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## Histological and histochemical studies on the reproductive tract of Kashmir faverolla chicken

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### Abstract

Kashmir Faverolla being one of the famous native chicken breed of Kashmir valley is reared primarily for meat and egg production and constitutes one of the major sources of animal protein. With such an importance of this breed the present study was undertaken to demonstrate the histological and histochemical nature of its ovary and oviduct involving ten adult mature female hens. The intact reproductive tracts were procured from local vendors selling slaughtered birds. After dissection, it was observed that the left functional oviduct was a long convoluted tube that occupied a large part of abdominal cavity and comprising of five parts viz; infundibulum, magnum, isthmus, uterus and vagina. The wall of the oviduct consisted of mucosa, derived from pseudostratified epithelium and a glandular lamina propria. The tunica serosa was observed to consist of a thin layer of loose connective tissue with good numbers of lymphocytes. Longitudinal folds in mucosa extended spirally down the length of the oviduct varying in height and thickness. Muscularis mucosa was found to be thickest in the uterus, where it formed a sphincter at the uterovaginal junction as well as in vagina. Acid mucopolysaccharide and metachromasia dominated the magnum whereas neutral mucopolysaccharide dominated the infundibulum followed by the uterus.

**Keywords:** poultry, reproductive tract, histology, histochemistry, kashmir faverolla

### 1. Introduction

Kashmir Faverolla is an important indigenous Kashmiri poultry breed reared in backyard that is well adapted to local extremes of temperature and is primarily reared for meat and egg production. In order to ensure its persistent and maximum production and to evolve better managemental practices, it had become essential to have in-depth study of its reproductive tract. Besides, there is underlying husbandry and/or management issues associated with the reproductive disease condition that need to be addressed. The hen's reproductive system consists of two parts: the ovary and the oviduct. The ovary contains thousands of ova which can develop into the yolk and eventually an egg. When ovulation occurs, the mature follicular oocyte is released from the ovary and is received by the oviduct. The ovum is then surrounded with the albumen, the shell membranes, and the shell to form the characteristic avian egg. In addition to forming and transporting the egg, the oviduct is the site of sperm storage, sperm transport, fertilization and early embryonic development<sup>[5]</sup>. The structure and function of the reproductive tracts have been documented in a variety of birds, such as the domestic fowl<sup>[2, 3, 8, 21]</sup>, the Japanese quail<sup>[6, 11]</sup>, the guinea fowl<sup>[24, 25]</sup>, the pied myna<sup>[14]</sup> and the ostrich<sup>[28]</sup>. There is a lack of proper information regarding the reproductive tract of this native kashmiri breed, therefore this investigation was aimed to describe the various histological and morphological aspects of the reproductive tract.

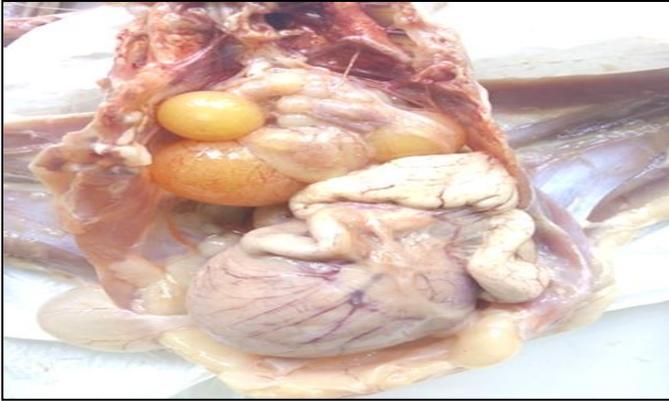
### 2. Material and Methods

The study material comprised of reproductive tracts of ten female Kashmir Faverolla birds of 10-12 months of age, collected from local poultry slaughter houses. Intact reproductive tracts were brought to the laboratory and were subjected to detailed morphological examination. Representative tissue pieces from various parts of the tract were collected and fixed in 10% buffered formalin for histological examination. The tissue samples were then processed for a routine paraffin embedding technique and 5 micron thin tissue sections were stained with Harris' Haematoxylin and Eosin<sup>[18]</sup>. Duplicate sections were stained with Masson's Trichrome

stain for demonstration of connective tissue [18] and Toluidine blue for demonstration of metachromasia [16]. For demonstration of Acid and Neutral Mucin activity, parallel tissue sections, were stained by Combined Alcian Blue PAS stain [4].

### 3. Results and Discussion

#### Gross Anatomy



**Fig 1:** Showing intact in situ reproductive system



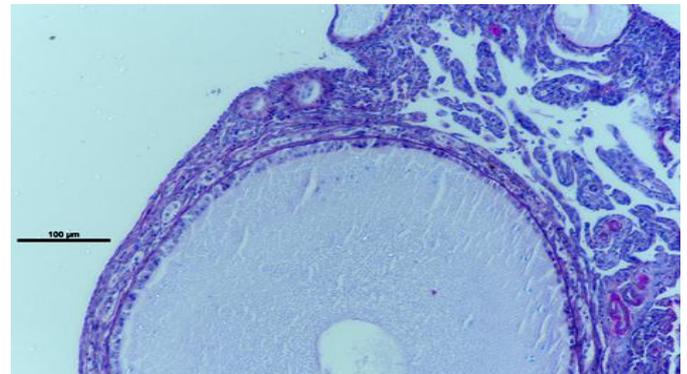
**Fig 2:** Showing internal view of female reproductive tract

The reproductive system consists of two parts: the ovary and the oviduct. The ovaries of the birds were found to be arranged as a cluster of follicles with variable size. There were four to six large yolk-filled follicles 2-4 cm in diameter, accompanied by a greater number of 2 to 10mm yellow follicles and numerous small white follicles (Fig.1). The oviduct consisted of five morphologically as well as functionally distinguishable regions: infundibulum, magnum, isthmus, shell gland, and vagina (Fig.2) and was a highly convoluted muscular structure. The first part being the infundibulum was funnel shaped, thin-walled and its mucosa had a rugged surface. The magnum was the largest part that depicted thick longitudinal folds and was clearly demarcated from the isthmus by thin, bright, narrow constricted translucent membrane. Isthmus was observed to be a narrow part having thin folds. Similar results were documented in fowl and in adult turkey [20], but on contrary in emu [29] the mucosal folds were similar to magnum. The shell gland (uterus) was an elongated dialed muscular sac with a number of longitudinal folds on its inner surface which was in agreement with the observation of various authors during the histopathology of reproductive tracts of domestic fowl [19], hens [23], ducks [20] and adult turkey [10] The vagina was studded with folds separated from the shell gland by the utero-vaginal sphincter muscle and terminated at the cloaca.

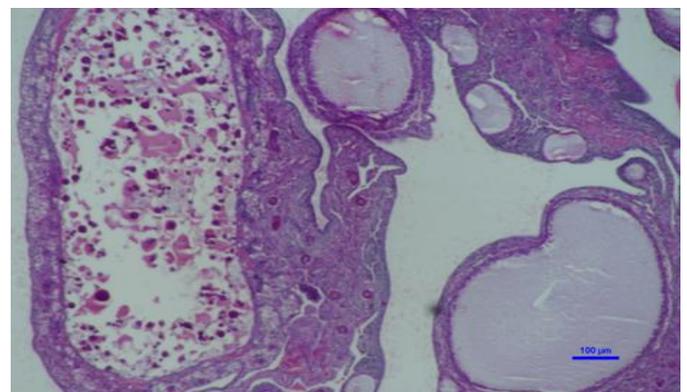
#### Histological and histochemical study

##### Ovary

The follicular wall of avian oocyte was a complex structure composed of several layers and surrounded by connective tissue. Each follicle consisted of a growing yolk-laden oocyte with a rounded nucleus. The oocyte was surrounded by several layers: the theca externa, theca interna, membrana granulosa and perivitelline membrane (Fig.3). The stroma comprised of follicles of varying sizes, postovulatory follicles, atretic follicles and a fibrovascular connective tissue with sinusoidal blood vessels where cells transport nutrients and provide mechanical support to the growing oocyte along with an important role in the synthesis of steroid hormones [20]. Atretic follicles were characterized by accumulation of lipid droplets of varying sizes (Fig.4). Bursting atretic follicle revealed disorganized granulosa, yolk, vacuolar cells as well as various cells in the ooplasm (Fig.5). Thecal hypertrophy, vacuolization in theca interna cells, collagenization of theca externa, disrupted basal lamina and perivitelline layer (zona pellucida) were evident. Disruptions of the basal lamina, loss of cell contacts and as a consequence, disintegration of the follicle-cell layer, were also characteristic signs of atresia. The increased height of the granulosa layer at the beginning of atresia is due to an increased cell proliferation [23]. The heterogeneity of atretic follicle may mean that the process of destruction is not strictly synchronized between the cells of the atretic follicle. The findings on follicular atresia in the ovary of the domestic hen were in agreement with the studies of earlier workers [8]. The basement membrane of the developed and developing follicles revealed positivity for neutral mucopolysaccharide (Fig.6).



**Fig 3:** Section of ovary showing the various layers of ovarian follicle. PAS stain

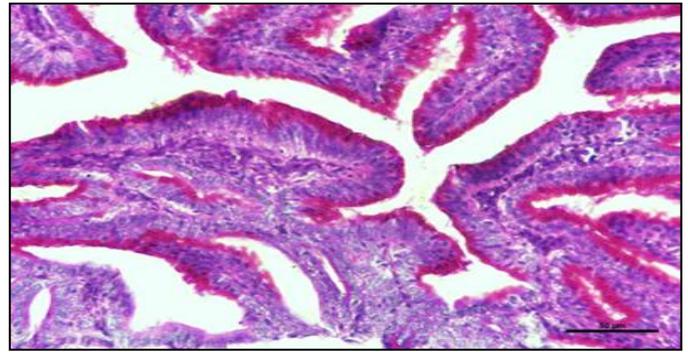


**Fig 4:** Showing atretic follicle with accumulation of lipid droplets. H&E stain

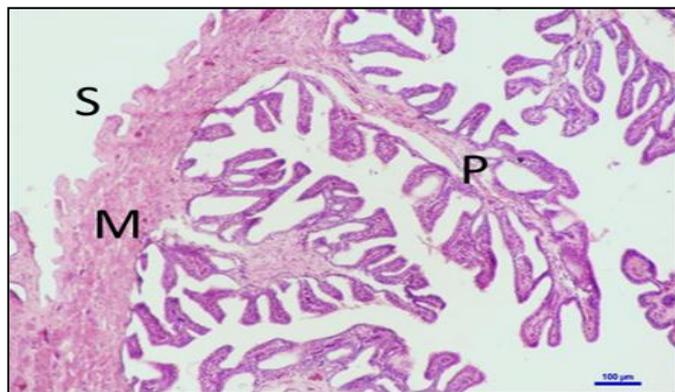
##### Infundibulum

The wall of the infundibulum was divided into the tunica mucosa which was thick with extensive folds, followed by

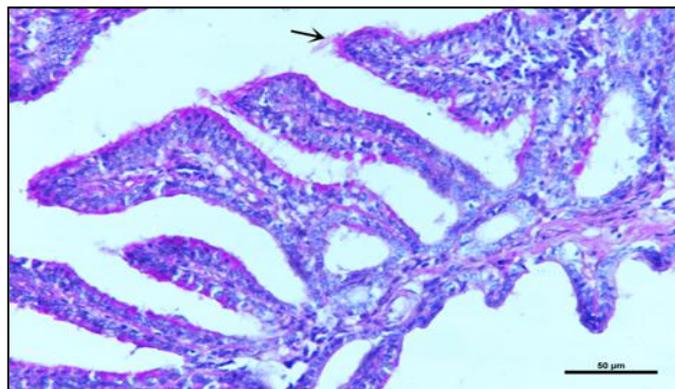
tunica muscularis consisting of an outer longitudinal layer and inner circular layer and tunica serosa which covered the funnel externally. The mucosa was quite rugged and was thrown into shallow ridges which increased in height as the funnel narrowed towards the neck region. The mucosal folds in the neck of infundibulum were made up of tall primary which divided into secondary and tertiary folds (Fig.7). The epithelium was ciliated simple columnar, while the bottoms of the grooves between folds were lined by non-ciliated secretory cells (Fig.8). The mucosal ciliation was very heavy in this region. Similar observations were made in pigeon [15, 9] in domestic fowl and turkey [17, 13]. Lamina propria was composed of vascularized connective tissue which extended into the core of the folds (Fig.9). The apices of the lined epithelium revealed strong positivity for neutral mucopolysaccharide (Fig.10).



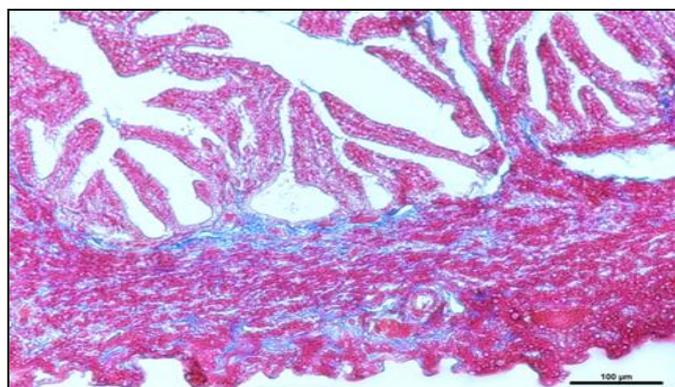
**Fig 10:** Showing neutral mucopolysaccharide activity in mucosal folds. PAS stain



**Fig 7:** Showing serosa (S), muscularis (M), primary, secondary (P) and tertiary folds. H&E stain



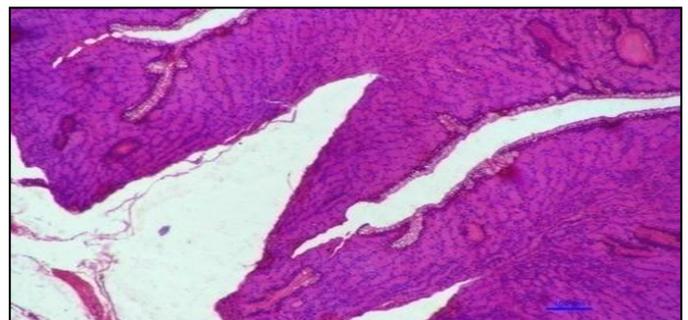
**Fig 8:** Showing ciliated epithelium (arrow) and non ciliated glandular grooves. PAS stain



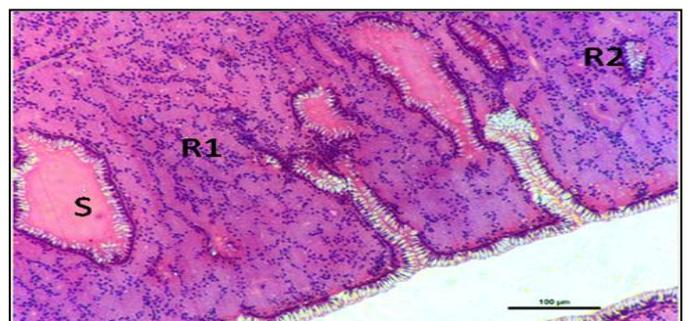
**Fig 9:** Showing blue coloured connective tissue in Lamina Propria. Masson's Trichrome stain

**Magnum**

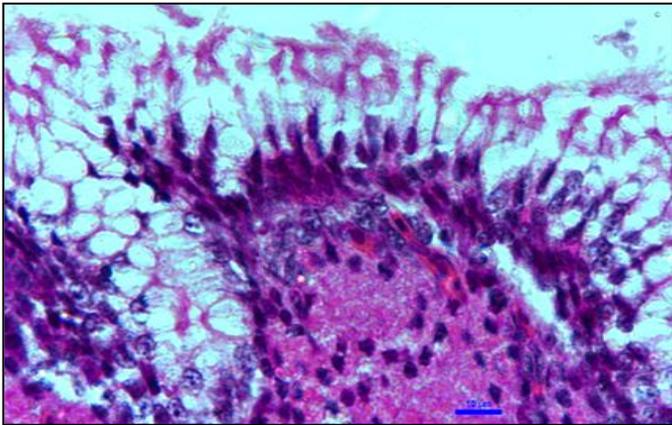
The tunica mucosa of magnum was composed of simple cuboidal to simple columnar epithelium. The primary mucosal folds were taller and broader due to the presence of enormous sub mucosal glands. Similar findings were reported in ostrich [28] and emu [29]. Vesicles containing protein secretions were easily seen by PAS-Alcian blue stain (Fig.11). The tubular glands of the magnum exhibited distinctive features. Three morphological phases of activity were recognized i.e regenerating, secretory and resting activity (Fig.12). The epithelium of magnum was pseudostratified columnar and was made up of ciliated columnar and secretory cells (goblet) (Fig.13). The nuclei of the secretory cells were round and located close to the base of the cell, whereas the nuclei of ciliated cells were oval and occupied the central apical region of the cell. The regenerating tubular glands had clearly defined lumen and their secretory cells were cuboidal. Mucosal epithelium of magnum showed strong positivity for acid mucopolysaccharide (Fig.14) and the same areas were positive for Metachromasia appearing pink on staining (Fig. 15). Similar findings have been reported in the magnum of hen [1]. The propria submucosa of the core of the mucosal folds was made up of loose fibrocellular connective tissue. Several capillaries were located between the connective tissue cell and the fibres (Fig.16).



**Fig 11:** Section showing mucosal folds of magnum. H&E stain



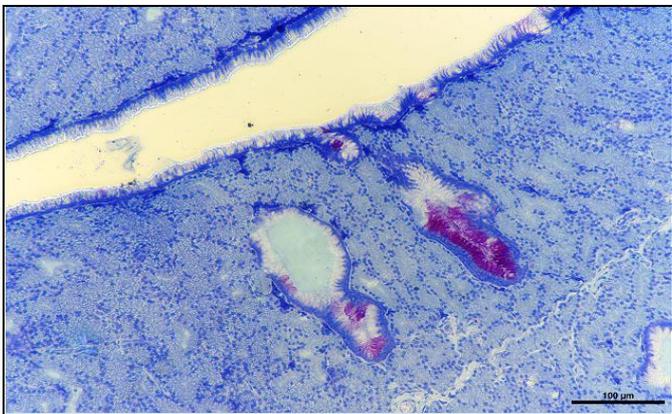
**Fig 12:** Section showing secretory (S) regenerating (R1), and resting gland (R2). H & E stain



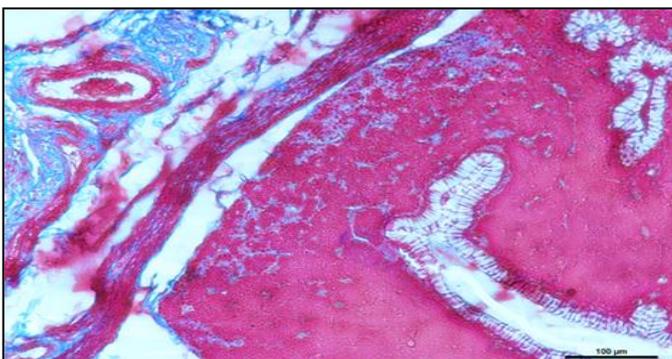
**Fig 13:** Section showing details of epithelium- ciliated columnar and secretory cells. Nuclei lying close to base of cell of secretory cell. H&E stain



**Fig 14:** Showing acid mucopolysaccharide activity in secretory glands. PAS Alcian blue stain



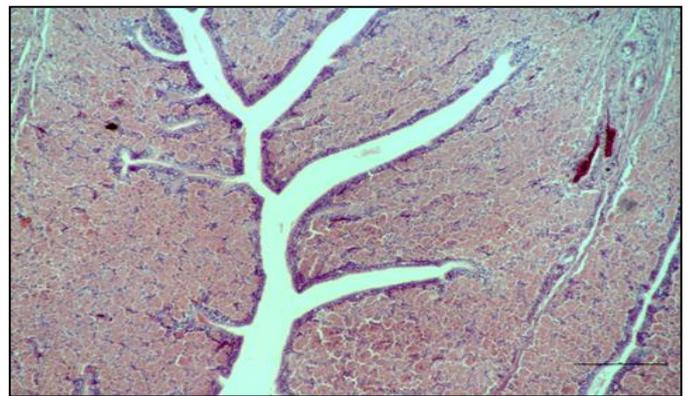
**Fig 15:** Showing metachromasia (presence of acidic material in secretory gland as well as mucosal epithelium). Toluidine blue stain



**Fig 16:** Section showing vasularized connective tissue in magnum. Masson's Trichome stain

### Isthmus

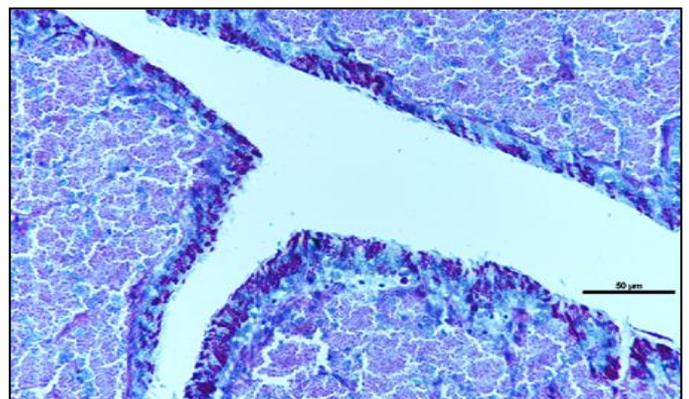
The primary folds of the isthmus were not as broad as those of the magnum. The folds of the isthmus were elongated and leaf-shaped folds (Fig.17). The surface epithelium of the isthmus was ciliated pseudo-stratified columnar. Ciliation was seen on the mucosal surface of the epithelium (Fig.18). Goblet cells were present among the columnar cells. The glands were numerous at the junctions between folds, whereas they were less densely arranged and resembled the glands of the magnum. The lining epithelium of isthmus showed the presence of both acidic and neutral mucopolysaccharides (Fig.19). As reported earlier in the pekin duck [16] and hen [1]. The tunica muscularis mucosa was arranged in two layers; inner longitudinal and outer circular. The lamina propria submucosa of isthmus was loose connective tissue housing branched tubular glands (Fig.20). These findings correlated with the histological studies in ducks. [28] Similar findings were reported in domestic fowl [17, 13], turkey [26] and emu [29].



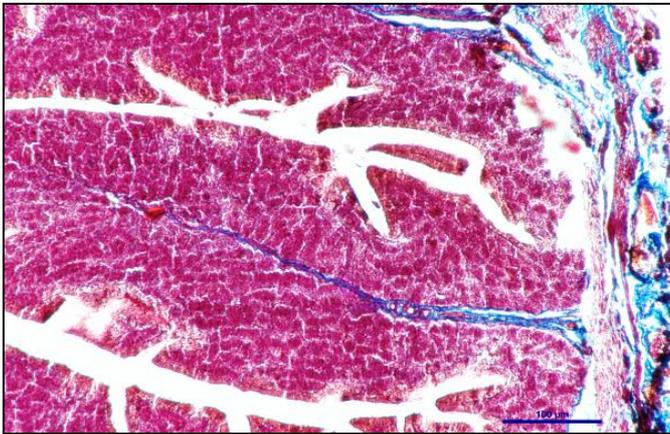
**Fig 17:** Section showing primary folds of isthmus. H & E stain



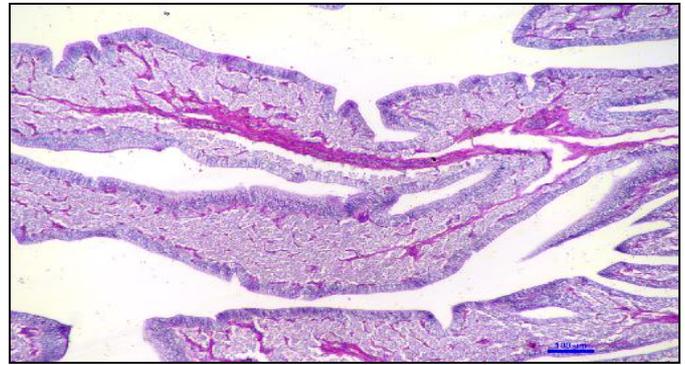
**Fig 18:** Section showing ciliation (arrows) on mucosal epithelium of isthmus and tubular glands. H & E stain



**Fig 19:** Section showing neutral polysaccharide activity in mucosal epithelium of isthmus. PAS Alcian blue stain



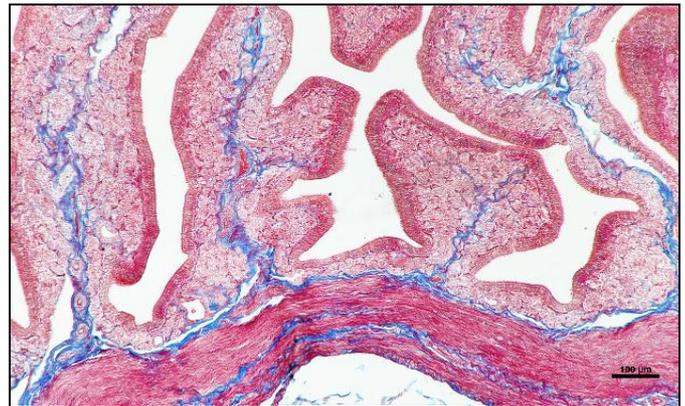
**Fig 20:** Section showing lamina propria submucosa of isthmus with loose connective tissue housing branched tubular gland. Masson's Trichome stain



**Fig 23:** Moderate acid mucopolysaccharide activity in the mucosal epithelium of shell gland. PAS Alcian blue stain

**Uterus/ Shell gland**

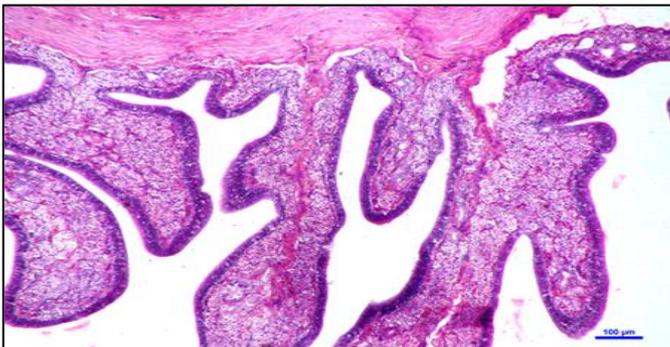
The folds of uterus were not as broad as those of isthmus and they were comparatively less glandular (Fig.21). The mucosal surface of the uterus was lined by ciliated as well as non-ciliated cells of pseudostratified epithelium with intermittent ciliated columnar cells (Fig.22). The ciliated cells contained secretory granules. The ducts were complex, branched and were formed from polygonal gland cells. Slight to moderate acid mucopolysaccharide activity was noticed in the mucosal epithelium of the shell gland (Fig.23). The current finding corroborates with the reports of previous workers [22] suggesting a single layer of short columnar cells over the whole length of the oviduct, and same findings were also described in turkey [27] as well. The lamina propria submucosa consisted of highly vascularized loose connective tissue housing branched tubular glands. The tunica muscularis mucosa was thicker than the preceding parts and constituted the thick inner circular and outer longitudinal (Fig.24).



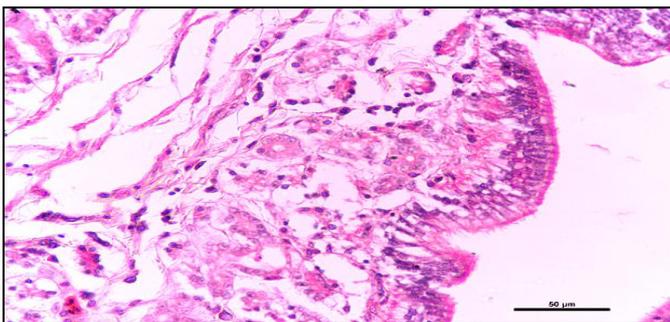
**Fig 24:** Section showing loose connective tissue housing branched tubular glands of shell gland and thick muscularis mucosa. Masson's Trichome stain

**Vagina**

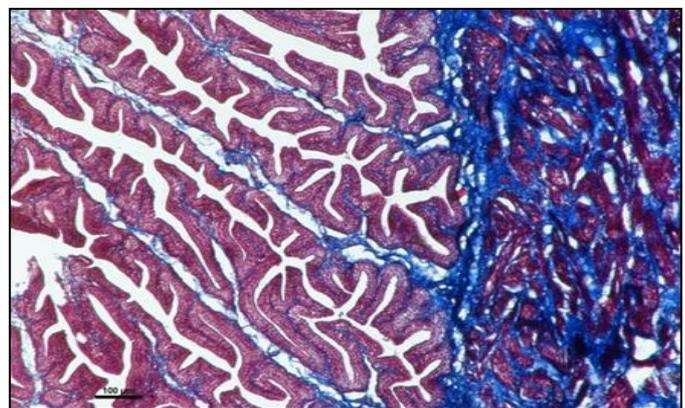
It was found that the mucosa was raised into numerous narrow longitudinal ridges, which were regular in the arrangement and narrow as compared to the other parts of the oviduct. Primary, secondary and tertiary folds were present (Fig.25). Vagina was lined by pseudo stratified columnar epithelium, which was well documented earlier [15, 29]. The muscularis was highly developed in this part of the oviduct, showed distinct inner circular and outer longitudinal layer separated by connective tissue fibres. The fimbriated region was densely populated with ciliated cells. At the anterior end of the vagina, there were sperm storage tubules/glands (Fig.26) lined by tall columnar cells. The occurrence of sperm host glands in the lamina propria of the vagina were described earlier by researchers [5, 16, 14] in duck and [13] in chicken.



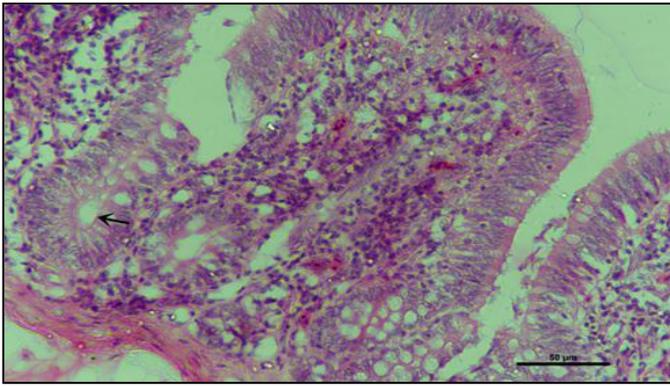
**Fig 21:** Section showing mucosal folds and muscularis of uterus. H&E stain



**Fig 22:** Section showing pseudostratified epithelium and polygonal gland cells. H&E stain



**Fig 25:** Section showing long, slender, primary folds bearing numerous secondary folds and loose connective tissue housing branched tubular glands. Masson's Trichome stain



**Fig 26:** Section showing sperm host gland within the mucosa of vagina near uterovaginal junction (arrow). H&E stain

#### 4. Conclusion

Histological and histochemical studies on the reproductive tract of Kashmir faverolla chicken showed that the reproductive tract comprised of ovary, infundibulum, magnum, isthmus, shell gland and vagina. Each region of the tract varied with respect to shape, size and number of the folds as well as muscular content, which made the birds adaptable to physiological needs for transmission of the eggs across the tube that was very well appreciated by the routine histological stains. All the parts of the tract were distinct from each other depending on their diverse function and role in egg production. Histochemically, neutral mucopolysaccharide was predominant in infundibulum while acid mucopolysaccharide dominated in magnum while the metachromasia dominated the magnum.

#### 5. Acknowledgement

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