Immunolocalization of Intermediate Filaments and Laminin in the Oviduct of the Immature and Mature Japanese Quail (*Coturnix coturnix japonica*)

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Summary

The present study describes the distribution of vimentin, desmin, smooth muscle actin (SMA) and laminin in the oviduct of the immature and mature Japanese quail. The cytoskeletal proteins vimentin, desmin and SMA have been shown to be involved in cellular support, differentiation, migration and contractility. Laminin is a major component of basement membranes. Luminal epithelia in the infundibular and magnal regions of immature and mature birds exhibited strong vimentin immunoreactivity. Luminal epithelial cells exhibiting strong vimentin immunoreactivity were present in the isthmus and shell gland regions of only mature quails. Infundibular glandular grooves displayed strong vimentin immunostaining. In

contrast, the glandular epithelia of the magnum, isthmus and shell gland were vimentin immunonegative. Fibroblasts and vascular endothelial cells in the lamina propria of the oviductal regions studied exhibited strong vimentin immunostaining. Smooth muscle cells forming the tunica muscularis and vascular tunica media displayed strong desmin and SMA immunostaining. Strong laminin immunostaining was demonstrated in the basement membranes associated with smooth muscle cells, as well as in the basement membranes underlying the luminal and glandular epithelia.

In conclusion this study has shown that the immunolocalization of desmin, SMA and laminin in the oviduct of the Japanese quail is similar to that in the domestic fowl. However, differences in the immunoexpression of vimentin in the LE of the two avian species were shown to exist. In addition, the study has shown that the immunolocalization of vimentin in the Japanese quail varies depending on the oviductal region, as well as the developmental stage of the oviduct.

Introduction

The avian oviduct is sub-divided into the infundibulum, magnum, isthmus, shell gland, and vagina (Solomon, 2002). In addition, the oviduct contains specialized regions in the infundibulum and utero-vaginal junction where spermatozoa are stored (Frieβ *et al*., 1978; Koyanagi and Nishiyama, 1981). Several studies have been carried out on the histology and ultrastructure of the oviduct in immature and mature birds (Wyburn *et al*., 1970; Fertuck and Newstead, 1970; Draper *et al*., 1972; Holm and Ridderstrale, 2002; Madekurozwa, 2005, 2007a; 2012; Madekurozwa, 2013).

The cytoskeletal proteins vimentin, desmin and smooth muscle actin (SMA) play a role in structural support, cellular differentiation, cell migration, and

contractility (Amsterdam and Aharoni, 1994; Goldman *et al.*, 1996; Galou *et al.*,1997; Fletcher and Mullins, 2010). Vimentin is present in cells of mesenchymal origin, as well as in certain epithelial cells (Lazarides, 1980; Kohnen *et al.*, 2000; Korgun *et al.*, 2007). SMA is a specific marker for smooth muscle, while desmin is expressed by all muscle types (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 present in the basement membranes underlying luminal and glandular epithelia (Tanaka *et al.*, 2009).

Avian diseases, such as infectious bronchitis (Chousalkar and Roberts, 2007), Newcastle disease (Solomon, 2002), mycoplasma synoviae infection (Feberwee *et al.*, 2009) and egg drop syndrome (Raj *et al.*, 2001), affect the normal functions of the various regions of the oviduct leading to a decrease in the quality and quantity of eggs produced. Thus far the pathogenesis of diseases affecting the oviduct have been studied using mostly histopathological and ultrastructural techniques (Chousalkar and Roberts, 2007; Chousalkar *et al.*, 2009). This is contrary to the situation in mammals where pathologies of the female reproductive tract are increasingly being studied using immunohistochemistry. In particular, antibodies against intermediate filaments and microfilaments are widely used to study the effect of inflammatory conditions and neoplasia on the cytoskeleton of cells forming the reproductive tract (Perez-Martinez *et al.*, 2001; Aupperle *et al.*, 2004; Gil da Costa *et al.*, 2009). Furthermore, antibodies against the glycoprotein laminin have been used to study the effects of various pathological conditions on the morphology of endometrial basement membranes (Faber *et al.*, 1986).

The Japanese quail has been successfully used as an avian model to study the effects of viruses and toxins on growth and reproductive activity (Rathnamohan, 1985; Bryan *et al*., 1989; Kamata *et al*., 2009**)**. Information gained from studies on the Japanese quail is used to assess the possible impact of viral and toxic agents on domestic and wild birds. Despite the extensive use of the Japanese quail in research, there is currently a lack of information on the immunolocalization of cytoskeletal proteins and basement membrane markers in the oviduct of this species. It is envisaged that such information will be used in investigations on the effects of toxins, viruses and neoplasias on the cytoskeleton and basement membranes 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 oviducts of immature and mature Japanese quails. In addition, basement membranes in the oviductal regions were studied using an antibody against laminin.

Materials and Methods

Animals and tissue preparation

Ten immature (4 weeks old) female Japanese quails (*Coturnix coturnix japonica*) were kept in individual cages under a light regime of 16 h light: 8 h dark. This light regime had been maintained from hatch as per the guidelines of the Agricultural Research Council, Pretoria. Ten actively laying quails, with an average bodyweight of 210.2 ± 5.6 g (mean ± SE), were maintained at a light regime of 16 h light: 8 h dark. Two females were housed in cages with one male. 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) at between 10 and 11am. All the procedures used in this study were approved by the Animal Use and Care Committee of the University of Pretoria.

Tissue samples were collected from the infundibulum (funnel and tubular regions), and the middle regions of the magnum, isthmus, shell gland pouch, utero-vaginal junction (UVJ) and vagina. The tissue samples were immersion-fixed in 4% phosphate buffered formaldehyde for 48 h.

Immunohistochemistry

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, while positive immunostaining for vimentin, desmin, SMA and laminin was observed in the control sections.

Results

The distribution and intensity of immunostaining in mature quails was unaffected by the location of the forming egg. The shell gland regions of 8 sexually mature quails contained eggs. Forming eggs were found in the magnal regions of the remaining 2 quails.

Infundibulum (funnel and tubular regions)

Luminal epithelium (LE)

The funnel region in immature and mature birds was arranged in primary folds. The tubular region of immature birds displayed primary folds, while primary, secondary and tertiary folds were observed in mature birds.

Mucosal folds in the infundibular regions of both immature and mature birds were lined by a simple columnar epithelium, composed of ciliated and non-ciliated cells (Figs 1a and 2a,c). Strong vimentin immunostaining in the funnel regions of immature and mature quails was confined to non-ciliated epithelial cells. These cells were located predominantly in the basal and lateral regions of the mucosal folds (Fig. 1b). In the tubular region vimentin immunoreactivity was demonstrated in both ciliated and non-ciliated cells (Figs 2b,c).

Vimentin immunostaining in the infundibular LE was observed throughout the cytoplasm of the cells.

Glandular Epithelium (GE)

Infundibulum (tubular region)

Glandular grooves were observed in the tubular infundibular regions of only mature quails. No spermatozoa were observed in the glandular grooves. The glandular grooves were lined by vimentin immunopositive simple cuboidal non-ciliated cells (Figs 2b,c).

Lamina propria

Moderate to strong vimentin immunostaining was displayed by fibroblasts in both the funnel (Fig. 1b) and tubular regions of the infundibulum.

Desmin, SMA (Fig. 2a) and laminin immunostaining was demonstrated in smooth muscle cells forming the tunica media of blood vessels within the lamina propria of infundibular mucosal folds.

Sub-epithelial capillary networks were observed in both regions of the infundibulum. Endothelial cells in the sub-epithelial capillary networks were immunoreactive for vimentin, but immunonegative for SMA and desmin. Encircling the endothelial cells were desmin and SMA immunopositive pericytes. Interposed between the endothelial cells and pericytes was a laminin immunopositive basement membrane.

Tunica muscularis

Smooth muscle cells forming the tunica muscularis were immunopositive for desmin, SMA and laminin. The tunica muscularis in the funnel region of immature and mature birds consisted of smooth muscle cells interspersed with connective tissue and blood vessels (Fig. 1a). Well-defined inner circular and outer longitudinal smooth muscle layers formed the tunica muscularis in the tubular infundibular region.

Tunica serosa

Underlying the tunica muscularis was a tunica serosa composed of loose connective tissue, and a layer of vimentin immunopositive mesothelial cells.

Magnum

Luminal epithelium (LE)

The mucosal layer of the magnum, in both immature and mature birds, was arranged in primary folds (Fig. 3b).

Strong vimentin immunostaining was demonstrated in the perinuclear areas of ciliated and non-ciliated cells forming the magnal LE (Fig. 3a). Vimentin immunostaining in immature and mature birds was similar.

Glandular Epithelium (GE)

Wide areas of loose connective tissue separated the tubular glands in immature birds (Fig. 3b). In contrast, tubular glands in mature birds filled the area between the LE and the centre of the primary mucosal folds (Fig. 3a). The tubular glands in both immature and mature quails were vimentin immunonegative (Fig. 3a). Coursing between the tubular glands were capillaries with vimentin immunopositive endothelial cells (Fig. 3a).

Lamina propria

The central regions of the primary folds were occupied by wide areas of loose connective tissue in immature birds, while narrow areas containing small amounts of connective tissue were observed in mature birds. Coursing through the central lamina propria cores were desmin (Fig. 3b), SMA and laminin immunoreactive smooth muscle bundles. In addition, desmin, SMA and laminin immunostaining was demonstrated in smooth muscle cells forming the tunica media of blood vessels within the lamina propria. Vimentin immunostaining was demonstrated in vascular endothelial cells (Fig. 3a), as well as fibroblasts in the lamina propria.

A prominent sub-epithelial capillary network was present in the magnal region (Fig. 3a).

Tunica muscularis

Inner circular and outer longitudinal smooth muscle layers formed the tunica muscularis in the magnal regions of immature and mature quails. Smooth muscle bundles from the inner layer of the tunica muscularis extended into the magnal primary folds (Fig. 3b).

Tunica serosa

Loose connective tissue and a layer of vimentin immunopositive mesothelial cells formed the tunica serosa.

Isthmus

Luminal epithelium (LE)

Primary and secondary folds, lined by a simple columnar LE, were observed in the isthmic regions of immature and mature quails. The LE in immature birds was vimentin immunonegative, while several immunopositive cells were evident in mature birds (Fig. 4). Strong vimentin immunostaining was demonstrated throughout the cytoplasm of the immunopositive cells.

Glandular Epithelium (GE)

The GE in immature and mature birds was vimentin immunonegative (Fig. 4). The tubular glands in immature quails were separated by areas of loose connective tissue, while glands in mature birds were compact (Fig. 4).

Lamina propria

The lamina propria in the central areas of the isthmic mucosal folds contained fibroblasts, blood vessels and smooth muscle bundles. A capillary network was observed below the luminal epithelium. Vascular endothelial cells (Fig. 4) and fibroblasts displayed strong vimentin immunostaining. The tunica media surrounding the endothelial cells was immunopositive for SMA, desmin and laminin.

Tunica muscularis

Well-defined inner circular and outer longitudinal smooth muscle layers formed the tunica muscularis in the isthmus. Smooth muscle bundles extended from the inner tunica muscularis layer into the mucosal folds. The smooth muscle bundles exhibited strong desmin, SMA and laminin (Fig. 9a) immunostaining.

Tunica serosa

The tunica serosa was composed of loose connective tissue and vimentin immunopositive mesothelial cells.

Shell gland pouch

Luminal epithelium (LE)

The mucosa of the shell gland pouch in immature and mature birds was arranged in primary and secondary folds. The secondary folds were less prominent in immature birds (Fig. 5a). A simple to pseudostratified columnar LE, composed of non-ciliated and ciliated cells, lined the mucosal folds of immature and mature quails. Occasional vimentin immunopositive cells were demonstrated in the LE of mature birds (Fig. 5c).

In these cells strong vimentin immunostaining was distributed throughout the cytoplasm.

Glandular Epithelium (GE)

Extensive areas of loose connective tissue separated the tubular glands in immature birds (Figs 5a,b), while in mature birds glands filled the area between the LE and the centre of the primary mucosal folds. Tubular glands in both immature and mature birds were vimentin immunonegative. Numerous capillaries with vimentin immunopositive endothelial cells coursed between the tubular glands (Fig. 5b).

Lamina propria

Strong vimentin immunostaining was demonstrated in the fibroblasts of both immature and mature quails (Fig. 5c). In addition, strong vimentin immunostaining was exhibited by the endothelial cells of blood vessels in the lamina propria core, as well as in the sub-epithelial area (Fig. 5c). The tunica media of blood vessels exhibited desmin, SMA (Fig. 5b) and laminin immunostaining.

Tunica muscularis

The inner and outer layers of the tunica muscularis were separated by a well-defined vascular zone. As in previous regions, smooth muscle bundles from the inner layer of the tunica muscularis extended into the primary mucosal folds (Fig. 5a).

Tunica serosa

The tunica serosa underlying the tunica muscularis was formed by loose connective tissue, and a layer of vimentin immunopositive mesothelial cells.

Utero-vaginal junction (UVJ)

Luminal epithelium (LE)

The UVJ of immature birds was characterized by the presence of short mucosal folds (Fig. 6a), while the folds in mature birds (Fig. 6b) were similar to those in the shell gland. The ciliated pseudostratified columnar LE, lining the mucosal folds of both immature and mature birds, was vimentin immunonegative (Fig. 6c).

Glandular Epithelium (GE)

Mucosal folds in the proximal UVJ regions of mature birds contained both sperm storage tubules (SST), and glands typical of the shell gland (Fig. 7a). Relatively few SST were observed in the mucosal folds of immature birds (Fig. 6a), while folds in mature birds displayed numerous tubules (Fig. 6b). Spermatozoa were observed in the SST of mature birds (Fig. 7b). SST in immature birds were lined by columnar cells, while cuboidal to columnar cells were present in mature birds (Figs 6c and 7b). The SST of both immature and mature quails were vimentin immunonegative.

Lamina propria

Vimentin immunopositive fibroblasts were present in the lamina propria of immature and mature quails (Fig. 6c).

Desmin (Fig. 6a), SMA (Fig. 6b) and laminin were demonstrated in smooth muscle bundles coursing through the central lamina propria cores of primary folds in the UVJ. In addition, smooth muscle cells forming the tunica media of blood vessels displayed desmin, SMA (Fig. 7b) and laminin immunostaining.

A well-developed sub-epithelial capillary network was present in the UVJ of both immature and mature quails (Fig. 6c).

Tunica muscularis

Desmin (Fig. 6a), SMA and laminin were demonstrated in the smooth muscle cells forming the tunica muscularis.

The tunica muscularis in both immature and mature birds was formed by thick inner circular and outer longitudinal smooth muscle layers. As in previous oviductal regions, smooth muscle bundles from the inner layer of the tunica muscularis extended into the primary folds of the UVJ (Fig. 6b).

Tunica serosa

Loose connective tissue and vimentin immunoreactive mesothelial cells formed the tunica serosa.

Vagina

Luminal epithelium (LE)

Non-glandular primary and secondary folds formed the vaginal mucosa in immature and mature birds (Fig. 8). The ciliated pseudostratified columnar LE lining the mucosal folds was vimentin immunonegative.

Lamina propria

Desmin (Fig. 8), SMA and laminin immunoreactive smooth muscle bundles coursed through the central lamina propria cores of primary folds in the vagina. The tunica media of blood vessels within the lamina propria displayed desmin (Fig. 8), SMA and laminin immunostaining.

Tunica muscularis

Thick inner circular and outer longitudinal smooth muscle layers formed the tunica muscularis in the vaginal region. As in previous oviductal regions the smooth muscle cells forming the tunica muscularis were immunopositive for desmin (Fig. 8), SMA and laminin. In addition, smooth muscle from the inner layer of the tunica muscularis extended into the primary folds of the vagina (Fig. 8).

Tunica adventitia

Underlying the tunica muscularis was a tunica adventitia composed of loose connective tissue.

Basement membranes

Strong laminin immunostaining was demonstrated in basement membranes underlying the epithelia in all oviductal regions studied (Figs 9b,c). In addition, basement membranes associated with smooth muscle cells of the tunica muscularis and tunica media were laminin immunopositive (Figs 9a,c).

Discussion

The results of the present study complement extensive histological and ultrastructural observations of the oviduct in the immature and mature Japanese quail (Fertuck and Newstead, 1970; Hoffer, 1971; Frieβ *et al.*, 1978; Holm and Ridderstrale, 2002).

During egg formation the oocyte is engulfed by the funnel region of the infundibulum. Fertilization of the oocyte occurs in the tubular region of the infundibulum (Brillard, 2003). The present study indicated that vimentin

immunostaining in the LE of the infundibulum was similar in immature and mature quails. In the infundibular funnel vimentin immunostaining was demonstrated in cells lining the basolateral aspects of the mucosal folds. Similar observations have been made in the infundibular funnel of the domestic fowl (Madekurozwa, 2013), as well as in the uterus of the pig (Bailey *et al.*, 2010). It is thought that the basolateral localization of vimentin is due to the presence of a high number of cells undergoing proliferation and remodeling (Wang and Stamenovic, 2000, 2002; Bailey *et al.*, 2010). Vimentin immunostaining in the LE of the infundibular funnel was similar in the Japanese quail and domestic fowl (Madekurozwa, 2013). However, variations existed in the immunostaining of the LE in the tubular infundibular regions. Vimentin immunostaining in the Japanese quail occurred throughout the LE of the infundibular tubular region, while immunostaining in the domestic fowl was restricted to a few apical cells (Madekurozwa, 2013).

Magnal LE cells are responsible for the synthesis of the egg white protein avidin (Kohler *et al.*, 1968). The intracellular distribution of vimentin immunostaining in magnal LE cells of birds in the current study was similar to that described in the domestic fowl (Madekurozwa, 2013). However, the distribution of vimentin immunopositive cells in the magnal LE of the Japanese quail varied markedly from that of the domestic fowl. A basolateral distribution of immunopositive cells was observed in the domestic fowl, while vimentin immunopositive cells in the Japanese quail were evenly distributed throughout the LE.

The morphology of infundibular glandular grooves and SST in the present study was similar to observations made in the domestic fowl (Burke *et al.,* 1972), duck (Pal, 1977), Japanese quail (Frieβ *et al.,* 1978), mallard (Sharma and Duda, 1989), rhea (Parizzi *et al.*, 2008) and turkey (Bakst *et al.,* 2010). The sperm storage

capacities of both infundibular glandular grooves, as well as SST in the UVJ are well documented (Bobr *et al.,* 1964; Bakst, 1981, 1998, 2011; Bakst *et al*., 2010). Interestingly, in the present study spermatozoa were observed only in the SST of the UVJ. This may be due to the low number of spermatozoa stored in the infundibular glandular grooves (Bakst, 1998). In the current study, strong vimentin immunostaining was demonstrated in the glandular grooves of the infundibulum, but not in the SST of the UVJ. The presence of strong vimentin immunostaining in infundibular glandular grooves has recently been reported in the domestic fowl (Madekurozwa, 2013).

In the current investigation there was a marked difference in the developmental stage and distribution of magnal, shell gland and isthmic glands in immature and mature birds. Tubular glands in immature quails were sparsely distributed, while glands in mature birds were compacted. Similar observations have been made in the oviductal regions of the immature and mature domestic fowl (Breen and De Bruyn, 1969; Palmiter and Wrenn, 1971), Japanese quail (Hoffer, 1971; Eroschenko, 1979; Pageaux *et al*., 1986; Berg *et al*., 2001), ostrich (Madekurozwa, 2002, 2004) and rhea (Parizzi *et al*., 2008). A study by Yu and Marquardt (1973) has shown that the egg white protein ovalbumin is secreted by magnal tubular glands. Tubular glands in the isthmus produce shell membranes (Hoffer, 1971; Draper *et al*., 1972), while the shell gland is responsible for eggshell formation (Jonchere *et al*., 2010). Magnal, isthmic and shell gland tubular glands of immature and mature quails in the present study, as well as in the domestic fowl (Madekurozwa, 2013) were vimentin immunonegative. Vimentin immunoexpression in the glandular cells of mammals is species dependent. Endometrial glands are

vimentin immunonegative in the rat (Korgun *et al*., 2007) and horse (Aupperle *et al*., 2004), but immunopositive in the human (Norwitz *et al*., 1991).

In the present study laminin immunopositive basement membranes lined the GE and LE in both immature and mature quails. Similar observations have recently been made in the domestic fowl (Madekurozwa, 2013). In addition, laminin has been demonstrated in basement membranes underlying endometrial LE and GE in the human (Iwahashi *et al*., 1996), baboon (Fazleabas *et al*., 1997) and rat (Korgun *et al*., 2007). Laminin is known to play a role in basement membrane formation, epithelial differentiation and cellular remodeling (Tennenbaum *et al*., 1996; Ekblom *et al*., 1998; Alcaraz *et al*., 2008; Yurchenco and Patton, 2009).

In the current investigation desmin and SMA were demonstrated in the vascular tunica media. The distribution and immunostaining intensity of the smooth muscle cell markers were similar in immature and mature quails. Desmin and SMA immunoreactivity in the tunica media of birds has previously been demonstrated in ovarian blood vessels of the Japanese quail (Van Nassauw *et al*., 1992; Madekurozwa, 2012) and emu (Madekurozwa, 2007b), as well as in the oviductal blood vessels of the domestic fowl (Madekurozwa, 2013). Desmin and SMA in the vascular tunica media are involved in the adaptation of blood vessels to alterations in blood flow (Loufrani *et al*., 2002; Stiebellehner *et al*., 2003).

In birds, the forming egg is transported distally by rhythmic contractions of the tunica muscularis (Crossley *et al*., 1975). The contractility of the tunica muscularis has been studied in the domestic fowl (Crossley *et al*., 1975) and Japanese quail (Talo and Kekalainen, 1976; Arjamaa and Talo, 1983). In the current study the arrangement of the layers forming the tunica muscularis was in agreement with previous studies on the avian oviduct (Hoffer, 1971; Sharma and Duda, 1989; Parizzi

et al., 2008). As observed in the domestic fowl (Madekurozwa, 2013), the tunica muscularis of the Japanese quail was immunopositive for both desmin and SMA. In addition, the basement membranes associated with the smooth muscle cells forming the tunica muscularis displayed strong laminin immunoreactivity. Similar observations of desmin, SMA and laminin immunostaining have been made in the human (Iwahashi *et al*., 1996; Leoni *et al*., 1990), pig (Persson and Rodriguez-Martinez, 1997), horse (Aupperle *et al*., 2004) and mouse (Mehasseb et al., 2009).

The results of this study indicate that the localization of SMA, desmin and laminin in the oviduct of the Japanese quail is similar to that reported in the domestic fowl (Madekurozwa, 2013). However, notable differences exist in the immunoexpression of vimentin in the LE of the two avian species. In addition, the immunolocalization of vimentin in the Japanese quail varies depending on the oviductal region and the developmental stage of the oviduct.

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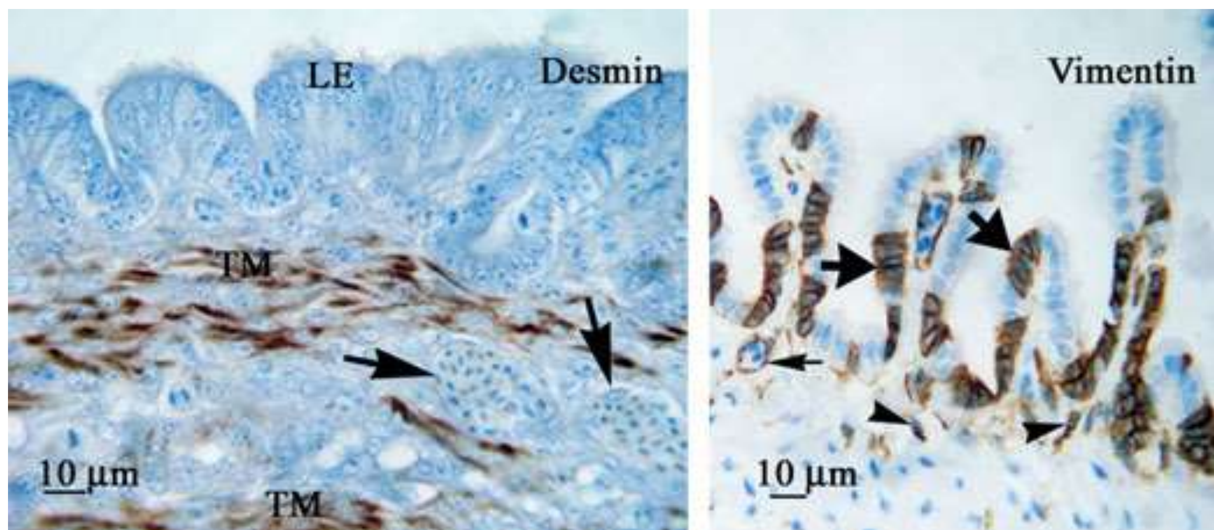

Figure 1. Infundibular funnel regions of immature **(a)** and mature **(b)** quails. **(a)** LE: Luminal epithelium. TM: Tunica muscularis. Arrows: Blood vessels. **(b)** Immunopositive non-ciliated cells (thick arrows), vascular endothelial cell (thin arrow) and fibroblasts (arrowheads).

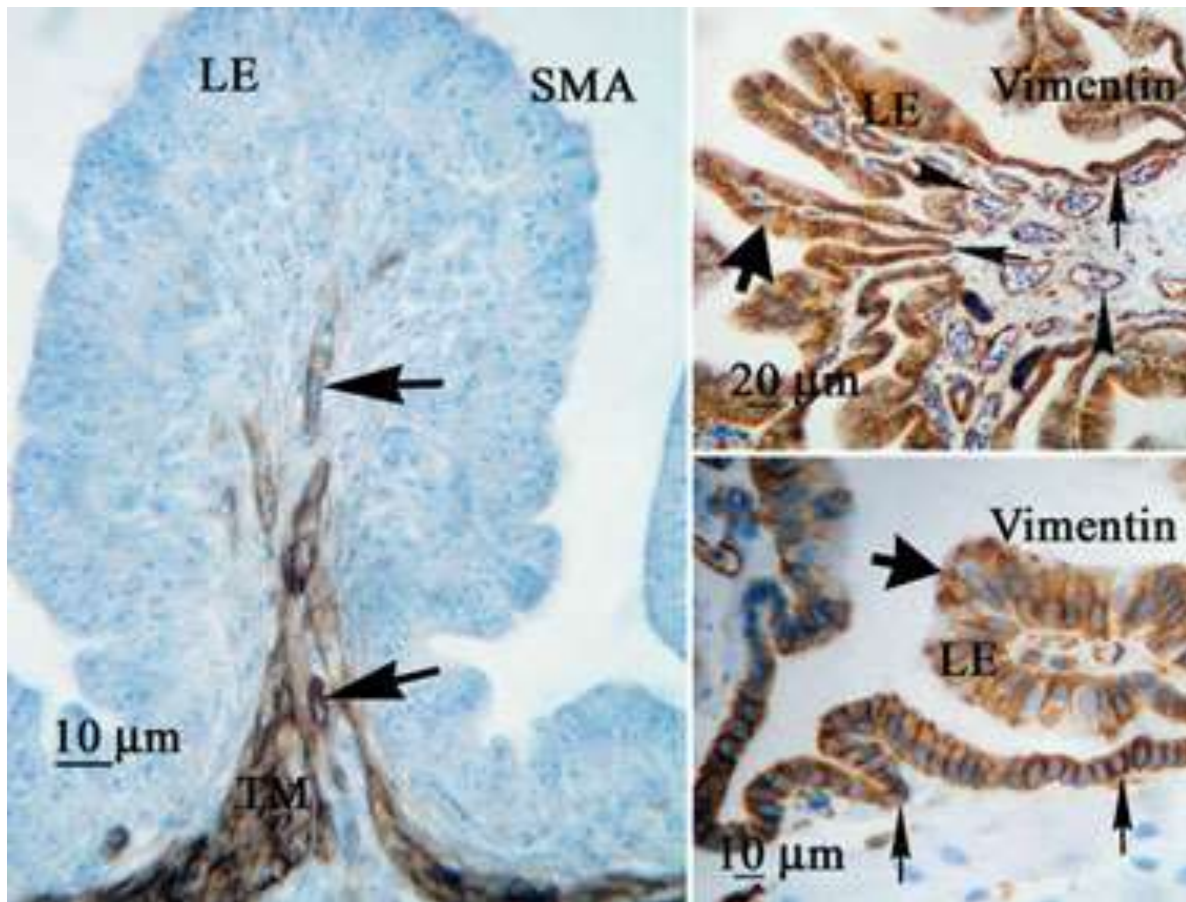

Figure 2. Infundibular tubular regions of immature **(a)** and mature **(b & c)** quails. **(a)** LE: Luminal epithelium. Arrows: Immunopositive vascular tunica media. TM: Tunica muscularis. **(b & c)** LE: Luminal epithelium. Immunopositive luminal (thick arrows), glandular (thin arrows) and vascular endothelial (arrowheads) cells.

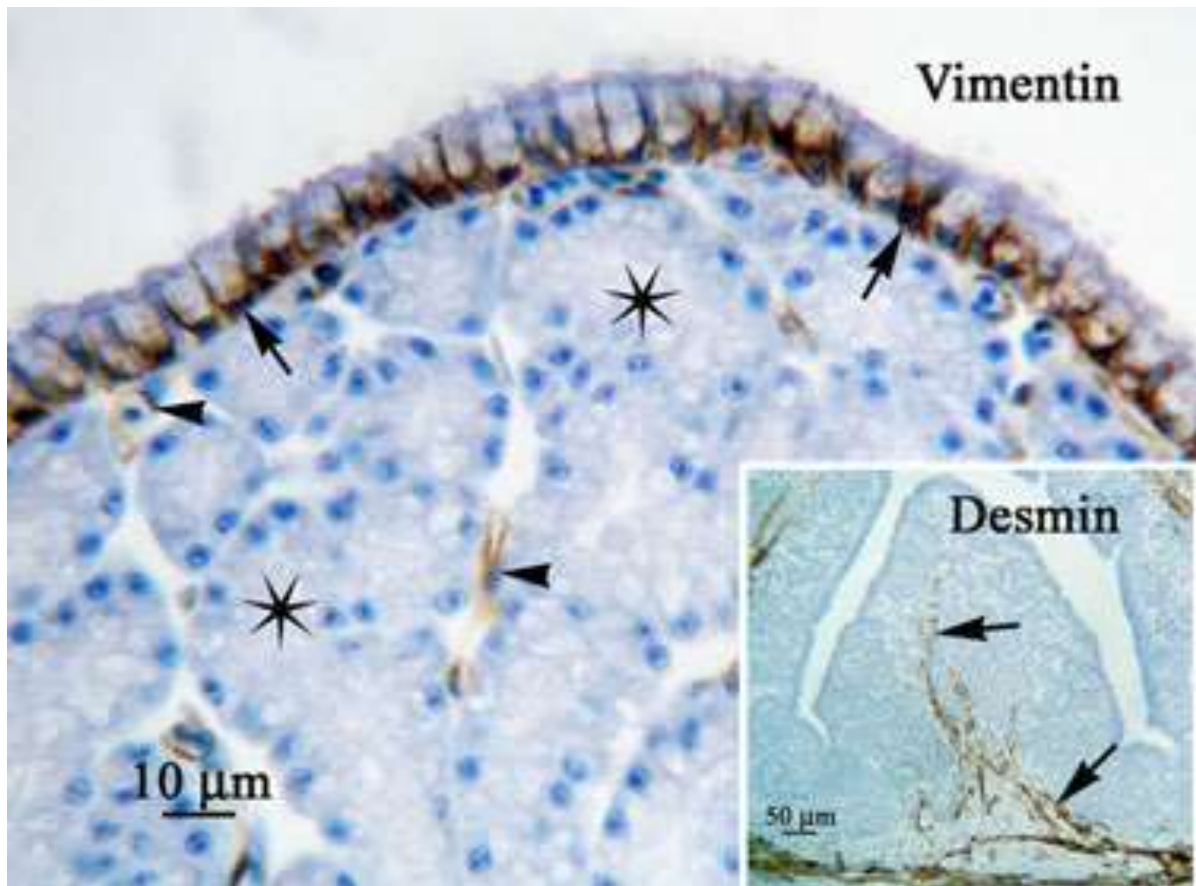

Figure 3. Magnal regions of mature **(a)** and immature **(b)** quails. **(a)** Immunopositive luminal epithelial (arrows) and vascular endothelial (arrowheads) cells. Asterisks: Immunonegative tubular glands. **(b)** Survey photomicrograph of a magnal mucosal fold. Arrows: Immunopositive smooth muscle cells.

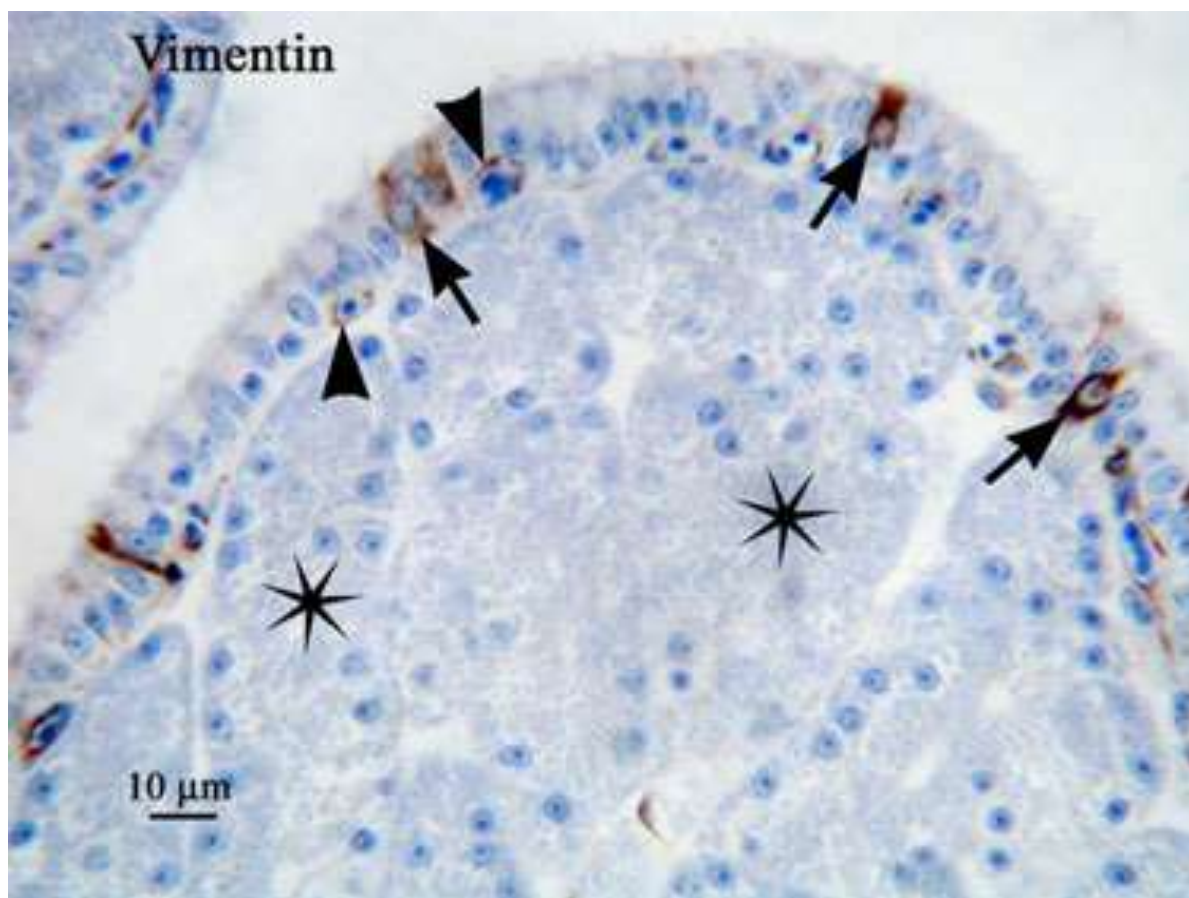

Figure 4. Isthmus of a mature quail with immunopositive luminal epithelial (arrows) and vascular endothelial (arrowheads) cells. Asterisks: Immunonegative tubular glands.

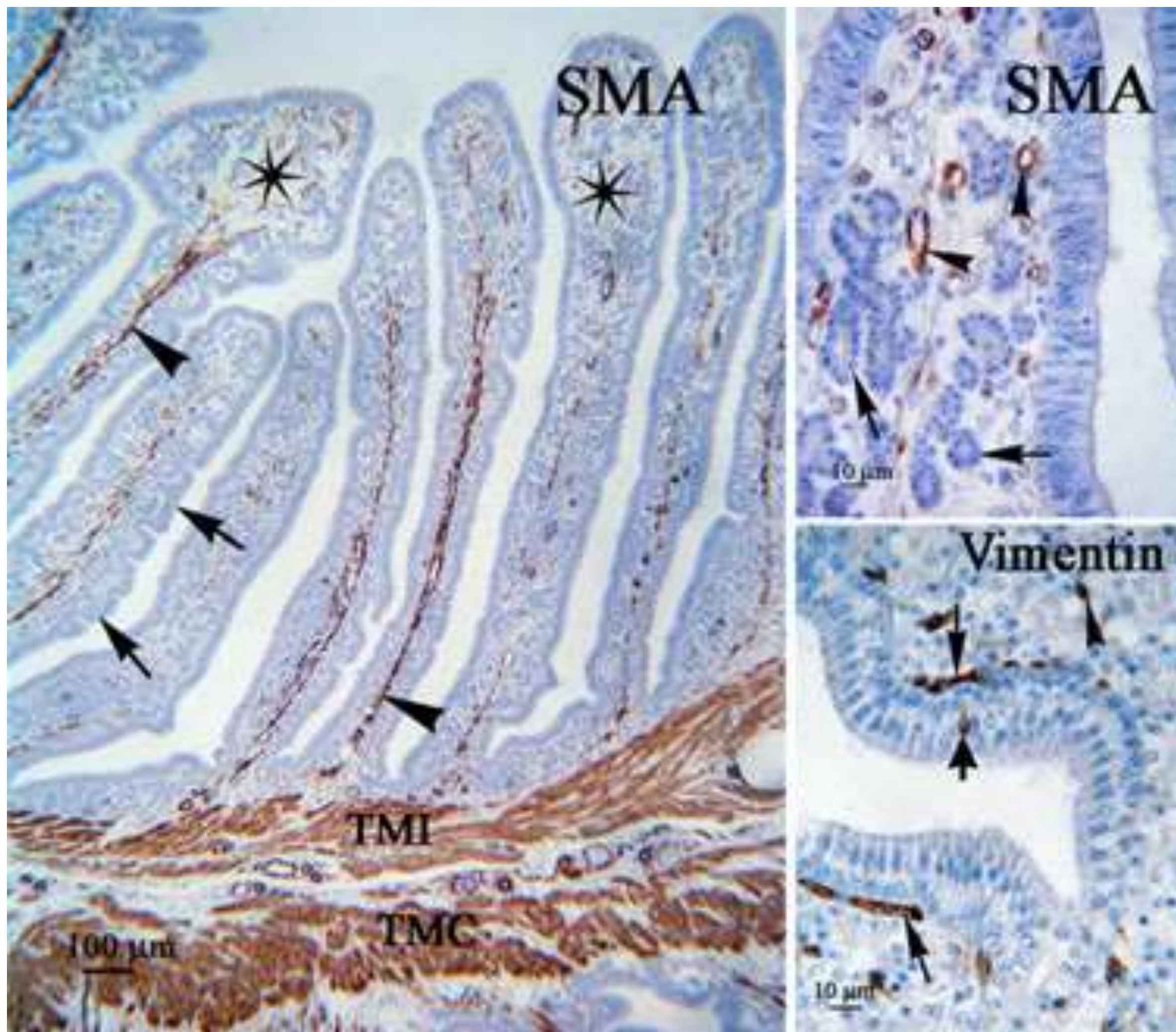

Figure 5. Shell gland regions of immature **(a & b)** and mature **(c)** quails. **(a)** Primary (asterisks) and secondary (arrows) mucosal folds. Arrowheads: Immunoreactive smooth muscle cells. TMI (inner) and TMC (outer) layers of the tunica muscularis. **(b)** Arrows: Tubular glands. Arrowheads: Immunopositive vascular tunica media. **(c)** Immunopositive luminal epithelial cell (thick arrow), vascular endothelial cells (thin arrows) and fibroblast (arrowhead).

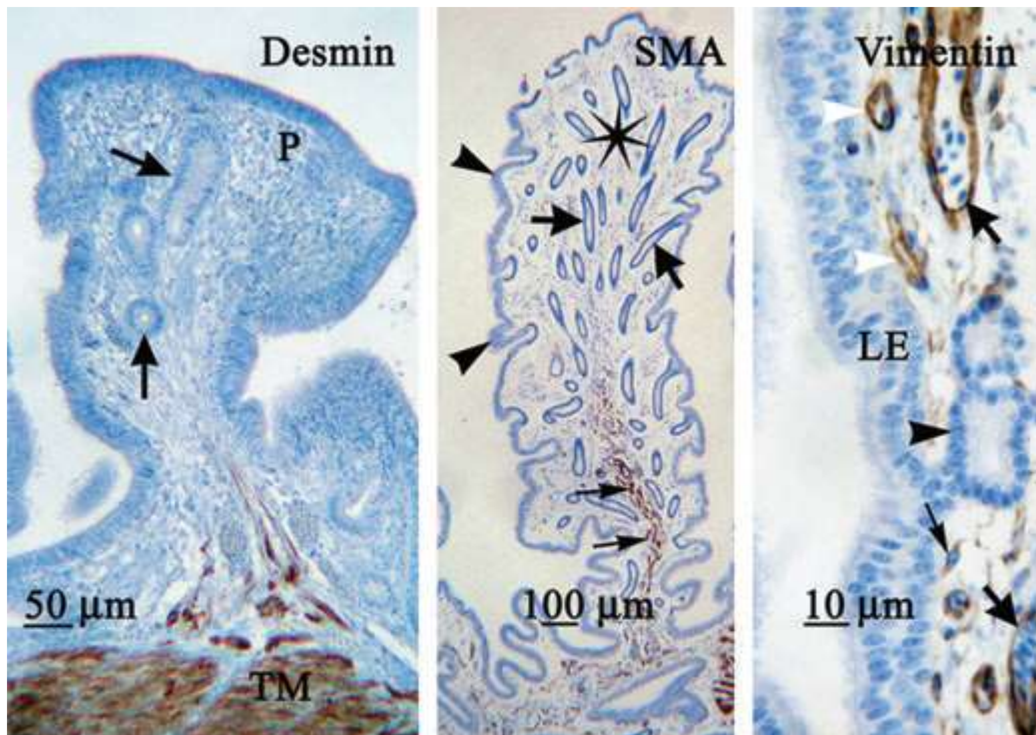

Figure 6. UVJ regions of immature **(a)** and mature **(b & c)** quails. **(a)** P: Primary fold. Arrows: SST. TM: Tunica muscularis. **(b)** Primary (asterisk) and secondary (arrowheads) folds. Thick arrows: SST. Thin arrows: Immunopositive smooth muscle cells. **(c)** Immunonegative luminal (LE) and SST (black arrowhead) epithelia. Immunopositive vascular endothelial cells (thick arrows) and a fibroblast (thin arrow). White arrowheads: Sub-epithelial capillaries.

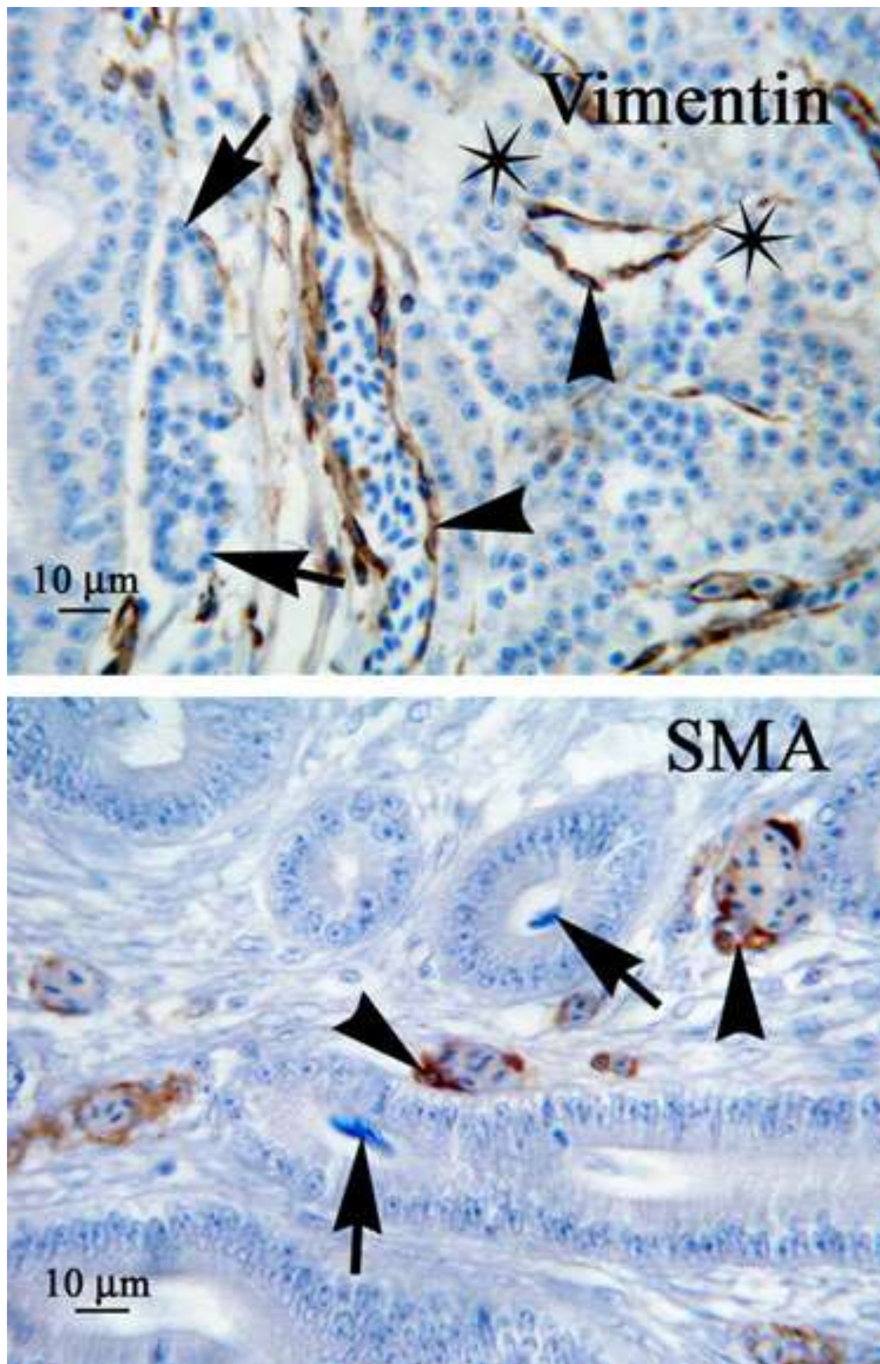

Figure 7. UVJ of a mature quail. **(a)** Mucosal fold with SST (arrows) and shell gland tubular glands (asterisks). Arrowheads: Immunopositive vascular endothelial cells. **(b)** SST with spermatozoa (arrows). Arrowheads: Immunopositive vascular tunica media.

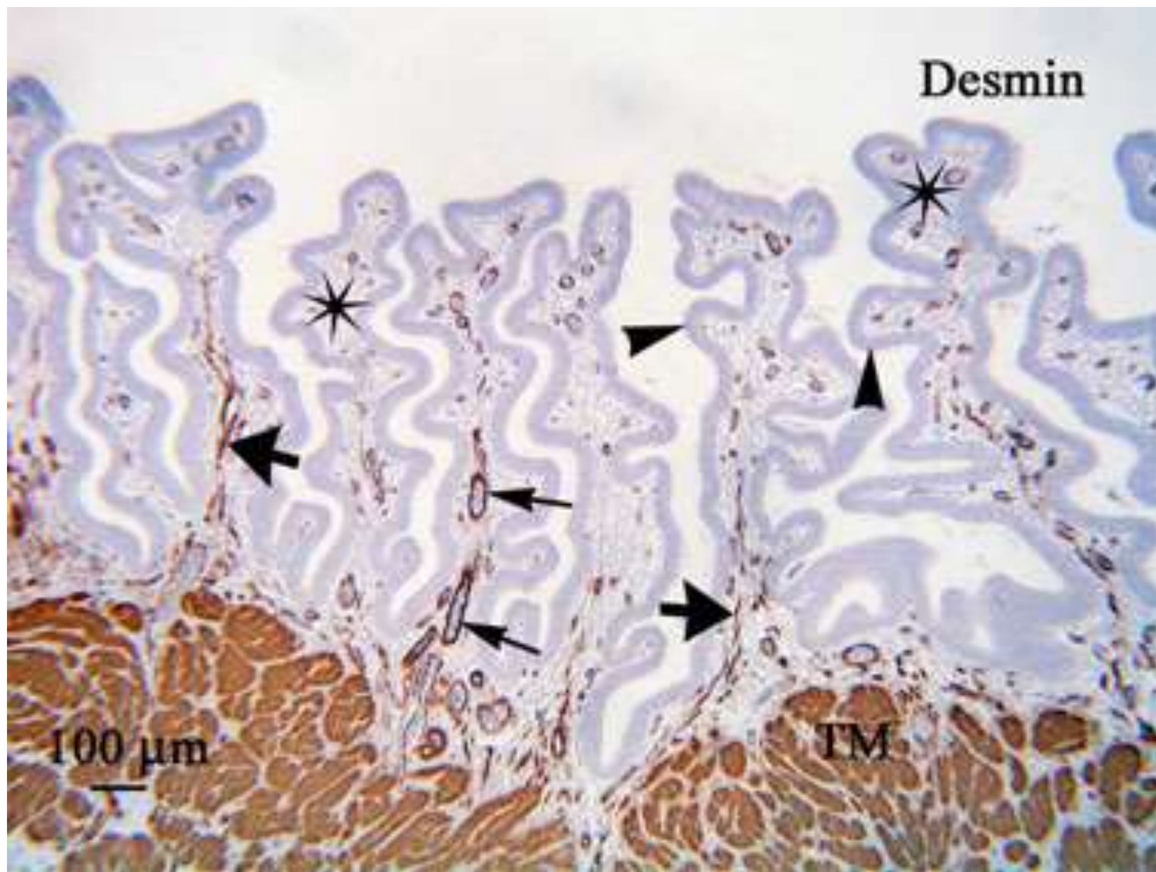

Figure 8. Vaginal region of a mature quail with primary (asterisks) and secondary (arrowheads) folds. Immunopositive smooth muscle cells are present in the tunica muscularis (TM), lamina propria (thick arrows) and vascular tunica media (thin arrows).

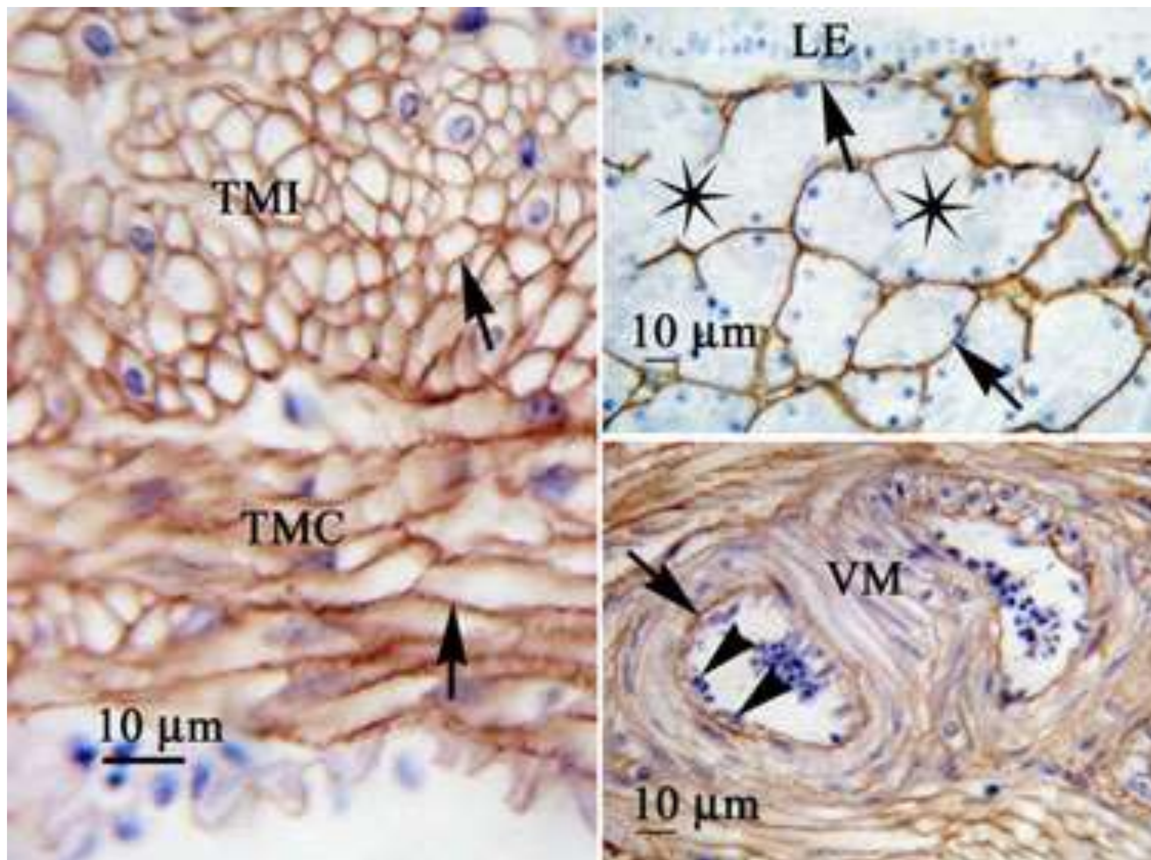

Figure 9. Isthmus (**a & b**) and magnum (**c**) regions of mature quails. Laminin immunostaining (arrows) in basement membranes associated with the luminal epithelium (LE), glandular epithelium (asterisks), vascular endothelial lining (arrowheads), vascular tunica media (VM), as well as the inner (TMI) and outer (TMC) layers of the tunica muscularis.

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