desmin and SMA by pericytes is an indication of the contractile ability of these cells (Fujimoto and Singer, 1987; Herman and D'Amore, 1985). It is known that pericytes are involved in the alteration of capillary diameter, which results in changes in capillary blood flow (Peppiatt et al., 2006; Kutcher and Herman, 2009).

The immunoexpression of vimentin by GE in the present study depended on the oviductal region. The GE was vimentin immunopositive in the tubular region of the infundibulum, but immunonegative in the rest of the oviduct. In mammals vimentin immunoreactivity of the GE is species dependent. The endometrial GE is vimentin immunopositive in the human (Norwitz et al., 1991), but immunonegative in the horse (Aupperle et al., 2004) and rat (Korgun et al., 2007).

A laminin immunopositive basement membrane lined the oviductal GE in the domestic fowl. The immunolocalization of laminin in the basement membrane underlying the GE clearly defined the profile, branching pattern and distribution of the tubular glands. In mammals laminin has been localized in basement membranes

underlying the endometrial luminal and glandular epithelia in the human (Iwahashi et al., 1996), baboon (Fazleabas et al., 1997) and rat (Korgun et al., 2007). Laminin influences a multitude of activities including epithelial differentiation, cellular remodeling, cell adhesion and basement membrane formation (Tennenbaum et al., 1996; Ekblom et al., 1998; Alcaraz et al., 2008; Yurchenco and Patton, 2009).

In the current study smooth muscle cells of the tunica muscularis and vascular tunica media demonstrated strong desmin and SMA immunostaining. Desmin immunoexpression has been reported in the myometrium of the human (Leoni et al., 1990), pig (Persson and Rodriguez-Martinez, 1997), horse (Aupperle et al., 2004), rat (Korgun et al., 2007) and mouse (Mehasseb et al., 2009). Smooth muscle cells in the myometrium of the the mink (Winther et al., 1999) and mouse (Mehasseb et al., 2009) exhibit SMA immunostaining. Desmin and SMA are involved in muscular contractions of the myometrium initiated during parturition (Taggart and Morgan, 2007). In birds contractions of the oviductal tunica muscularis are responsible for the transportation of the egg distally (Crossley et al., 1975).

Immunohistochemical studies on the mammalian uterus have localized desmin in the tunica media of the human (Leoni et al., 1990), pig (Persson and Rodriguez-Martinez, 1997), horse (Aupperle et al., 2004), rat (Korgun et al., 2007) and mouse (Mehasseb et al., 2009). SMA immunostaining has been reported in the tunica media of uterine blood vessels located in the mink (Winther et al., 1999) and mouse (Mehasseb et al., 2009). Desmin and SMA in vascular smooth muscle cells have a role in the adaptation of blood vessels to alterations in blood flow (Loufrani et al., 2002; Stiebellehner et al., 2003).

In addition to desmin and SMA smooth muscle cells are characterized by the presence of laminin. In the present study laminin was immunolocalized in the

basement membrane of smooth muscle cells in the tunica muscularis, as well as in the vascular tunica media. Laminin associated with smooth muscle cells has been demonstrated immunohistochemically in the human (Iwahashi et al., 1996; Tanaka et al., 2009), rat (Korgun et al., 2007) and mouse (Mehasseb et al., 2009).

The results of the present study indicate that the cellular localization of desmin and SMA in the oviduct of the domestic fowl is similar to that reported in the vasculature and myometrium of mammals. Furthermore, the localization of laminin in the basement membranes of the epithelial and smooth muscle cells is as reported in mammals. Further studies are needed to ascertain the developmental stage of epithelial oviductal cells in the domestic fowl expressing vimentin.

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