POSTNATAL DEVELOPMENT OF THE OVIDUCT IN THE KUTTANAD DUCK (*Anas platyrhynchos domesticus*)

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(*Anas platyrhynchos domesticus*)

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DECLARATION

I hereby declare that this thesis, entitled **"POSTNATAL DEVELOPMENT OF THE OVIDUCT IN THE KUTTANAD DUCK (*Anas platyrhynchos domesticus*)"** is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

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CERTIFICATE

Certified that this thesis, entitled **"POSTNATAL DEVELOPMENT OF THE OVIDUCT IN THE KUTTANAD DUCK (*Anas platyrhynchos domesticus*)"** is a record of research work done independently by **Dr. Patki Harshad Sudhir** under my guidance and supervision and it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to him.

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From the day we arrive on this planet…And blinking steps into the Sun…There is more to see that can ever be seen…more to do that can ever be done…There is far too much to take in here…more to find than can ever be found…But the Sun rolling high…through the Sapphire sky…keeps the great and small on endless round…It's the Circle of Life…and it moves us all…through despair and hope…through faith and love…till we find our place…On the path unwinding…in the Circle…The Circle of Life.

Title song of great animation movie "The Lion King"

(Walt Disney Pictures)

These lines exactly capture my feelings at this moment and this is what I learned through the genesis of my thesis, so far the most significant scientific accomplishment in my life. Even though I sufficiently comprehend that this is not even a glint of light in the gigantic aura of science. Its materialization would have been impossible without the people who supported and believed in me. More often our words are incapable of expressing the heart's language. Within the limits of lexicon, I am humbly expressing the deepest sense of acknowledgment to all concerned.

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Dedicated To

My Beloved family

And

Respected Teachers

Introduction

1. INTRODUCTION

On the occasion of 150th anniversary of the best seller in the field of biology 'The Origin of Species' by 'Charles Darwin', it may be recalled that, ducks were one of the waterfowl species that he passionately studied in detail and made one of the first statement supporting the recently established fact that the common domestic duck was derived from the wild mallard. Domestication of duck occurred during New Stone Age between 4000 to 10000 years ago in China. Ducks belonging to the order Anseriformes, diverged from the fowl (Galliformes) about 90 to 100 million years ago (Tuinen and Hedges, 2001). Today next to domestic chicken in rank, ducks play vital role in food security of mankind worldwide.

In Asia, especially in humid tropics and subtropics, duck production is closely associated with wetland paddy farming and said to be mutually beneficial and eco-friendly. India has a long coastline and extensive inland areas which are suitable for duck production. Duck population in India has increased from around six million in 1961 to over 35 million in 2007 and India ranks seventh in duck meat production globally (FAO STAT, 2009). Valavan *et al.* (2009) stated that in the year 2008, India had 11.91 million Desi layer ducks which produced 13633 lakhs of eggs.

Kerala is blessed by nature with numerous backwaters and low lying waterlogged areas where duck farming is being integrated excellently with pisciculture and paddy cultivation. Kerala is the home tract of the Kuttanad breed of ducks which are good egg producers giving nearly 150 to 180 eggs per annum with attractive egg size (Jalaludeen *et al.*, 2004). According to Kerala state livestock census 2003, the duck population in Kerala was 6.61 lakhs. Kuttanad ducks are so named from their place of origin 'Kuttanad' region, the rice bowl of Kerala. These ducks are also known for their disease resistance and adaptability to different climatic conditions.

The egg is one of the most nutritious, natural, unadulterated and easily digestible food on earth with the highest biological value and is considered as the golden standard to measure the quality and nutritive value of any other human food. Senthilkumar *et al.* (2009) reported that duck eggs had higher energy value, better protein and lipid profile than chicken eggs and were richer in vitamins and minerals.

Jalaludeen *et al.* (2009) reported that duck eggs were different from chicken eggs in their internal quality parameters also, which was evident from the thicker vitelline membrane and albumen, and higher percentage weight contribution of the shell membranes to the total egg weight in the duck eggs.

Duck farmers in Kerala have favoured native Kuttanad ducks over Khaki Campbell ducks, due to attractive egg size and more disease resistance (Jalaludeen *et al.,* 2004).

In order to ensure persistent and maximum production of these indigenous ducks and to evolve better managemental practices, a sound knowledge on the developmental aspects of reproductive tract is essential. Oviduct acts as the final assembly room where the albumen and egg envelopes are added and final steps of egg formation occurs resulting in the calciferous porous egg specific for each avian species. Although research works have been conducted on the oviduct of domestic fowl and Japanese quail, information regarding the developmental pattern of the oviduct in duck is scanty.

Therefore, a comprehensive study on the postnatal development of the oviduct in the Kuttanad duck was undertaken with following objectives:

1. To study the normal postnatal development of the oviduct in Kuttanad ducks at different ages

2. To find out the relationship of the developmental pattern of the oviduct with the age and body weight

Review of Literature

2. REVIEW OF LITERATURE

2.1 DEVELOPMENT AND GROSS MORPHOLOGY OF THE OVIDUCT

2.1.1 Topography of the Oviduct

Surface (1912) observed in the laying hen that the much convoluted oviduct occupied the left dorsal quadrant of the coelom completely and also the ventral quadrant to some extent.

According to Bradley and Grahame (1960) in domestic fowl, the oviduct was suspended from the left side of abdominal cavity by the dorsal ligament, a thin, folded, peritoneal membrane attached dorsally to the inner surface of abdominal cavity, running caudally from the region of the fourth thoracic rib to the region of the cloaca. The ventral ligament, was not however, connected to the body wall, but showed presence of a thick muscular band along its lower edge that fused posteriorly with the vagina.

According to Kern (1963) the oviduct in domestic fowl was related dorsally to the left kidney, laterally to the left lateral wall, ventrolaterally to the intestines and ventrally on the left, to the ventriculus and spleen. The oviduct extended backwards against the dorsal part of the left body wall in relation to the ilium and ischium and emptied into the cloaca lateral to the left ureter (King, 1975).

Lucy and Harshan (1999a) noted that in non laying Japanese quails, the oviduct occupied the left half of the coelom ventral to left kidney, but, the oviduct containing a developing egg in laying period also extended towards the right side and displaced the intestines in right ventral direction considerably.

Soley and Groenewald (1999) described that in young ostrich, the oviduct was a straight, narrow tube, pale in colour and lay ventral to left kidney whereas, in adult ostrich hen, it was a very long, convoluted organ richly supplied with blood.

2.1.2 Development of the Left and Right Paramesonephric Ducts in Aves

It has been generally accepted that in most of the avian species, the left paramesonephric duct alone develops fully and the right one ceasing to do so. However, rudimentary forms of the right oviduct were detected in the adult domestic hen (Bradley and Grahame, 1960; Aitken, 1971; King and Mc Lelland, 1975 and Paneerselvam *et al*., 1989).

Bradley and Grahame (1960) underlined the fact that, although initially in the embryo the provision was made for two ovaries and two oviducts, only the left ovary and its corresponding duct reached maturity and the right oviduct was usually detectable in the adult hen as a rudimentary organ of 2-3 cm in length joining the cloaca at the same level as the left functional oviduct. They also noted that much larger persistent oviduct, which if present, was usually cystic and suggested endocrine influence to be responsible for the acceleration or inhibition of growth of the right oviduct.

Kamar and Yamani (1962) recorded presence of two oviducts and left ovary in the case of immature Pekin duck and observed that both the oviducts had separate orifices in the cloaca. According to them, increased weights of these oviducts along with corresponding increase in weight of endocrine glands signified a potent endocrine control over the occurrence of right oviduct.

Occurrence of fully developed right oviduct might be considered as a rare phenomenon even in the domesticated aves and more so in wild, wherein the chances of inbreeding are the rarest with the exception of endemic populations. But, occurrence of both the functional ovaries was recorded in at least 34 avian species belonging to 13 different orders as reported by Kinsky (1971) who stated that the carinates like falcons, hawks and vultures contributed 52 per cent of the total number of species in which paired ovaries were documented and successful ovulation from right as well as left ovary or from both ovaries alternatively in the same laying season was a normal finding in kiwi, a ratite belonging to order Apterygiformes. He suggested that the reduction in the right oviduct of the birds

might well have preceded the reduction of right ovary during their evolutionary history.

Tripathi *et al.* (1981) recorded the occurrence of a bifunctional oviduct in a two year-old white leghorn chicken and stated that although the left ovary was fully developed there was no evidence of presence of the right ovary and both the well developed oviducts appeared to be supplied by the same ovary.

Onyeanusi *et al*. (1986) reported the occurrence of the right oviduct in the guinea fowl (*Numida meleagris*) and noted that the lumen of the oviduct was patent as far as the caudal one-third of the vagina, but had no evident opening into the cloaca.

Wakamatsu *et al.* (2000) found that persistence of right oviduct was a hereditary phenomenon which could be attributed to expression of two pairs of autosomal recessive genes which were relatively stable in homozygous condition.

2.1.3 Structural Classification of Different Regions of the Oviduct

Romanoff and Romanoff (1949), Hodges (1974), King (1975) and Gilbert (1979) in domestic fowl, Chakravorti and Sadhu (1961) in the laying kite, Fitzgerald (1969) and Lucy and Harshan (1999a) in the Japanese quail, Das and Biswal (1968) and Muwazi *et al.* (1982) in mature ostrich, Sharma and Duda (1986), Rao (1994) and Ozen *et al*.(2009) in the duck and Mohammadpour and Keshtmandi (2008) in turkey and pigeon described five regions of the oviduct in mature birds, namely, the infundibulum, magnum, isthmus, shell gland and vagina.

The infundibulum was observed to be divisible into a funnel and a tubular neck region in case of the domestic fowl by (Aitken and Johnston, 1963; Hodges, 1974; King, 1975 and Nickel *et al.,* 1977). According to Hodges (1974), the funnel region of the infundibulum consisted of a thin walled, funnel-shaped opening which was flattened in the dorso-ventral direction with its flared lips lying in close proximity of the ovary. Walls of the funnel converged rapidly to

form the infundibular neck, a narrow thin-walled tube which increased in size and thickness to form the magnum.

Romanoff and Romanoff (1949) found that the magnum region in fowl had the maximum number of coils and convolutions. Hodges (1974), King and Mc Lelland (1975), Solomon (1975) and many other workers found in domestic fowl and other birds that the magnum was by far the longest and most coiled part of the oviduct. Because of its extensiveness, the magnum region was referred to as the oviduct in the strict sense by Nickel *et al.* (1977).

Hodges (1974), King and Mc Lelland (1975), Solomon (1975) and Gilbert (1979) described the macroscopic limits of the isthmus region in the domestic fowl as a translucent region that extended from the aglangular zone, which separated it from magnum, to the tubular shell gland, which to the naked eye was marked by a distinct colour change from brown to off-white.

According to Johnston *et al.* (1963), Solomon (1975) and Gilbert (1979), the uterus of the domestic fowl was subdivided into a short, comparatively narrow, anterior portion through which the egg passed rapidly, and a pouch-like posterior portion in which it was lodged during the greater part of the period of shell formation. This pouch existed independently of the egg, and, although reduced in size, could be distinguished in the oviduct of the non-laying hen.

Bradley and Grahame (1960), Bobr *et al.* (1964), Hodges (1974) and King and Mc Lelland (1975) observed that the vagina was a relatively short, much convoluted, S-shaped tube that extended from a well developed muscular sphinctor at the posterior end of shell gland and it terminally opened into the urodeum of cloaca. Moraes *et al.* (2007) while studying the morphology of the oviduct of nothura spotted quail observed whiter opaque coloured mucosa of relatively shorter vaginal region. Bakst and Akuffo (2009) described that in turkey, the tough, opaque tunica serosa that bound the sac-like uterus merged with the convolutions and angularity of vagina and at the same time, the coprodeum was also observed merging with the connective tissue that masked the juncture of

the vagina and urodeum, all these resulted in the condensed nature of the vagina prior to its opening into the urodeum.

2.1.4 Postnatal Developmental Pattern of the Oviduct

Hafez (1955) compared the differential growth rate of visceral organs of domestic fowl and observed that the ovary had the highest growth rate followed by the oviduct and spleen.

Hafez and Kamar (1955) found that the size and weight of the hen's ovary and oviduct were influenced by age as well as by the reproductive phase. The oviduct was small and non-functional before sexual maturity while it attained a bigger size at the sexual maturity. Once the sexual maturity was attained there was only an increase in the oviduct thickness and it weighed about 13 times heavier during laying period than during resting phase.

The anatomical and histological observations of Fitzgerald (1969) and Pageaux *et al.* (1984) indicated that in the coturnix quail, the functional maturity of the oviduct was attained by six to seven weeks of age.

Nickel *et al.* (1977) reported that in a day-old chick, the left oviduct was present as a tiny tube just visible to the naked eye which increased gradually in size until puberty and in the laying hen it was more than double the length of the bird's trunk and expanded from the ovary to the cloaca in a massive coil.

Krapu (1981) in common mallard and Tome (1984) in migratory Ruddy ducks observed that body weights were increased during arrival to breeding site and in pre-laying period, primarily as a result of the deposition of stored lipids whereas, a decline in body weight was evident between the laying and early incubation phase as a result of lipid utilization associated with egg formation and regression of the oviduct in late incubation phase.

Vernerova and Burda (1984) studied the development of genitalia in ducks and they found that the female genitalia showed maximum development between 130 and 160 days of age.

Elashi and Horst (1985) noticed that the oviduct weight was significantly correlated with egg weight and albumin weight, but not with the egg shell weight.

The morphology and weight of genitalia in female turkeys and hens was compared by Vernerova and Firmanova (1985) and found that the weight of the ovary and the oviduct increased along with the increase in body weight and age.

Kelany *et al.* (1992) compared the postnatal development of the oviduct in high (Hy-line) and low (Dandrawi) egg producing domestic fowl and noted that the length of the oviduct increased slowly until 16th week of age and a markedly longer oviduct was present at the 20th week. At 24 weeks of age, the oviduct length for Hy-line breed was higher than that of the Dandrawi breed.

Lucy and Harshan (1999a) reported that in the Japanese quail, the oviduct witnessed rapid developmental changes between 30 to 40 days of age, with the weight of the oviduct being increased about 17 times between 40 to 50 days of age and finally it contributed 4.05% of the total body weight at the age of maturity.

Rahman *et al.* (1999) compared the developmental pattern of the oviduct in high producing ISA brown strain and low producing indigenous chicken in Bangladesh and observed that the total mass of the oviduct was significantly higher in high producing hens than in low producing indigenous hens and with the advancement of age, the size and length of the oviduct of ISA brown chicken increased more significantly than in indigenous chicken with a proportionately greater number of mucosal folds in the magnum and vagina. Similar observations were also made by Banerjee *et al.* (2006) in the Rhode Island Red birds.

Khokhlov and Kuznetcov (2007) found that in the domestic fowl development was more rapid in the caudal regions of the oviduct than in cranial regions.

Mohammadpour (2007) compared the morphology of oviduct of the domestic fowl with that of the duck in egg-laying phase and concluded that, the total weight and length of the oviduct was significantly higher in the hen than in the duck.

2.1.5 Segmental Variation in the Developmental Pattern of the Oviduct

2.1.5.1 Infundibulum

In chicken and turkey, Woodard and Mather (1964) observed a relatively short infundibulum. Lucy and Harshan (1999b) noticed that in the Japanese quail, the infundibulum was undifferentiated in the day-old chicks but in adult quails it was relatively longer and contributed 17.1% of the total length of the oviduct in Japanese quail. Khokhlov (2008) conducted an extensive study on the development of infundibulum of the domestic fowl from 150 days to 540 days of age and observed regular fluctuations in weight and length of the infundibulum with respect to total weight and length of the oviduct and noted that these fluctuations were synchronized with the functional stage of the oviduct, such that during the egg-laying period the weight-wise and length-wise contribution of infundibulum was found to be more than that in non-laying period.

2.1.5.2 Magnum

Rao Saheb and Iyengar (1945) and Marshall (1961) recorded that in the domestic fowl, the magnum made up more than 50% of the total length of oviduct.

Yu and Marquardt (1972) demonstrated that in the domestic fowl the rate of growth of magnum was much greater than other portions of the oviduct and these changes were primarily associated with hyperplasia as shown by increased amount of total DNA, and to a much lesser extent with the cellular hypertrophy as shown by increased ratios of dry matter/DNA.

According to Pageaux *et al.* (1986) in Japanese quail, the most interesting aspects of magnum growth (proliferation, differentiation, initiation of the

synthesis of specific proteins) took place in the beginning, when the oviduct weighed less than one gram and further development of the magnum involved only the accumulation of secretory products.

According to Lucy and Harshan (2000), the magnum was not differentiated in the early stages of life in the case of Japanese quail and in the differentiated oviduct, the magnum was found to be the longest and most coiled segment and contributed 48.3% of the total length of the oviduct.

2.1.5.3 Isthmus

Woodard and Mather (1964) observed that in chicken and turkey, the isthmus region was relatively short. Lucy and Harshan (1999c) in Japanese quail and Moraes *et al*. (2007) in nothura spotted quail found that the coturnix egg had thicker shell membrane when compared to that of chicken which could be attributed to increased length and the more time spent by the developing egg in this segment. Lucy and Harshan (1999c) in Japanese quail and Moraes *et al.* (2007) in nothura spotted quail reported that isthmus region contributed 17.7% and 16.32% to the total length of the oviduct, respectively.

2.1.5.4 Uterus (Shell gland)

In day-old Japanese quail, the uterine region could be distinguished as a small dilatation located between the 14^{th} lumbosacral segment and the third coccygeal vertebra (Lucy and Harshan, 1998) and the uterus of the adult quail was a sac-like distended region which was relatively shorter than that of chicken and turkey. The wall of the uterus was thin when compared to that of the magnum and isthmus but was more distensible.

Mohammadpour (2007) compared the morphology of uterus of the domestic fowl with that of the duck in egg-laying phase and observed that the uterus weighed less than that of fowl and was of shorter length.

2.1.5.4 Utero-vaginal Junction

Holm and Ridderstrale (2002) studied the development of utero-vaginal sperm storage tubules in the Japanese quail during sexual maturation. The sperm storage tubules started to develop at around 28 days of age with low columnar cells and at 35 days of age, small invaginations consisting of tall columnar cells with basal nuclei were observed. With the onset of extremely rapid growth of the oviduct at about 40 days of age, sperm storage tubules also exhibited rapid phase of development and by 49 days of age, when all the birds were in egg-laying phase, the presence of functional sperm storage tubules also became evident. They also speculated that although the development of the sperm storage tubules coincided with the oviductal development, the utero-vaginal sperm storage was possible even before the complete maturation of the oviduct in a few birds.

2.1.5.5 Vagina

Lucy and Harshan (1999d) observed that the vagina in the Japanese quail was relatively shorter than in the domestic fowl and turkey and the developmental sequence of vaginal region in quail was almost similar to that of the more cranial regions, but no glandular development could be noticed.

2.2 SEGMENTAL VARIATION IN THE DEVELOPMENTAL HISTOMORPHOLOGY OF OVIDUCT

2.2.1 Infundibulum

Surface (1912) gave the term ‘glandular grooves’ which referred to the grooves lined by both few ciliated cells and group of non-ciliated secretory cells between the two successive mucosal folds of the neck region of the infundibulum, thus functionally acting as secretory region. King and Mc Lelland (1975) reported the presence of plates of glandular cells at the bottom of the grooves in the wall of the funnel region. They also observed that some convoluted branched tubular glands were present in the tubular part of the infundibulum but were more confined to the region adjoining the magnum. According to Parida *et al*. (2000) in

the Japanese quail, the tubular glands were mainly found at the origin of the secondary folds.

Bradley and Grahame (1960) described the formation of the tubular glands and described that in the neck of the infundibulum, passing caudally; the mucosal folds became more complex with more deeper glandular grooves which eventually gave rise to small tubular glands from their corners.

Aitken and Johnston (1963) summarized four types of epithelial cells in the mucosa of infundibulum namely non-secretory ciliated cells located essentially in the surface epithelium; non-ciliated mucous-secreting goblet cells found amongst the ciliated cells; secretory cells other than goblet cells which were located in the glandular grooves and lining cells of the tubular glands of the caudal region of the infundibulum. They also proved that the cells of the infundibular glands of the oviduct in domestic fowl participated in the formation of chalazae.

The lining epithelium at the opening of the lips of the funnel was flat ciliated type which rapidly changed into ciliated columnar in the distal funnel and neck of the infundibulum (Dominic, 1960 in pigeon and Hodges, 1974 in domestic fowl). Muwazi *et al.* (1982) stated that the Infundibulum in the ostrich was internally lined by a tall, simple columnar epithelium with some areas of pseudostratification.

Hodges (1974) and Naragude *et al.* (1999) in domestic fowl, Lucy and Harshan (1999b) in Japanese quail and Moraes *et al.* (2007) in nothura spotted quail and Das and Biswal (1968), Rao (1994) and Ozen *et al.* (2009) in duck observed that the ciliated cells were tall columnar cells with an oval nucleus lying at or above the centre of the cells and possessed an apical tuft of cilia whereas, the goblet cells showed a basal nucleus and apical granular cytoplasm. On the contrary, Parida *et al*. (2000) reported the absence of goblet cells in the infundibulum proper and described their sparse presence at the infundibulum-magnum junction in the Japanese quail.

Hodges (1974) stated that the longitudinally oriented low mucosal folds appeared to increase in depth as the funnel approached the neck region whereas within the neck of the infundibulum, the spirally oriented longitudinal folds increased in depth and exhibited secondary folds. According to Hodges (1974) and Gilbert (1979) in domestic fowl and Lucy and Harshan (1999b) in Japanese quail, the neck region showed ill-defined longitudinal and circular muscle bundles and at the posterior end of the neck where the infundibulum joined the magnum, both the muscular and mucosal layers increased in thickness and complexity.

Khokhlov and Kuznetcov (2007) reported that in the domestic fowl infundibulum showed the least height and width of mucosal folds throughout the postnatal development. The height and width of mucosal folds were found to be increased rapidly during the period of intensive development until the period of stable functioning of the oviduct was attained.

Chousalkar and Roberts (2008) were of the opinion that, in domestic fowl, non-ciliated cells in the infundibulum region contributed to the secretions during egg formation, whereas the ciliated cells showed little evidence of secretion. Khokhlov (2008) conducted an extensive study on the developmental morphology of the infundibulum of the domestic fowl from 150 days to 540 days of age and observed that the size of the epithelium during this period changed within the limits of 10 to 18 μm, depending on the functional condition of the organ. Analysis of the wall of the infundibulum revealed that tunica mucosa occupied the maximum thickness (64%) then followed by the tunica muscularis (29%) and tunica serosa (7%). The serosal tunic was found to be thin with folds, blood vessels and nervous plexuses.

Mohammadpour and Keshtmandi (2008) conducted histomorphological study of infundibulum in turkey and pigeon, and found that, the mucosal lining of the folds of funnel region showed extensive ciliation and speculated that, the rhythmic beating of these cilia and the gross oblique arrangement of the mucosal folds created a vortex to pull and transport the egg in both the species. Mucosal

folds in the infundibulum of pigeon were much taller than those in turkey and in pigeon these mucosal folds were leaf-shaped with secondary folds.

2.2.2 Infundibulum-Magnum Junction

According to Aitken (1971), at the infundibulum-magnum junction, group of infundibular glands intermingled with tubular glands of the magnum, but the epithelial types lining the glands were always distinct.

The surface of the oviduct of *Gallus domesticus* was examined by Bakst and Howarth (1975) using cryofracture followed by scanning electron microscopy and found that the short, randomly oriented mucosal folds of the infundibulum increased in height and longitudinal orientation as they approached the magnum region. Similar observations were also made by Lucy and Harshan (1999b) and Parida *et al.* (2000) in the Japanese quail and Rao (1994) and Ozen *et al.* (2009) in duck.

2.2.3 Magnum

According to Richardson (1935), in the last three to four centimetres of the magnum in the domestic fowl, the surface epithelium contained very large number of secretory cells and thus this area was named as ‘mucous region’.

Yu and Marquardt (1972) carried out an investigation to illustrate the biochemical and histomorphological pattern of development in the magnum of fowl and showed that these developmental changes were identified histologically as the formation, proliferation and subsequent growth of cytoplasmic mass of the tubular gland cells and observed that these cells, containing secretary granules attained their maximum size in the laying hens.

Hodges (1974) noted that the overall diameter of the magnum was considerably greater than that of the infundibular neck, which was mainly due to a marked increase in the thickness of the wall. This was partly as a result of an increase in the development of muscle layers but was mainly due to the increased thickness of mucosa. Mucosal folds of magnum when compared to infundibular

region were considerably increased in their number, height and width. It has been speculated that, development of glandular layer although increased the size of the mucosal folds, tended to decrease the degree of secondary folding.

Hodges (1974) also reported that the muscular layers of the magnum were somewhat thicker and the bundles were more clearly arranged in definite layers than in the infundibular funnel. However the musculature of this region was less developed than in the posterior parts of the oviduct. He also demonstrated that the mucosa of the magnum was lined by an epithelium consisting of ciliated columnar cells and secretory goblet cells. Before the passage of yolk through the magnum the goblet cells which appeared to dominate in the epithelium were distended with secretory granules whereas, ciliated cells appeared as narrow structures; but once the secretory material was discharged, the goblet cells appeared to be obscured by the ciliated cells and the surface had a ciliated appearance. Gilbert (1979) described the secretory granules and confirmed their incorporation in the albumin deposition.

Pageaux *et al.* (1984) stated that in the quail, the most dramatic growth and differentiation of the magnum began between 21 to 28 days of age and the birds started egg-laying about 20 days later.

Studies on the morphogenesis of magnum in the Japanese quail by Pageaux *et al.* (1986) indicated that immature magnum was characterized by low mucosal folds with densely packed cells of connective tissue lined by a single layer of undifferentiated luminal epithelial cells and the occurrence of large number of mitotic cells in the epithelium. Subsequent increase in the height of mucosal folds indicated the phase of rapid proliferation which was followed by gradual invasion of proliferating epithelial cells in to the subepithelial stroma to form tubular glands. It was also evident from their study that the tubular gland formation and ciliogenesis occurred simultaneously. Electron microscopy suggested that the appearance of mucous granules in the luminal cells preceded the appearance of secretory granules in tubular gland cells.

Yoshimura and Ogawa (1998) compared the magnum region of the guineafowl with that of domestic fowl and observed that in the magnum of the guineafowl, the secondary mucosal folds were well developed forming many large duct-like structures in the lamina propria. Madekurozwa (2005) stated that ciliogenesis in the magnum region in immature ostriches with active ovaries was patchy. Hutchison (2008) investigated the development of the magnum region of the oviduct in relation to the reproductive cycle of the Barbary dove (*Streptopelia roseogrisea*) and observed that the tubular glands in the mucosal folds increased in size along with increase in precursor albumin granule content of glandular epithelium during the pre-egg-laying phase which was a result of an increase in the plasma progesterone levels highlighting the endocrine control of growth of the oviduct during postnatal development.

Mohammadpour and Keshtmandi (2008) compared histomorphology of magnum region in the oviduct of turkey and pigeon, and found that, the height of mucosal folds of magnum in pigeon was much greater than those in the turkey.

2.2.4 Magnum-Isthmus Junction

Hodges (1974) in domestic fowl, , Rao (1994) in duck, Lucy and Harshan (2000) in Japanese quail and Moraes *et al.* (2007) in nothura spotted quail found that at the posterior end of the magnum the longitudinal mucosal folds became reduced in height and each fold became thinner with the concurrent development of more secondary folds. It was also observed that almost immediately, however, the mucosal folds increased in height again as they entered the isthmus proper but did not regain the height or breadth found in the magnum.

2.2.5 Isthmus

The ciliated cells of the surface epithelium retained their columnar shape with an apical nucleus throughout the secretory activity, being unconstricted by the adjacent glandular cells. When the isthmus was secreting the shell membranes the gland epithelium showed maximum activity and each cell became full of

spherical, deeply stained granules. The secretory material in their lumina was either of similar granules or fused secretory masses which after passing out of the tubular glands frequently took the form of threads or twisted strands (Richardson, 1935). The nuclei were basal, rounded and pale staining. During resting phase the glands stained more evenly and due to reduction in the number of granules, large and clear areas appeared in the cytoplasm of cells and their nuclei became large and vesicular in appearance.

Romanoff and Romanoff (1949) stated that the overall diameter of the isthmus was less than that of the magnum.

Draper *et al.* (1972) reported that in the domestic fowl, surface epithelium of isthmus had approximately equal number of ciliated and non-ciliated cells which were separated from an underlying layer of tubular glands by a thin glycoprotein basement membrane and a thin connective tissue containing blood vessels.

The mucosal folds of the isthmus region were rather angular in the appearance and the apertures of the tubular glands were situated in the depressions of the surface epithelium, which were much more numerous than in any other part of the oviduct. Tubular glands within the mucosal folds were similar to that seen in the magnum, but were not so well developed (Hodges, 1974). Mehta *et al.* (2005) were of the opinion that the secretory granules in the cells of isthmian glands were fine and smaller than those of magnal glands.

Hodges (1974) described the lining epithelium of the isthmus to contain both ciliated and secretory cells. Regular alteration of these cells was noticed and according to him, these cells were having approximately the same width.

Histomorphology of the isthmus region during non-laying and laying stage in common mallard was studied by Sharma and Duda (1992). According to them, during the development only the tunica serosa remained unchanged while the tunica mucosa and tunica muscularis exhibited significant increase in thickness.

According to Sharma and Duda (1992), each tubular gland was lined by six to eight cuboidal cells with centrally placed nuclei and sparse cytoplasm in non secretory phase. This was transformed into pyriform cells with basal nuclei filled with coarse secretory granules in the secretory phase.

Hoffer (2005) studied the ultrastructure and cytochemistry of the shell membrane secreting region of the Japanese quail and noted that the tubular glands were made up of only a single cell type, the principal cells that contained numerous secretory granules. Chousalkar and Roberts (2008) identified two types of glandular cells in the isthmus region of domestic fowl during egg-laying cycle electron microscopically.

2.2.6 Isthmus – Uterus Junction

The initial portion of the shell gland was short, tube-shaped and showed different type of glands when compared to either the isthmus or the shell gland proper, thus this portion was referred to as isthmo-uterine region by Richardson (1935). Hodges (1974) showed that in the isthmo-uterine junction, there was no abrupt change in gland architecture between the adjacent regions, but an area of intermingling of glands could be observed within the lamina propria.

2.2.7 Uterus or Shell Gland

Romanoff and Romanoff (1949) stated that the sac-like shell gland proper showed distensible walls, which were not as thick as those of the magnum or isthmus. The musculature of the wall was well developed, particularly the outer longitudinal layer, and in general these layers were found to be better developed than in the more anterior segments of the oviduct.

Johnston *et al.* (1963) located a third type of glands to be present at the junction between isthmus and uterus whose granules much smaller and less numerous than those of isthmus and cytoplasm was frequently vacuolated.

According to Breen and Bruyn (1969), at ultramicroscopic level, the uterine tubular glands also possessed irregular frond-like microvilli, which during

the egg shell formation formed large blebs and were speculated to be correlated to the elaboration of a watery, calcium-containing fluid. They also found that the free surface of the apical cells possessed numerous cilia interspersed with long slender microvilli whereas, the luminal surface of the basal cells showed presence of only microvilli. These microvilli formed an extensive network of cytoplasmic processes in presence of a fully calcified egg in the uterus and were much shorter and less in number in presence of an incompletely formed egg. They also demonstrated that at the final stages of shell formation, these microvilli resembled stereocilia which underwent cyclical changes similar to the microvilli of apical cells.

Secretory granules were a constant feature of the supranuclear cytoplasm of the apical cells. Active secretion of these granules could be seen during the earlier phases of shell formation and during the latter half of shell secretion many secretory granules migrate to supranuclear cytoplasm. Here, a series of events were described by Breen and Bruyn (1969) and Hodges (1974). According to them, the secretory granules were subjected to a process of disintegration in supranuclear cytoplasm with concurrent formation of a large membraneless space, which was termed as vacuoloid, which subsequently regressed and the space was re-occupied by developing endoplasmic reticulum. This suggested that material in the vacuoloid originating from the disintegrating granules was re-synthesized and utilized for the formation of secretory product. Similar observations by Chousalkar and Roberts (2008) confirmed that at electron microscopic level the non-ciliated glandular cells of the shell gland contained more vacuoles which regressed during the onset of egg formation with the subsequent replacement by extensive rough endoplasmic reticulum.

Hodges (1974) observed that when the shell gland was empty the gland cells became more cuboidal in appearance surrounding an enlarged lumen. According to him, underlying layer of complex branched tubular glands contained within the mucosal fold opened onto the surface of the folds by means of short, less conspicuous ducts that were lined by the gland cells. The tubular gland ducts

were closely packed together and in the cross-section, they consisted of five to seven or more polygonal cells enclosing a lumen which was empty during non laying phase. But, prior to and during secretory phase cells contained pale-staining granules and after the shell formation the cytoplasm was found to be markedly vacuolated, with relatively large nucleus located towards the base of each cell.

Hodges (1974) was of the opinion that in the cross-section the mucosal folds were of lower height than those of the magnum and were less distended by the glandular layer such that some secondary folding was visible. According to Bakst and Howarth (1975) these mucosal folds were narrow when compared to those of the more cranial segments.

Lucy and Harshan (1998a) noted that the uterus was wider and thinner than in the cranial portions of the oviduct in the day-old quail chicks. In the adult birds, low primary mucosal folds were transformed into numerous long, spatula-shaped folds covered by ciliated apical and non-ciliated basal cells similar to the domestic fowl. They also observed that the tunica muscularis was very well developed and consisted of inner circular and irregular bundles and the outer thick longitudinal layer.

Yoshimura and Ogawa (1998) compared the shell gland region of the guineafowls with that of domestic fowl and found that the distribution of the tubular glands in the lamina propria at the bottom region of the mucosal folds was significantly denser than at the apical region in both species. Height of the mucosal folds was significantly greater in the shell gland region than in the magnum and isthmus in guineafowl, whereas it was the greatest in the magnum in domestic fowl.

Khokhlov and Kuznetcov (2007) observed that throughout the developmental period, the shell gland showed the greatest height of the epithelium in domestic fowl.

Madekurozwa (2007) noticed that in immature ostriches with active ovaries, the surface epithelium of the uterus showed a single layer of columnar cells with alternating apical and basal nuclei. Accordingly these were termed as apical and basal cells, with the apical cells being ciliated and the basal cells having restricted apical surface.

Mohammadpour (2007) compared the histomorphology of the uterus of the domestic fowl with that of duck in egg-laying phase and stated that instead of the usual continuous mucosal folds, the shell gland mucosa was thrown into longitudinally numerous flat, discontinuous, leaf-shaped folds. Height and number of the primary mucosal folds were greater in duck than in hen but secondary mucosal folds were larger in the case of hen.

2.2.8 Utero-vaginal Junction

Bobr *et al.* (1964) reported that the normal residence sites for spermatozoa in the avian oviduct were the tubular glands within the mucosa of utero-vaginal junction, the utero-vaginal glands. According to them, these glands were present in a thick ring of pushed-in mucosal folds found in the lumen with the vaginal folds on one side and uterine folds on the other.

The microscopic structure of sperm storage tubules was described by Fujii (1963), Bobr *et al.* (1964), Schindler *et al.* (1967), Van Krey *et al.* (1967), Gilbert *et al.* (1968), Burke (1972) and Tingari and Lake (1973) in domestic fowl, Van Krey *et al*. (1974), Schuppin *et al.* (1984), Bakst and Richards (1985), Bakst (1992), Bakst (1994), Bakst and Akuffo (2008), Bakst and Akuffo (2009) and Miranda *et al.* (2009) in the turkey, Bakst and Bird (1987) in American kestrel, Pal (1977) and Rao (1994) in the domestic duck and Renden *et al*. (1981) and Lucy and Harshan (1999e) in the Japanese quail. Of the 27 recognized orders of aves, sperm storage tubules have been identified histologically only in selected species of Galliformes (Fujii and Tamura, 1963), Anseriformes (Pal, 1977) and Falconiformes (Bakst and Bird, 1987). The sperm host glands were more sparsely distributed and less convoluted than the uterine glands but had

larger diameter as observed by Gilbert *et al.* (1968). According to Tingari and Lake (1973), there was no evidence of smooth muscle fibres or other contractile elements associated with sperm host glands and these were surrounded by connective tissue elements only.

Pal (1977) in the domestic duck and Bakst and Bird (1987) in the American kestrel (*Falco sparverius*) reported that the utero-vaginal junction that connected the uterus and vagina differed histologically from the adjacent zones. He observed that this region was characterized by low, longitudinally arranged mucosal folds which were lined by tall columnar ciliated cells with the apical nuclei alternating with mucous secreting goblet cells containing basal nuclei. The lamina propria of the mucosa housed numerous tubular glands called the sperm host glands which were responsible for sperm storage after copulation.

Ultrastructural studies were performed on the utero-vaginal sperm storage tubules of the quail by Frieb *et al.* (1978). The true glandular epithelium was composed only of columnar cells with microvilli on their luminal end. The characteristic luminal feature was a large lipid droplet in the perinuclear region.

Brillard (1990) suggested that in the absence of secretory cycle, the microvilli bordering the lumen of the utero-vaginal glands could participate in the purification of this micro-environment, thus allowing prolonged survival of spermatozoa in this region. They also indicated that the mechanism controlling the filling and/or storage of spermatozoa in the utero-vaginal glands was immuno-dependent and was closely related to the functional integrity of the glycocalyx surrounding the spermatozoon.

Bakst (1994) used nuclear fluorescent bisbenzimide stained sperms to evaluate the distribution of sperms in the oviduct of turkey hens before or after the onset of egg production and found that lower percentage of filled sperm storage tubules were associated with the onset of egg production, an indication that sperm storage capacity of the sperm storage tubules was diminished with the onset and continuation of egg production.

In domestic duck, Rao and Vijayaragavan (2000) found that the sperm host glands were restricted in between two muscular ridges, cranial and caudal utero-vaginal ridges. They also observed few mucosal folds on the uterine side of the cranial utero-vaginal ridge to contain transitional glands intermediate in appearance between sperm host glands and uterine glands. The transitional glands were larger than uterine glands but were smaller in diameter than sperm host glands.

Freedman *et al.* (2001) in turkey and Das (2003) in domestic fowl reported histological evidence for the innervations of sperm storage tubules at the utero-vaginal junction of the oviduct by bright field microscopy and suggested that a previously unrecognized neural factor may function in the oviductal sperm storage in, and release of spermatozoa from the sperm storage tubules.

Breque *et al.* (2006) investigated the antioxidant status of the lower oviduct and found a higher ascorbic acid content in utero-vaginal junction as compared to other regions of the lower oviduct of domestic fowl which indicated that utero-vaginal junction provided a complex defense barrier against lipid peroxidation of the sperm plasma membrane during prolonged in vivo storage unique to avian species.

Bakst and Akuffo (2008) considered that sperms that traversed the vagina and populated the sperm storage tubules were a result of an intensive process of selective elimination or by cryptic female choice or both. They localized serotonin reactive cells immunohistochemically and found that such cells appeared to populate vaginal and utero-vaginal mucosa in the turkey but not the sperm storage tubule epithelium. Even though the source of serotonin was not made clear in this study, they speculated that viable sperms facing the vaginal and utero-vaginal epithelium might be triggering the serotonin release and the released serotonin in turn could augment cilia and sperm tail beat frequencies to facilitate smooth muscle contraction and indicated a local control mechanism responding to sperm storage and motility in the oviduct.

Miranda *et al.* (2009) studied the sperm storage tubules in turkey and mentioned that the lining epithelium was slightly different from fowl in that in laying stage, it consisted of light and dark prismatic cells with vesicular nucleus and basal as well as apical microvilli. According to them, in the reproductive phase, the total number, length and inner and outer diameter of sperm storage tubules were increased when compared to quiescent phase.

2.2.9 Vagina

The height of the epithelium over the crest of the vaginal folds was greater than that found in the shell gland (Richardson, 1935). However, in the depressions between the folds, the cells were shorter with a predominance of the non-ciliated gland cells (Fujii, 1963).

Fujii (1963) observed that the vaginal duct was not straight but, was having a distal initial double flexure followed by further slight convolutions which were closely bound together and to the shell gland by an outer well developed connective tissue layer.

In vagina, there were tubular glands within the corium of mucosal folds and the surface epithelium was thrown into many secondary folds and consisted of alternating ciliated cells, with apical nuclei and non ciliated mucous secreting glandular cells possessing basal nuclei (Aitken, 1971 and Hodges, 1974).

King and Mc Lelland (1975) noted that the shell gland was separated from vagina by a well developed muscular sphincter. The vagina extended from this muscular sphincter to the cloacal wall. The vaginal wall was observed to be very thick due to thicker tunica muscularis comprising of moderately developed outer longitudinal layer and strongly developed inner circular layer being thicker than in any other part of the oviduct.

Brennan *et al*. (2007) were the first to describe the complex anatomy of the vagina in various waterfowl species and they correlated the length and configuration of the vagina to the morphology of the male's intromittant organ

and subsequently arrived at an explanation describing the coevolution of the waterfowl genitalia.

Madekurozwa (2008) compared the gross anatomy and histomorphology of the vagina of the emu (*Dromaius novaehollandiae*) and ostrich (*Struthio camelus*) and found a series of broad annular mucosal folds bearing convoluted primary folds in this region. The lining epithelium was a combination of ciliated and non-ciliated cells wherein, the non-ciliated and few ciliated cells lining the crypts contained mucin droplets, function of which was unclear.

2.2.10 The Immune Status of the Avian Oviduct During Postnatal Development

Biswal (1954), Trautmann and Fiebiger (1957), Bradley and Grahame (1960) and Kimijima (1989) in domestic fowl, Rao (1994) in duck, Lucy and Harshan (1999d) and Parida *et al.* (2000) in the Japanese quail, Moraes *et al.* (2007) in nothura spotted quail and Mohammadpour and Keshtmandi (2008) in turkey and pigeon reported that lymphocytes were present in the all the segments of the oviduct in either diffused form or in aggregates along with many plasma cells. Contrary to this, Das and Biswal (1968) could not locate lymphocytes in the oviduct of duck.

Mast cells were demonstrated by Rao (1994) throughout the oviduct in the domestic duck with majority of cells present in the infundibular region.

Khan *et al.* (1996) studied postnatal development of T-cell subpopulations in the oviduct in the Dekalb strain of the White Leghorn chicken by using an immunohistochemical method. T-lymphocytes first infiltrated the oviduct at five weeks. The number of T cells peaked at 15 weeks in the magnum, isthmus and uterus, and at 19 weeks in the infundibulum and vagina. The epithelium of the oviduct contained both granular and agranular lymphocytes. The relative frequency of T-cell subpopulations was found to be higher in the vaginal part than in other parts of the oviduct. These results suggested that the postnatal

developmental changes of T-cell subpopulations depended on regions of the oviduct and on the age of the bird.

Yoshimura and Zheng (1999) localized the macrophages in different regions of the oviduct of domestic fowl by immunohistochemistry. Macrophages could be located in the mucosal epithelium and stroma of all oviductal segments in immature and laying hens and the population of macrophages was significantly higher in laying hens than in immature hens. Laying hens had a significantly higher population of the macrophages in the vagina than immature hens.

2.2.11 General Histomorphology and Developmental Changes in the Oviduct

Richardson (1935) remarked that though the limits of the sub-divisions of the oviduct were primarily set by external, macroscopic criteria, they were finally determined by the changes in the structure of the tubular glands. Bradley and Grahame (1960) stated that oviduct of domestic fowl was devoid of any muscularis mucosae.

Yu and Marquardt (1972) stated that the tubular gland formation occurred in the developing oviduct of fowl weighing 1gm and that the egg albumin synthesis was initiated when the oviduct weighed approximately 3 gm.

Hodges (1974) described seven layers in the wall of the avian oviduct viz., tunica serosa an outer covering of peritoneal epithelium, an outer longitudinal smooth muscle layer covering an inner circular smooth muscle layer with an intervening connective tissue layer in which blood vessels and nerves were present, a layer of connective tissue internal to the circular muscles followed by the mucosa consisting of a lamina propria containing glands in most regions of the oviduct and an inner epithelial lining.

Scanning electron microscopic studies were conducted on the surface of the oviduct of *Gallus domesticus* by Bakst and Howarth (1975) who demonstrated the predominance of ciliated epithelial cells throughout the oviduct

with non-ciliated cells approaching an equal proportion in the magnum and isthmus.

King and Mc Lelland (1975) observed that in spite of the distinctive contribution made by each region of the oviduct, the mucosal folds were more or less continuous throughout the oviduct, though varying in height and thickness. They ascribed the function of upstream transport of spermatozoa and downstream transport of the egg to the peristaltic activities of tunica muscularis in the wall of the oviduct and noted that the tunica muscularis was thickest in the vagina followed by uterus and thinnest in the infundibulum.

There had been very few attempts to correlate nesting behaviour with reproductive development in birds. Hutchison (1977) correlated the development of nesting behaviour in female budgerigars with changes in the tubular glands of magnum and found that the oviducts were small with tubular glands being formed in the magnum region on the first and second days of nest box entry (i.e., eight to nine days before the egg laying) but, showed an increase in oviduct weight with albumin formation in the tubular glands of the magnum region, just within three to four days after the nest box entry. He concluded that such temporal relationships between oviduct and ovarian development and nesting behaviour were similar to those of nest building species such as the canary. Gupta and Maiti (1987) reported that during the nesting cycle of pied myna (*Sturnus contra contra*), the greatest growth in all the regions of the oviduct could be observed from early nest building to the actual egg laying period and this was followed by rapid involution during incubation period.

Arjaama and Talo (1983) were able to observe bundles of collagen between the muscle cells in the magnum and to a lesser extent in the isthmus of quail. Geetha *et al.* (1992) reported the presence of elastic and reticular fibres in the lamina propria of all the regions of the adult quail oviduct.

Evencioneto *et al.* (1997) compared the luminal oviductal epithelium of the laying and non-laying Muscovy duck (*Cairina moschata*) and noticed that

while in the laying period the mucosal folds and its lining epithelium were much more developed, with a certain predominance of non-ciliated cells; the non-laying period showed a great predominance of ciliated cells, except in the funnel region of infundibulum where they observed equilibrium between the two cell types.

With the aid of electron microscopy, Ozen *et al.* (2009) in Pekin duck proved that the organization of the monolayered ciliated and secretory cells forming the lamina epithelialis, particularly in the regions of uterus and vagina created an illusion or erroneous impression of cellular stratification because of the differential positioning of the nuclei of these cells. They also found that in the case of immature ducks and ducks in quiescent phase of reproductive cycle, the ciliated and secretory cells lining the epithelium and the proprial glands were not fully developed.

2.2.12 Role of the Oviduct in Formation of the Egg Components

Richardson (1935) related shell mineral secretion to the tubular glands of uterus. This function was also been suggested by the fine structural studies done by Johnston *et al.* (1963) and Breen and Bruyn (1969) in fowl. However, Schraer and Gay (1971) conducted autoradiographic localization of radioactive calcium by intravenous administration of ^{45}Ca and concluded that the columnar epithelium of the uterus had a greater affinity for ^{45}Ca than the tubular uterine glands or mucosal cells from other regions of the oviduct, which suggested that the columnar epithelium was the most active in translocation of calcium from the blood vessels to the lumen of the shell gland. Johnston *et al.* (1963) and Aitken (1971) attributed the cuticle formation by the apical cells of uterine mucosa. Makita *et al*. (1983) detected calcium in the secretory granules of both ciliated cells and tubular gland cells of isthmus, shell gland as well as magnum regions of the avian oviduct by scanning electron microscopy and energy dispersive X-ray microanalysis techniques which were otherwise not stainable with conventional calcium staining

methods. Thus, conclusive affirmation with regards to calcium secretion from different cell types of the oviduct is not yet fully established.

Burke (1972) believed that the infundibulum was the site of fertilization and that the sperms could not penetrate the ovum after it was covered by albumin.

The time relationships between ovulation, egg formation and oviposition in khaki Campbell ducks were investigated by Simmons and Hetzel (1983). It was estimated that ovulation occurred on an average 10 minutes after oviposition, and the ovum spent 15-30 minutes in the Infundibulum, 2.5 to 3 hours in the magnum, and 2 to 2.5 hours in the isthmus and 18.6 hours in the shell gland. Thus the mean ± S.D. time interval between two consecutive ovipositions was estimated to be 24.0 ± 0.3 hours.

Different proteins and extracellular matrix macromolecules were discovered and localized in various segments of the oviduct which were thought to be contributing to avian-type egg formation by many workers. Immunological studies of specific secretary proteins in the oviduct of hen by Yu and Marquardt (1972) indicated that the pattern of formation of these proteins was different and conalbumin could be detected in all regions of oviduct in all stages of development, whereas the ovalbumin was present only in the magnum in laying stage and not in any other region of the oviduct.

In order to establish the role of Calmodulin and related peptides in the activation of calcium transporting system in the avian egg shell gland, Lundholm (1990) determined the calmodulin content in the mucosa of different regions of the oviduct and found that the highest content was in the shell gland mucosa both in laying ducks and hens and also noted high content of calmodulin even in isthmus region, where the shell formation begins.

Ancel and Girard (1992) observed that certain birds like guinea fowl produced very heavy and thick shelled eggs. Panheleux *et al.* (1999) compared the anatomical features of the eggshell formation in guinea fowl and domestic

fowl and found that the rate of egg shell formation in guinea fowl was similar to that of domestic fowl but, in case of guinea fowl, the duration of linear shell deposition was increased by 2.1 hours relative to that in domestic fowls. Thus they concluded that the kinetics of egg shell deposition largely explained the increased egg shell weight in the guinea fowl.

Sharma and Duda (1992) were able to demonstrate that the isthmus region gave rise to a finely granulated secretory material which mixed with the glandular secretory product and this mixture later coalesced to form aggregates which organized into fibre like structures which contributed to shell membrane formation.

Hincke *et al*. (2000) identified lysozyme as a component of the uterine fluid by microsequencing and used immunocytochemistry to document its localization in the perialbumin layer surrounding the albumin, egg shell membranes and the shell matrix and speculated that in addition to its well known antimicrobial properties which could add to the protective function of the egg shell during embryonic development, it might also be a structural protein which in soluble form influenced calcium carbonate deposition during calcification.

Fernandez *et al.* (2001) and Wang *et al.* (2002) used immunohistochemical and *in situ* hybridization techniques to localize the type-X collagen in the isthmus region of the oviduct and postulated that in contrast to other matrix proteins, type-X collagen did not alter with the position of the egg in the oviduct. It was a short chain collagen present in the egg shell membranes fibres and contributed to structural integrity of egg and according to them, avian egg shell membranes played a key role in the formation and final structure of the egg shell.

Nys *et al*. (2001) electrophoretically classified egg shell matrix proteins as egg white proteins (lysozyme, ovalbumin, conalbumin, ovotransferrin and clusterin), bone protein (osteopontin), and proteins specific to the uterus (ovocleidins-17 and -116; ovocalyxins-32 and -36) and suggested that the egg shell matrix influenced the process of crystal growth by controlling size, shape

and orientation of the calcite crystals which contributed to the mechanical strength of the egg shell substantially.

Fernandez *et al.* (2003) defined the avian egg shell as an acellular bioceramic containing organic and inorganic substances that were sequentially assembled during the transit of egg along the oviduct. They demonstrated the occurrence of osteopontin, an extracellular matrix protein in each region of the oviduct which coincided with the concomitant presence of the egg in such region and concluded that this molecule could be a part of the mechanism regulating the egg shell calcification.

Fernandez *et al.* (2004) suggested that along with extracellular matrix molecules, the carbonic anhydrase was crucial for the normal egg shell formation since, its presence in the oviductal mucosa increased the velocity of the calcium crystal growth and eventually contributed to the fusion of these crystals to form aggregates.

Until recently the asymmetry of avian egg was being attributed to coiled nature of the oviduct, muscular contractions and rotation of egg *in vivo.*

Mao *et al.* (2006) described the role of the magnum-isthmus junction in the formation of the shell membrane in domestic fowl (*Gallus domesticus*). According to them, the narrow width of the lumen at the junction indirectly participated in the determination of the asymmetrical ellipsoidal shape of the eggs that were encased by the egg white and subsequently by perialbumin layer and shell membrane.

Mao *et al*. (2007) explained the asymmetry of egg shape as an adaptation specific to aves to reproduce in dry environment for which air chamber formation was a prerequisite and immunohistochemically correlated inner, medial and outer shell membrane formation to isthmus region which according to them, was responsible for formation of air chamber and thereby altered the centre of gravity to result in asymmetric ellipsoid shape of avian egg.

Rahman *et al.* (2007) studied the mechanism of chalaza formation in the oviduct of the Japanese quail and revealed that chalazae and chalaziferous layers were composed of the same materials as those produced by both types of secretory cells in the luminal and glandular epithelia at the infundibulum.

2.3 HISTOCHEMISTRY

2.3.1 Mucopolysaccharides

Fujii (1963) confirmed the accumulation of the non-ciliated gland cells in the depressions between the vaginal mucosal folds by the presence of acid mucopolysaccharides in these cells.

Johnston *et al.* (1963) noted that P.A.S. positive granules in the isthmo-uterine junctional zone glands to be smaller and less numerous than the isthmian glands.

Johnston *et al.* (1963) demonstrated the presence of P.A.S. positive granules in supranuclear region of basal cells of uterine mucosa and observed that the goblet cells in the tubular part of the uterus gave more P.A.S. positive reaction than those in the shell gland proper. Basal cells of the anterior shell gland stained for sulphated mucopolysaccharides, whilst those in the pouch stained for mucoproteins. According to Hodges (1974) in domestic fowl, Rao (1994) in duck, Lucy and Harshan (1998) in Japanese quail and Madekurozwa (2007) in immature ostriches with active ovaries, apical cells of the uterine epithelium gave a negative reaction to all stains for mucopolysaccharides.

The goblet cells found throughout the oviduct showed intense P.A.S. positive reaction in fowl (Aitken and Johnston, 1963), in duck (Pal, 1977 and Rao, 1994), in quail (Renden *et al.,* 1981 and Lucy and Harshan, 1998) and in immature ostriches with active ovaries (Madekurozwa, 2007).

Aitken (1971) demonstrated the presence of P.A.S. positive, slightly alcian blue-positive and toluidine blue metachromatic secretory material in the infundibular region of domestic fowl and also observed that the cells lining the

glandular grooves in this region showed presence of very fine P.A.S. positive granules which were alcian blue-negative. In the magnum, lining epithelium consisted of an acidic mucopolysaccharide. The isthmus region showed the presence of neutral mucopolysaccharides and sulphur-containing proteins in the lamina epithelialis. Similar observations were also made by Draper *et al*. (1972).

The transitional glands along with the sperm host glands showed intense P.A.S. positive reaction but were negative for acid mucopolysaccharides in fowl (Aitken and Johnston, 1963), in duck (Pal, 1977 and Rao, 1994) and in quail (Renden *et al.,* 1981 and Lucy and Harshan, 1998).

Ozen *et al.* (2009) reported that in case of Pekin ducks, particularly in the region of the isthmus, secretory cells were ascertained to be rich in P.A.S. positive material. The proprial glands of the isthmus also contained P.A.S. positive granules while the glands positioned near the epithelium stained lightly and those situated more deeply were observed to stain darker. Such a reaction was found to be very weak in immature ducks. They also demonstrated the presence of neutral and acid mucopolysaccharides using P.A.S. and Alcian blue (pH 2.5) method in the epithelium of isthmus region; while in magnum, only the acid mucopolysaccharides were seen whereas, vaginal region gave a negative reaction for neutral as well as acid mucopolysaccharides.

2.3.2 Lipids

Large amount of lipid was observed in the mucosal epithelium of the shell gland, transitional glands and the sperm host glands of domestic fowl (Fujii, 1963; Gilbert *et al*., 1968 and Tingari and Lake, 1973), ducks (Pal, 1977 and Rao, 1994), turkeys (Schuppin *et al*., 1984) and quails (Lucy and Harshan, 1998).

The significance of lipids in maintaining sperms was suggested by the absence of lipid containing vacuoles from the utero-vaginal gland cells of infertile turkeys (Gilbert *et al*., 1968).

2.3.3 Glycogen

Glycogen could be located in the sperm host glands of domestic fowl **(**Gilbert *et al*., 1968 and Tingari and Lake, 1973), turkeys (Schuppin *et al*., 1984), but was completely absent in ducks (Pal, 1977) and quails Lucy and Harshan (1998). On the contrary, Ozen *et al.* (2009) demonstrated that in case of Pekin ducks, utero-vaginal junction as well as infundibulum bearing the sperm host glands did show the presence of glycogen rich secretion.

Chousalkar and Roberts (2008) observed a predominance of glycogen particles in the tubular shell gland when compared to other regions of the oviduct in domestic fowl.

Lucy and Harshan (1999b) and Ozen *et al.* (2009) traced the presence of glycogen in the various regions of the oviduct in Japanese quail and in Pekin ducks, respectively and found that only the epithelial lining of isthmus region showed a positive reaction for glycogen.

2.3.4 Alkaline Phosphatase (ALP)

Kar (1950) studied the ALP activity in immature and in-lay pigeons and observed that in juvenile pigeons of four month age, ALP was traceable in very sparse amounts whereas, in adult in-lay pigeon, activity of the enzyme was appreciable in tubular glands of the various regions of the oviduct.

Wilcox and Cloud (1965) observed that ALP activity was markedly higher in the ovary than in the oviduct of hen. They also observed that, the ALP activity was the maximum in the vagina followed by the infundibulum, shell gland, isthmus and the least in the magnum.

The ALP activity in the oviduct of the domestic fowl was restricted to the endothelial lining of the blood vessels in entire oviduct (Solomon, 1975), the vaginal epithelium (Fujii, 1963) and the sperm host glands (Gilbert, 1979).

Lucy and Harshan (1998) observed that in the Japanese quail, alkaline phosphatase activity was seen in the uterus, isthmus and magnum in the decreasing order of intensity whereas, the infundibular and vaginal epithelium showed only a mild positive reaction for alkaline phosphatase. It was also observed that the apical region of the lining epithelium of sperm host glands gave a positive reaction for alkaline phosphatase.

Chaudhuri and Maiti (1999) examined the oviductal enzyme activity during annual ovarian cycle of the Indian tree pie (*Dendrocitta vagabunda*) and found that ALP value was low during the non-breeding phase (August to January), increased during the progressive phase (February to March), became maximum during breeding season (April to May) and decreased in the regression phase (June to July).

Bakst and Akuffo (2007) located scattered ALP positive cells in the epithelia of the vagina and utero-vaginal sperm host glands of turkey and observed an intense activity in apical borders of sperm storage tubules. They suggested that, ALP activity might reflect cell differentiation and proliferation in the vagina and sperm storage tubules and possibly a mechanism for the transfer of lipids from the sperm host glands to resident sperms.

2.3.5 Acid Phosphatase (ACP)

The ACP activity was demonstrated in the epithelium and tubular glands of isthmus, shell gland and vagina of the domestic fowl (Fujii, 1963; Gilbert *et al*., 1968 and Tingari and Lake, 1973), ducks (Pal, 1977 and Rao, 1994) and quails (Lucy and Harshan, 1998).

Pal (1977) and Rao (1994) observed intense ACP activity in the sperm host glands of duck.

Chaudhuri and Maiti (1999) examined the oviductal enzyme activity during annual ovarian cycle of the Indian tree pie (*Dendrocitta vagabunda*) and

found that ACP value followed similar pattern of seasonal changes in activity as that of ALP.

Materials and Methods

3. MATERIALS AND METHODS

In all, 78 Kuttanad ducks were used for the present study. The birds were selected randomly from a single hatch and reared at the University Poultry Farm, Mannuthy under semi-intensive system of management. Feed and water were provided *ad lib*. The ducklings were not given any vaccination.

The study was carried out in birds of different age groups, ranging from day-old to 24 weeks as shown in table 1. The material was collected from six birds in each group at fortnightly intervals.

Table 1. Age and number of birds used for the experiment

Sl. No.	**Age of birds**	**Number of birds**
1	Day-old	6
2	2 weeks	6
3	4 weeks	6
4	6 weeks	6
5	8 weeks	6
6	10 weeks	6
7	12 weeks	6
8	14 weeks	6
9	16 weeks	6
10	18 weeks	6
11	20 weeks	6
12	22 weeks	6
13	24 weeks	6
	Total	**78**

The live body weight of the birds was recorded and then they were anaesthetized and bled to death. The birds were dissected and the topography of the oviduct including its position and relationship were noted.

In the day-old duckling, the infundibulum, magnum and isthmus regions were undifferentiated, but the uterus and vagina were recognizable with an overall increase in the total diameter of uterine region of the oviduct. Morphologically, the same picture was noticed up to the seventh group. From the 12th week onwards all the segments of the oviduct were clearly differentiated, which were as follows.

1) Infundibulum:	From the tip of the funnel to the base of the neck.
2) Magnum:	From the base of the neck of the infundibulum to a narrow translucent zone at the magnum-isthmus junction.
3) Isthmus:	From the narrow translucent zone to the isthmo-uterine junction.
4) Uterus:	From the isthmo-uterine junction to the utero- vaginal junction.
5) Vagina:	From the utero-vaginal junction to the cloaca.

The morphometry including weight, length and diameter of the oviduct and its segments was recorded. After recoding the biometry and gross features, the material was fixed in neutral buffered formalin. Different segments of the oviduct were processed and paraffin sections of 5μm thickness were taken for histological studies. For alkaline and acid phosphatases (ALP and ACP), the material was fixed using chilled acetone. For histochemical demonstration of

lipids and phosphatases, frozen sections of 10μm thickness were used. Micrometric observations were recorded using an ocular micrometer.

The following staining techniques were employed:

1. Haematoxylin and Eosin (H & E) staining technique for routine histological studies (Luna, 1968).
2. Van Gieson's method for collagen (Luna, 1968).
3. Gomori's rapid one step trichrome method (Luna, 1968) for connective tissue.
4. PAS and Alcian blue method for mucopolysaccharides (Luna, 1968)
5. Best's carmine method for glycogen (Bancroft and Stevens, 1977).
6. Gomori's alkaline phosphatase cobalt method (Singh and Sulochana, 1996).
7. Gomori's method for acid phosphatase (Singh and Sulochana, 1996).
8. Oil Red O in propylene glycol method for fat (Luna, 1968).

The data on the following physical parameters were analyzed statistically (Snedecor and Cochran, 1994) to find out the relationship between the following, if any:

1. Age and the whole oviduct parameters (weight and length of the oviduct).
2. Age and the oviduct segmental parameters (weight, length, width and thickness of different segments of the oviduct).
3. Body weight and the whole oviduct parameters (weight and length of the oviduct).
4. Body weight and the oviduct segmental parameters (weight, length, width and thickness of different segments of the oviduct).
5. Age and the changes in the thickness of various layers of the oviduct
6. Height and width of mucosal folds and the diameter of tubular glands of different regions of the oviduct in various age groups in order to trace their growth pattern.

Results

4. RESULTS

4.1 DEVELOPMENT AND GROSS MORPHOLOGY OF THE OVIDUCT

4.1.1 Topography of the Oviduct

In the day-old duckling, the left oviduct was thin, thread-like, translucent, straight, narrow tube which occupied left side of the coelomic cavity (Fig. 1). It originated caudolateral to the left ovary and extended posteriorly along the ventral aspect of the left kidney and terminally joined the cloaca (Fig. 2). At this age, it was poorly supplied by blood vessels as evident from its generalized pallor.

The oviduct was suspended from the left lateral body wall by the dorsal ligament, a thin fold of peritoneal membrane attached dorsally to the inner surface of abdominal cavity, running caudally from the region of the fourth thoracic rib to the cloaca. The dorsal ligament also carried left ureter dorsolateral to the oviduct and both the oviduct and ureter ran parallelly in the caudal direction. The ventral ligament was attached to the ventral surface of the oviduct and showed a gradually thickening muscular band along its lower free edge that fused posteriorly with the caudal most region of the oviduct, the vagina.

In the adult laying bird without an egg, the oviduct was highly convoluted tubular structure and occupied the left half of the coelom almost completely (Fig. 3). In laying phase, however, the oviduct containing a developing egg extended towards the right side also and displaced the intestines in right ventral direction (Fig. 4). Dorsal surface of the oviduct was related to the ventral surface of the left kidney and left lateral body wall. Ventrally on the left, it was related to the proventriculus, gizzard and spleen towards the cranial aspect and intestines in the caudolateral part.

4.1.2 Development of the Left and Right Paramesonephric Ducts

The left paramesonephric duct was much more developed and functionally advanced than the right one during the postnatal period. The occurrence of right oviduct was difficult to trace out during the period from day-old to eight weeks of

age. From 10th week onwards, the presence of the right oviduct could be detected in two to three birds in each age group. Total number of birds with persistent right oviduct was recorded to be 20 and the average length of the rudimentary right oviduct ranged from 0.70 to 2.91 cm. Out of the 20 birds, an exceptional case was observed at 18th weeks of age, wherein, the right oviduct attained a maximum length of 13.80 cm (Fig. 5). Mean length of the left oviduct at same age was 29.23 ± 0.28 cm (Table.2).

Right oviduct was a richly vascular blind sac-like tube with rounded tip and showed two to three convolutions along its length before it joined the cloaca exactly at the same level as that of the left functional oviduct. The width of right oviduct was maximum in the cranial region and minimum in middle portion. The diameter slightly increased where it opened into cloaca and this region was opaque white in colour and was the toughest region of the right oviduct.

4.1.2 Structural Classification of Different Regions of the Oviduct

In the present study, five different segments of the oviduct namely, infundibulum, magnum, isthmus, uterus and vagina could be clearly distinguished from 12th week onwards (Fig. 6).

Infundibulum, the first segment of the oviduct, was held in position by the dorsal and ventral ligaments and its funnel-like opening was stretched and flattened in the dorso-ventral direction in longitudinal plane with its flared lips lying in close proximity of the ovary (Fig. 7). The infundibulum showed a distinct funnel and a tubular neck. Walls of the funnel converged rapidly to form the infundibular neck, a narrow thin-walled tube which increased in size and thickness to form the magnum.

The magnum, the second segment was by far the longest and most coiled segment of the oviduct and appeared darker than rest of the oviduct with a distinct brown to dark brown shade (Fig. 7).

A narrow translucent zone, the magnum-isthmus junction separated the magnum from the isthmus region and here the colour changed from brown to off-

white. The Isthmus region was narrower than the magnum and diameter gradually increased at the isthmo-uterine junction (Fig. 7).

The uterus or shell gland was subdivided into a short, comparatively narrow, cranial portion and a pouch-like caudal portion in which developing egg spent much of its time during the shell formation (Fig. 7). The existence of this pouch region was independent of the presence of the egg, and, although it was much reduced in size, it could be distinguished in the oviduct of the non-laying duck.

Vagina, the last segment of the oviduct was a relatively short, much convoluted, S-shaped condensed tube that extended from a well developed muscular sphinctor at the posterior end of the uterus and it terminally opened into the urodeum of cloaca dorsomedial to the left ureter (Fig. 7).

4.1.3 Postnatal Developmental Pattern of the Oviduct

4.1.3.1 Beginning of Coiling of the Oviduct

From day-old upto eight weeks of age there were no signs of the coiling of the oviduct and oviduct remained as a straight tube with collapsed lumen. From 10^{th} week onwards, slight coiling of the oviduct began (Fig. 8). By 14^{th} week, the oviduct showed much coiling and exhibited turgidity instead of collapsed nature throughout its length.

4.1.3.2 Phase of Partial Differentiation to Phase of Complete Differentiation of the Oviduct

In the day-old duckling itself, the caudal regions of the oviduct could be differentiated into dilated uterine region and a more distended opaque white vaginal region (Fig. 2). However, the cranial segments like infundibulum, magnum and isthmus were difficult to differentiate from gross appearance.

The phase of partial differentiation persisted until 10^{th} week of age. From 12th week onwards, all the segments of the oviduct were distinguished including the subdivisions of infundibulum.

Egg-laying started from 18^{th} week onwards and the oviduct was morphologically and functionally differentiated at this stage as evident from

concurrent development of shell gland pouch region and occurrence of developing egg within. All the birds started laying by 20 weeks of age.

4.1.3.3 Relationship between age and body weight

Average body weights of birds of different age groups are given in table 2. The mean body weight of the day-old Kuttanad duckling was 38.64 ± 0.34 g. The body weight progressively increased upto 16th week of age, before the beginning of egg-laying and the maximum mean weight of 1783.33 ± 89.85 g was attained at 18th week, when egg laying started (Table. 2). The body weight increased by 46 times from the day of hatch to 18th week of age. From 20th week onwards, the body weight showed a decreasing trend and at 24th week of age, the mean body weight was 1476.67 ± 28.05 g (Table. 2). From day-old to 24th week of age, body weight exhibited highly significant correlation with the age (r = 0.873) at 1% level of significance (Table. 9).

4.1.3.4 Relationship Between Age and Weight of the Oviduct

Mean weigh and length of oviduct at different ages are presented in table 2. In the initial stages, the increase in weight of the oviduct was in accordance with the growth of the bird (Fig. 9). In the day-old duckling, the oviduct weighed 0.05 ± 0.00 g and showed more than six times increase within two weeks (Table. 2). From 3rd week onwards, weight of the oviduct doubled at fortnightly intervals and showed only a slight increase from 8th to 10th week (Table. 2).

At 12th week of age, when all the segments were differentiated morphologically, the mean oviduct weight increased two and half times than that of the previous age group.

Mean oviduct weight showed a gradual increase from 12th week to 16th week and almost doubled by 18 weeks of age. A spurt in growth was noticed from 18th (12.91 ± 0.02 g) to 20th week (46.66 ± 0.23 g) as shown in figure 9. By this age, all the birds started laying. From this age onwards the increase in weight of the oviduct was gradual and maximum mean weight of the oviduct recorded was 51.28 ± 0.05 g at the age of 24 weeks (Table. 2).

During postnatal period, weight of the oviduct showed highly significant correlation with the age (r = 0.832) at 1% level of significance (Table. 9).

4.1.3.5 Relationship Between Age and Length of the Oviduct

Length of the oviduct increased about 30 times from 2.07 ± 0.03 cm in day-old birds to 63.03 ± 0.24 cm at 24th week of age (Table. 2).

The mean length of the oviduct increased about four times in four weeks and thereafter, the increase was gradual upto 10th week (Table. 2). From 10th week onwards, the lengthwise growth of the oviduct was rapid upto 18th week (Fig. 10). This was accompanied by the morphological differentiation of the oviduct segments. A spurt in growth was recorded from 18th week to 20th week, when all the birds started laying. Thereafter, the increase was gradual and maximum mean length of the oviduct was recorded at 24th week (63.03 ± 0.24 cm).

During postnatal period, length of the oviduct showed a highly significant correlation with the age (r = 0.920) which was more than that with the weight of the oviduct and age at 1% level of significance (Table. 9).

4.1.3.6 Relationship Between the Body Weight and Oviductal Parameters

Oviduct contributed 0.13% of the body weight in day-old ducklings and in adult birds (at 24th week), it contributed 3.47% of the body weight. But, weight of the oviduct showed no significant correlation with the body weight (r = 0.517) at 1% level of significance during postnatal period (Table. 9). On the other hand, the body weight showed a significant correlation with the length of oviduct (r = 0.657) at 5% level of significance (Table. 9).

4.1.3.7 Relationship between the weight and length of the oviduct

During the postnatal period, the weight of the oviduct showed highly significant correlation with the length of oviduct (r=0.979) at 1% level of significance (Table. 10).

4.1.4 Segmental Variation in the Developmental Pattern of the Oviduct

4.1.4.1 Infundibulum

From day-old to 10th week of age, infundibulum was not differentiated from the magnum and isthmus regions and morphological development was negligible during this period. From 12th week onwards, the five segments of the oviduct including the infundibulum were differentiated and the infundibulum was morphologically divided into cranial funnel and caudal neck regions.

Age related parameters of the funnel and neck regions of the infundibulum are given in tables 3 and 4. At 12th week of age, the weight of the funnel region of the infundibulum was 0.03 ± 0.00 g and was greater than that of the neck (Tables. 3 and 4). This relationship remained constant for all succeeding age groups and at 24th week of age, funnel region weighed 1.49 ± 0.00 g which was higher than that of the neck 1.16 ± 0.00 g (Tables. 3 and 4). Length of the neck region was more than that of the funnel in all age groups. The funnel was much wider than the neck throughout the postnatal period (Tables. 3 and 4). In adult birds (at 24th week), the infundibulum contributed 11.42% of the oviduct length (Fig. 11). The thin walled funnel was flattened dorsoventrally and its flared lips were in close proximity to the ovary in adult birds (Fig. 7).

From 12th week to 24th week of age, the weight, length and width of the funnel region of the infundibulum showed highly significant correlation with that of the age with corresponding 'r' values as 0.970, 0.900 and 0.965 respectively at 1% level of significance (Table. 9). But no significant correlation was noticed between funnel parameters and the body weight. Similarly, the weight, length and width of the neck region of the infundibulum also showed highly significant correlation with age but not with the body weight.

Weight and length of the funnel as well as the neck regions of the infundibulum showed highly significant positive correlation with the weight and length of the oviduct at 1% level of significance (Table. 10). Whereas, width of the infundibulum showed significant correlation with the weight and length of the oviduct at 5% level of significance only (Table. 10).

4.1.5.2 Magnum

Magnum was not differentiated grossly from the infundibulum and isthmus upto 10th week. Magnum was the longest and the most coiled segment of the oviduct (Fig. 7). Contribution of magnum to the total length was 38.55% at 24th week (Fig. 11). The overall diameter of magnum was greater than that of the neck of infundibulum and this increase was mainly due to a marked increase in the thickness of walls. In the caudal most region, the diameter gradually decreased to that of the isthmus.

Weight, length and width of magnum from 12th week of age are given in table 5. Weight of magnum increased about four times from 16th to 18th week and five times from 18th to 20th week. Thereafter, the increase in weight was gradual. Magnum contributed 37.40% of the oviduct weight. Length and width of magnum showed maximum increase between 18 to 20 weeks of age (Table. 5).

In differentiated oviduct, the weight, length and width of the magnum region showed highly significant positive correlation with the age and weight and length of oviduct at 1% level of significance (Tables. 9 and 10). But no significant correlation was found between parameters of magnum and the body weight.

4.1.5.3 Isthmus

Junction between magnum and isthmus was marked by a narrow translucent zone (Fig. 7). Differentiation of the isthmus region occurred at the same time as that of more cranial segments of the oviduct. Its overall diameter was lesser when compared to that of the magnum.

Age related changes in the weight, length and width of isthmus are given in table 6. Maximum growth rate in all the dimensions of the isthmus region was noted between 18 and 20 weeks of age. Isthmus contributed 8.61% of the total

weight of oviduct (Fig. 9). It was 12.31 ± 0.17 cm long and was about 19.53% of the total length at 24th week (Fig. 11).

From 12th week to 24th week of age, the weight, length and width of the isthmus region showed highly significant correlation with the age and weight and length of oviduct at 1% level of significance but, no significant correlation was found with the body weight (Tables. 9 and 10).

4.1.5.4 Uterus (Shell Gland)

In the day-old duckling itself, the uterus was a well differentiated segment of the oviduct (Fig. 2). The cranial part was short and tube-shaped whereas, the caudal part was a sac-like pouch region, the shell gland proper (Fig. 4). The wall was thin when compared to that of magnum and isthmus, but was more distensible to accommodate and pass the developing egg mass to the next segment, the vagina.

Age related changes in the uterine parameters are given in table 7. Uterine weight increased about three times from 16 weeks to 18 weeks of age and six times by 20 weeks. The shell gland contributed 31.08% of the total oviduct weight at 24th week (Fig. 9). Contribution to the total length was 14.47% (Fig. 11). Rapid increase in the length and width of uterus was observed between 18 and 20 weeks of age (Table. 7).

During the postnatal period, the weight, length and width of the uterus region showed highly significant correlation with the age, length and weight of the oviduct at 1% level of significance (Tables. 9 and 10).

Weight of the uterus showed no significant correlation with the body weight, whereas, length and width of the uterus showed significant correlation with the body weight with corresponding 'r' values 0.748 at 1% level of significance and 0.561 at 5% level of significance, respectively (Table. 9).

4.1.5.5 Vagina

In the day-old duckling itself, similar to the uterus, vagina was a well differentiated segment of the oviduct. The annular rings, nearly seven to eight in number, appeared over the external surface of the vagina at about 10th week of age (Fig. 12) and thereafter, these annular rings became nearly indistinct because of the more rapid development of the connective tissue which not only covered the external surface of the vagina but also bound the shell gland and cloaca with the vagina which resulted in condensed nature of the vagina in the adult Kuttanad duck.

Weight, length and width of vagina at various stages of postnatal life are shown in table. 8. Unlike in the case of other regions weight of vagina increased about five times from 10th week to 12th week. In the remaining age groups, the growth of vagina was gradual. Vagina contributed 17.75% of the oviduct weight at 24 weeks of age (Fig. 9). It was 10.08 ± 0.07 cm long at this age and contributed 15.99% of the total length of oviduct (Fig. 11).

From day-old to 24th week of age, the weight, length and width of the vagina showed significant positive correlation with the age, body weight and length and weight of oviduct (Tables. 9 and 10).

4.2. THE DEVELOPMENTAL HISTOMORPHOLOGY OF OVIDUCT

Although the limits of the sub-divisions of the oviduct were primarily set by external, macroscopic criteria, they were finally determined by the changes in the structure of the mucosal folds and tubular glands within.

4.2.1 Infundibulum

In the day-old ducklings, the cranial end of the undifferentiated oviduct corresponded to the infundibulum and consisted of the innermost epithelium and subepithelial tissue (Fig. 13). The oviduct wall was not differentiated into different tunics distinctly. The lumen was flanked by characteristic low mucosal folds which were lined by simple columnar epithelium. The total number of

mucosal folds was in the range of 10 to12. Caudally number of folds increased upto 16 to 18. The sub-epithelial connective tissue was made up of densely packed cells with fine collagen fibres and was rich in capillaries.

Both dorsal as well as ventral ligaments were attached to the oviduct and were abundantly supplied by blood vessels. Ventral ligament showed the presence of smooth muscle in addition to the connective tissue (Fig. 14).

At two weeks of age, the secondary mucosal folds started appearing in the cranial end of the undifferentiated oviduct corresponding to the infundibulum. By 6^{th} week, secondary folds started developing rapidly. At this age, a very thin tunica muscularis appeared as a single layer without any differentiation into inner circular and outer longitudinal muscle layers. Lining epithelium was simple columnar without any cilia and was clearly basophilic when compared to the lamina propria and thus was easily distinguishable.

At 12^{th} week of age all the segments of the oviduct were differentiated macroscopically but mucosa of the infundibulum was unaltered in the funnel region whereas, tubular glands first appeared in the lamina propria of the neck region. Figure 15 shows the low mucosal folds and very thin tunica muscularis of the funnel region. At 14^{th} week, neck region of the infundibulum showed rapid development of the mucosal folds into primary, secondary and tertiary folds with consequent increase in their height and width. Total number of mucosal folds was about 15 to 16. At this age, height and width of mucosal folds in the funnel measured 67.50 µm and 81.00 µm respectively, which were lesser than that of neck region. Ciliogenesis in the infundibulum began at this age and the lining epithelium also showed presence of few non-ciliated glandular cells and occasional vacuolation (Fig. 16). Tunica muscularis was thinner than more caudal segments of the oviduct. Glandular development progressed as neck region showed number of tubular glands as shown in figure 17.

In the adult Kuttanad duck (20 weeks of age), the mucosa of funnel region was thrown into primary and secondary folds (Fig. 18). At this age, there were

four types of epithelial cells in the mucosa of infundibulum namely non-secretory ciliated cells located primarily in the lining epithelium; non-ciliated mucous-secreting goblet cells found in between the ciliated cells; secretory cells other than goblet cells which were located in the glandular grooves and lining cells of the tubular glands of the caudal region of the infundibulum. The lamina propria of the funnel region of the infundibulum was devoid of any glands and contained collagen fibres along with fine reticular and a few elastic fibres.

The lining epithelium at the opening of the lips of the funnel was low columnar ciliated type which rapidly changed into tall ciliated columnar in the distal funnel and neck of the infundibulum. Ciliated cells possessed oval nuclei lying at or above the centre of the cell. Goblet cells showed a basal nucleus and apical granular cytoplasm.

Continuous series of non-ciliated glandular cells with a few ciliated cells in between were present at the bottom of the grooves between the two successive mucosal folds in the wall of the infundibulum. In the neck of the infundibulum, passing caudally, the mucosal folds became more complex with deeper glandular grooves which eventually gave rise to small tubular glands from their corners (Fig. 19).

The deeper regions of the mucosal folds where the transformation of glandular grooves into tubular glands occurred, the lining cells lacked cilia and were cuboidal with eosinophilic supranuclear cytoplasm (Fig. 20). The proprial glands were lined by cuboidal to columnar cells with indistinct boundaries, basally located light stained round nuclei, eosinophilic supranuclear cytoplasm and enclosed a large lumen. The proprial glands in the neck of the infundibulum were larger in diameter (Fig. 21), which increased in number as well as width gradually and attained maximum width at 24^{th} week of age. In the caudal most region of the neck of the infundibulum, goblet cells increased in number in the lining epithelium (Fig. 22).

The funnel region showed mainly primary mucosal folds with some secondary folds. The depth of longitudinally oriented low mucosal folds appeared to increase as the funnel approached the neck region whereas within the neck of the infundibulum, the spirally oriented longitudinal folds increased in depth and gave rise to numerous secondary and tertiary folds. Among different segments of the oviduct, the infundibulum showed the least height and width of mucosal folds throughout the postnatal development. At 24th week of age, the height and width of mucosal folds in funnel region were 261.00 ± 5.69 µm and 103.50 ± 2.85 µm respectively, which gradually increased in the neck region and measured 618.75 ± 2.07 µm and 126.00 ± 2.85 µm, respectively.

The muscular tunic of the neck region was thicker than that of the funnel and was differentiated into ill defined inner circular and outer longitudinal muscle bundles (Fig. 23). At the infundibulum-magnum junction, both the mucosal and muscular layers increased in thickness and structural complexity.

The collagenous serosal tunic was found to be thin with folds, blood vessels and nervous plexuses. Distinct serosal tunic was visible only in adult birds. In adult birds, tunica mucosa of the infundibulum occupied the maximum thickness (81%) followed by the tunica muscularis (16%) and tunica serosa (3%).

4.2.2 Infundibulum - Magnum Junction

Short mucosal folds which characterise the infundibulum increased in height and were more longitudinally oriented as they approached the magnum region at 10 weeks of age (Fig. 25).

In the adult Kuttanad duck, the branched tubular glands which were present in the tubular part of the infundibulum were more prominent in the region adjoining the magnum.

At about 20 weeks of age it was observed that, the eosinophilic supranuclear secretory granules of the infundibular glandular epithelium were invariably smaller, less numerous and more denser than those in the tubular

glands of the magnum. At the infundibulum-magnum junction, group of infundibular glands intermingled with tubular glands of the magnum, but the epithelial lining of the two types of glands were always distinct (Fig. 25).

4.2.3 Magnum

From day-old to four weeks of age, the magnum region was histologically similar to the infundibulum except in that the more number of mucosal folds were seen in magnum than in the infundibulum. At 4th week, secondary mucosal folds started appearing and total number of primary folds was in the range of 18 to 20. Thereafter the height and width of mucosal folds increased gradually. At 10th week (i.e. two weeks prior to external differentiation of oviduct into segments), each magnal fold was wide in nature with slightly narrow base lined by simple columnar epithelium (Fig. 26). Proprial glands were not developed. At the region where magnum was supported by dorsal and ventral ligaments, the mucosal folds appeared higher comparatively.

The most dramatic growth and differentiation of the magnum began at the age of 12 weeks when all the segments of the oviduct were differentiated morphologically. Lining epithelium of magnum started infolding and consequently the tubular glands appeared as invaginations of this surface epithelium (Fig. 27). At this age, glands did not extend to the deeper parts of the lamina propria, which were made up of loosely arranged connective tissue with many blood vessels. At this age, the mucosal folds became broader and higher comparatively with the total number of folds ranging from 20 to 22 with lesser secondary folds. It was observed that, development of glandular layer although increased the size of the mucosal folds, tended to decrease the degree of secondary folding. Ciliogenesis was observed almost at the same time of glandulogenesis. Total thickness of the wall of magnum was 121.50 ± 0.00 µm with very thin tunica muscularis which consisted of circularly arranged muscle fibres. Tunica serosa was made up of loose connective tissue and was richly supplied by large blood vessels and nerves (Fig. 28). Thereafter, the glandular

development continued and glands extended towards the core of the lamina propria.

At 18th week, with the beginning of egg-laying in a few birds, the blood capillary network which was limited to the core region of the lamina propria extended towards the margins of mucosal folds and was observed just beneath the basement membrane of the lining epithelium. At this age, secretory end piece of each tubular gland in the cross section was lined by 9 to 10 tall columnar cells with eosinophilic supranuclear cytoplasm. The diameter of magnal gland, at this age was 41.42 ± 0.58 µm with its lining columnar epithelium measuring 15.88 ± 0.67 µm in height. The maximum diameter of 51.33 ± 0.74 µm was attained at the age of 24 weeks.

In the adult Kuttanad duck, the overall diameter of the magnum was considerably greater than that of the infundibular neck, which was mainly due to a marked increase in the thickness of the wall. This was partly as a result of an increase in the development of muscle layers but was mainly due to the increased thickness of mucosa.

At 20th week, mucosal folds became wider and higher than the previous age groups (Fig. 29) and were lined by monolayered simple columnar ciliated epithelium with a few goblet cells (Fig. 30). Even in H&E staining, the epithelium had a peculiar grayish, dark appearance unlike previous groups (Fig. 30). Average height of lining epithelium was 21.00 ± 0.00 µm. Capillaries were seen in large number just beneath the lining epithelium. The lamina propria was filled with tubular glands lined by cuboidal to columnar epithelium with deeply eosinophilic cytoplasm and the lumen of the glands was difficult to identify. Glands were arranged in a radiating manner from the core to the periphery of the fold (Fig. 29). Tunica muscularis was thin and made up of inner circular and outer very thin longitudinal muscle layers (Fig. 31). The connective tissue core of each mucosal fold was also very thin from which connective tissue fibres were extending towards the periphery.

In the caudal most region of the magnum, the surface epithelium became taller (26.25 ± 0.64 μm) and contained very large number of goblet cells (Fig. 32 and 33). At 24th week, the lining epithelium at and above the base of the mucosal fold showed peculiar invagination (Fig. 33). Before the passage of yolk through the magnum the goblet cells which appeared to dominate in the epithelium were distended with secretory granules (Fig. 34) whereas, ciliated cells appeared as narrow structures; but once the secretory material was discharged, the goblet cells appeared to be obscured by the ciliated cells and the surface had a ciliated appearance. Tubular gland cells containing secretary granules attained their maximum size of 16.63 ± 0.39 μm in the laying period.

4.2.4 Magnum - Isthmus Junction

At 12th week of age, this region also was distinctly identifiable. At the posterior end of the magnum, the longitudinal mucosal folds became reduced in height and each fold became thinner with the concurrent development of more secondary folds (Fig. 35). It was also observed that almost immediately, however, the mucosal folds increased in height again as they entered the isthmus proper but did not regain the height or breadth found in the magnum.

The most noteworthy feature of this region was the complete absence of tubular glands in with subsequent occupation of the core of lamina propria by relatively dense connective tissue (Fig. 35). Thickness of tunica muscularis gradually increased from magnum to isthmus region with development of thicker inner circular layer.

4.2.5 Isthmus

The overall diameter of the isthmus was less than that of the magnum. In undifferentiated oviduct, the isthmus region was similar to the magnum without any secondary folds. Tunica muscularis was present as a continuous layer at two weeks. At about 4th week, the primary folds attained angular shape characteristic to the isthmus and the secondary folds started appearing (Fig. 36). At this age total number of mucosal folds was 9 to 10. Thereafter upto 8th week of age, all the

tunics remained unchanged. At eight weeks of age, tunica muscularis was differentiated into inner circular and outer longitudinal layers.

At 12th week, the glands started developing from the surface epithelium. The glands filled the lamina propria and compared to magnum glands, they extended to deeper regions of the lamina propria (Fig. 37). The core of the mucosal fold showed numerous blood vessels (Fig. 38). The lamina propria blended with the submucosa and no muscularis mucosa was present. Muscularis mucosa was absent throughout the oviduct. Tunica muscularis consisted of inner circular and outer longitudinal muscle layers separated by loose connective tissue rich in blood vessels. Tunica serosa was made up of loose connective tissue with blood vessels and nerves.

At 14th week, the glandular development continued and each mucosal fold was filled completely by tubular glands and was left with a very thin core of lamina propria. Glands were vacuolated. Cross sectional profile of tubular glands showed lining epithelium as cuboidal to columnar and enclosed a distinct lumen measuring 9.92 ± 0.37 µm. Thereafter, in succeeding groups, the glandular development continued and simultaneously ciliogenesis followed. Vacuolation of the glandular epithelium decreased.

In the adult Kuttanad duck, compared to all other segments of the oviduct, the glands in the isthmus were more loosely arranged (Fig. 39). Each secretory end piece was lined by pyramidal cuboidal cells with a large round nucleus towards the base of the cell and supranuclear deeply eosinophilic cytoplasm. Cross section of each secretory end piece was lined by six to eight cells.

The diameter of the tubular glands gradually decreased in the caudal portion of the isthmus and possessed wider lumen. Tubular glands within the mucosal folds were similar to those of the magnum, but were not so well developed. The secretory granules in the cells of isthmian glands were fine and smaller than those of magnum. Each tubular gland showed transformation of cuboidal cells with centrally placed nuclei and sparse cytoplasm in non secretory

phase into pyriform cells with basal nuclei filled with coarse secretory eosinophilic granules in the secretory phase (Fig. 40).

Compared to cranial portion, height of the epithelium (30.92 ± 0.58 μm) gradually increased in more caudal regions of the isthmus (34.42 ± 0.58 μm). Lining epithelium of the isthmus contained both ciliated and secretory non-ciliated columnar cells (Fig. 40). Regular alteration of these cells was noticed and these cells were having approximately the same width.

There were two types of non-ciliated glandular cells in the surface epithelium along with ciliated cells (Fig. 40). Some non-ciliated cells were having more eosinophilic cytoplasm and darkly stained nuclei while, the other type was lightly eosinophilic with large lightly stained nuclei. Occasionally certain surface epithelial cells of the isthmus showed vertically elongated darkly stained nuclei.

The ciliated cells of the surface epithelium retained their columnar shape with an apical nucleus throughout the secretory activity, being unconstricted by the adjacent glandular cells. When the isthmus was secreting the shell membranes, the gland epithelium showed maximum activity and each cell became full of spherical, deeply eosinophilic granules. The secretory material in their lumina was either of similar granules or fused secretory masses which after passing out of the tubular glands frequently took the form of threads or twisted strands.

The mucosal folds of the isthmus region were characteristically angular in the appearance (Fig. 39) and the apertures of the tubular glands were situated in the depressions of the surface epithelium, which were much more numerous than in any other part of the oviduct (Fig. 41). During the development only the tunica serosa remained unchanged while the tunica mucosa and tunica muscularis exhibited significant increase in thickness.

4.2.6 Isthmus – Uterus Junction

In differentiated oviduct isthmus - uterine junction showed no abrupt change in gland architecture between the adjacent regions, but an area of intermingling of glands could be observed within the lamina propria.

In adult birds, the isthmus- uterine junction also showed some sparsely distributed type of glands which were different when compared to either the isthmus or the shell gland proper. These glands had much smaller and less numerous secretory granules than those of the isthmus and cytoplasm was frequently vacuolated.

4.2.7 Uterus or Shell Gland

In the day-old duckling itself, towards the caudal portion of the oviduct, shell gland region was observed as dilated portion (Fig. 2). The mucosal folds were higher (40.33 ± 0.11 μm) and separated by deep furrows (Fig. 42). Most of the mucosal folds were cuboidal with equal height and width. At 2nd week, height of the mucosal folds increased and a few secondary folds also started to develop. One of the noteworthy features was the appearance of smooth muscle towards the periphery of uterine wall indicating the developing tunica muscularis. The lining epithelium was simple columnar with darkly stained nuclei.

At 4th week of age, height of mucosal folds increased and more secondary folds started appearing. In terminal portion of uterus, height of the mucosal folds decreased and the wall became thicker with more well developed tunica muscularis (Fig. 43). Two types of cells, light cells and dark cells were visible. Lamina propria was made up of loose connective tissue and was devoid of any glands. Tunica muscularis was thin and a fine network of collagen fibres could be observed in between the bundles of muscle fibres (Fig. 44). Ventral ligament was well developed with numerous blood vessels and nerve bundles and showed a continuous attachment of uterine region with the vagina indicating a close association between the two segments even at an early stage (Fig. 45).

Thereafter, not much change was seen in the uterine region upto 12 weeks of age. At 12th week, in the initial portions of the uterus, height of the mucosal folds was comparatively less and it increased gradually along with increase in the thickness of tunica muscularis. Mucosal folds at this age were thin, narrow and high (1215.00 ± 0.02 µm) as shown in figure 46. At this age, surface epithelium showed invagination to form tubular glands which started developing as a continuous layer just beneath the lining epithelium similar to magnum (Fig. 47) and showed some degree of vacuolation (Fig. 48). Lamina propria was similar to that of isthmus but glands did not extend to deeper portions of the lamina propria and were smaller than isthmian glands with average diameter of 28.00 ± 0.00 µm. The inner circular layer of tunica muscularis was thicker than outer longitudinal layer.

At 14th week, the folds increased in height and were comparatively thinner and with few secondary mucosal folds than the previous age group. They were spatula shaped. Ciliogenesis was in progress and appearance of cilia was more evident in apical regions of the mucosal folds (Fig. 49). Lamina propria was completely filled with tubular glands and central core showed numerous capillaries. Glands towards the base of the mucosal folds showed vacuolated cytoplasm (Fig. 50). However, no vacuolation was evident at the apical region or towards the central region of the mucosal folds. Each secretory end piece was lined by five to six cells with their nuclei arranged towards the apical portion and cytoplasm appeared eosinophilic. The basal portion of cells showed vacuolated appearance.

At 16th week, glandular development continued and towards the apex of uterine folds glandular end pieces were more distinct (Fig. 51). Similar to previous group, basal portion of mucosal fold showed vacuolated glandular end pieces. Tubular glands were closely packed in the mucosal folds and were empty during non laying phase. But, prior to and during secretory phase, cells contained pale-staining granules and after the shell formation, the cytoplasm was found to

be markedly vacuolated, with relatively large nucleus located towards the base of each cell.

By 18 weeks, mucosal folds acquired their characteristic leaf-like appearance and lamina propria was filled with glands with loose arrangement at the central core (Fig. 52). Towards the base of the fold, the vacuolated appearance of glands was reduced and glands had a uniform appearance throughout. Uterine glands were similar to isthmian glands, but were more closely packed and the lining epithelium was cuboidal to columnar with more closely packed cells having basally placed nuclei and eosinophilic cytoplasm.

In adult laying ducks, tunica muscularis was very thick with an inner circular and thicker outer longitudinal layers separated by loose connective tissue showing numerous vessels and nerves (Fig. 53). The most important feature was that at this age, smooth muscle fibres were observed to be extending to the core of the mucosal fold along with numerous collagen fibres (Fig. 54). Clear cells were also identified in the lining epithelium (Fig. 55).

The surface epithelium of the uterus showed a single layer of columnar cells with alternating apical and basal nuclei (Fig. 56). Accordingly these were named as apical and basal cells, with the apical cells bearing cilia and the basal cells having restricted apical surface.

Secretory granules were a constant feature of the supranuclear cytoplasm of the apical cells. Active secretion of these granules could be seen during the earlier phases of shell formation and during the latter half of shell secretion many secretory granules migrated to supranuclear cytoplasm. The secretory granules were subjected to a process of disintegration in supranuclear cytoplasm with concurrent formation of a large membraneless space, which was termed as vacuoloid, which subsequently regressed. Non-ciliated glandular cells of the shell gland contained more vacuoles which regressed during the onset of egg formation.

In the uterus containing the egg mass, the region opposing the surface of the developing egg showed flattened mucosal folds (Fig. 57). In the surface epithelium, apical portion of the cells were denuded as part of secretory process and thus in some cells only basal half could be seen. Secretory granules towards the lumen could also be located in the lumen (Fig. 58). Apart from usual round to elliptical secretory end pieces of the tubular glands, some uterine glands were also in the shape of 'U' or 'Y' and their nuclei were seen projecting from basal portion of cells (Fig. 59). The peculiar appearance of nuclei could be seen throughout the glandular epithelium (Fig. 59).

4.2.8 Utero-vaginal Junction

At utero-vaginal junction the sperm storage tubules appeared at 12 weeks of age and were located in a thick ring of pushed-in mucosal folds found in the lumen with the vaginal folds on one side and uterine folds on the other. The sperm storage tubules were thus observed to be limited in between two muscular ridges, cranial and caudal utero-vaginal ridges. The mucosal folds were low and lined by tall ciliated columnar cells with the apical nuclei alternating with mucous secreting goblet cells containing basal nuclei. The lamina propria of the mucosa housed numerous tubular glands called the sperm storage tubules which were responsible for sperm storage after copulation (Fig. 60).

There was no evidence of smooth muscle fibres or other contractile elements associated with sperm host glands and these were surrounded by connective tissue elements only.

At 12^{th} week and in all the succeeding age groups, mucosal folds towards the uterine side of the cranial utero-vaginal ridge contained transitional glands intermediate in appearance between sperm storage tubules and uterine glands (Fig. 61). The transitional glands were more densely packed compared to sperm host glands and were lined by large cells with large oval nucleus at the base and enclose a narrow lumen. At this age and in all succeeding groups, the transitional

glands were (31.50 ± 1.28μm) larger than uterine glands 28.00 ± 0.01 μm but were smaller in diameter than sperm storage tubules measuring 56.00 ± 0.03 μm.

The sperm storage tubules were lined by simple cuboidal to low columnar cells with centrally placed nuclei and at 14th week of age, these glands could be seen as small invaginations of surface epithelium lined by tall columnar cells with round nuclei placed in the basal halves of cells (Fig. 62). These sperm storage tubules were more sparsely distributed and less convoluted than the uterine glands and transitional glands but had larger diameter than both the gland types.

With the beginning of egg-laying phase, at about 18th week of age, sperm storage tubules also exhibited rapid phase of development and more number of cross sectional profiles of the glands were visible indicative of increased number of sperm storage tubules. The number of transitional glands was also increased. In adult laying Kuttanad ducks, the sperm storage tubules were lined by tall columnar cells with eosinophilic cytoplasm and basally placed nuclei (Fig. 63). In the reproductive phase, the total number and inner and outer diameters of sperm storage tubules were increased when compared to pre-laying phase.

4.2.9 Vagina

In the day-old duckling itself, the vagina, the caudal most portion of the oviduct was observed to be morphologically distinct. At two weeks of age, the vagina showed roughly triangular seven to eight primary folds lined by tall columnar cells interspersed with numerous goblet cells (Figs. 64 and 65). Even at this age, tunica muscularis was well developed and was differentiated into moderately developed outer longitudinal layer and well developed inner circular layer (Fig. 66). Vagina showed the thickest tunica muscularis among different regions of oviduct (Fig. 67). Caudal portions of the vagina showed low mucosal folds compared to cranial region in four weeks-old birds. Tunica serosa was also well demarcated.

By 10 weeks of age, the number of goblet cells reduced and typical secondary mucosal folds started appearing (Fig. 68). Cilia also appeared in the lining epithelium. At 12th week, folds were characteristically filiform or narrow and pointed in appearance with a few secondary folds. The lamina propria was devoid of any glands.

At 18th week, mucosal folds were taller but the height of the epithelium reduced considerably and in between the goblet cells and ciliated cells, some clear cells were also identified (Fig. 69). The core of mucosal fold consisted of bundles of collagen fibres with fibrocytes in between (Fig. 70).

In adult laying birds, lining epithelium of vagina showed large number of goblet cells and the ciliated cells occurred only rarely (Fig. 71). The height of the epithelium over the crest of the vaginal folds was greater than that of the shell gland. However, in the depressions between the folds, the cells were shorter with a predominance of the non-ciliated goblet cells. At this age, inner circular muscle layer extended into the core of the mucosal fold as in the case of uterine region (Fig. 72). Figure 73 shows low mucosal folds in the caudal end of vagina.

4.2.10 The Immune Status of the Oviduct During Postnatal Development

Lamina propria of mucosal folds of the vagina showed large number of lymphocytes along with diffused blood cells from 4 weeks-old birds (Fig. 74). At 16th week of age, core of the mucosal folds of uterus showed more eosinophilic areas along with blood cell infiltration. At 18th week of age, the connective tissue core of the magnum showed infiltration of blood cells including lymphocytes and heterophils (Fig. 75). The uterine region showed large immature lymphocytes at the base of the mucosal folds. Lamina propria of the utero-vaginal junction also showed accumulation of lymphocytes. At 20th week, lamina propria of the neck region of the infundibulum (Fig. 76) and magnum-isthmus junction (Fig. 77) showed loose aggregates of lymphocytes. At 22nd week, loose aggregations of lymphocytes were also evident in the lamina propria of the funnel region of the infundibulum, infundibulum-magnum junction and isthmus region (Fig. 78) of the

oviduct where lymphocytes were also observed in the tunica muscularis (Fig. 79). The loose aggregations of lymphocytes in the lamina propria were a common feature in the vaginal region in adult egg-laying Kuttanad duck (Fig. 80).

4.2.11 General Histomorphology and Developmental Changes in the Oviduct

During the postnatal development of the oviduct it was observed that in all age groups the wall of the oviduct was made up of seven layers viz., tunica serosa an outer covering of peritoneum made of connective tissue and mesothelium, an outer longitudinal smooth muscle layer covering an inner circular smooth muscle layer with an intervening connective tissue layer rich blood vessels and nerves, a layer of connective tissue internal to the circular muscle layer followed by the mucosa consisting of a lamina propria containing glands in most of the regions and an inner epithelial lining. The oviduct of Kuttanad duck throughout the developmental period was devoid of any muscularis mucosae. The mucosal folds were seen more or less continuous throughout the oviduct, although they varied in height and thickness.

In the case of immature ducks, the ciliated and secretory cells lining the epithelium and the proprial glands were not fully developed and during this pre-laying period, ciliated cells showed a great predominance in the lining epithelium while, in the laying period the mucosal folds and its lining epithelium were much more developed, with a certain predominance of non-ciliated cells. The cellular architecture of the monolayered ciliated and secretory cells which formed the lamina epithelialis, particularly in the regions of uterus and vagina was responsible for creating a false impression of cellular stratification or pseudostratification, because of the differential positioning of the nuclei of these cells.

The glandulogenesis started in the developing oviduct of Kuttanad duck at about 12^{th} week of age when the oviduct weighed around 5.59 ± 0.02 g (Table. 3). From the day-old to 24 weeks of age, the vagina had the thickest tunica muscularis while the infundibulum had the thinnest. Bundles of collagen fibres

were observed between the muscle cells and in the core of lamina propria in almost all regions. Elastic and reticular fibres were also present in the lamina propria of all the regions of the adult Kuttanad duck oviduct.

4.3 HISTOCHEMISTRY

4.3.1 Mucopolysaccharides

The goblet cells found throughout the oviduct showed intense P.A.S. positive reaction (Fig. 81).

In the magnum, lining epithelium consisted of an acidic mucopolysaccharide (Fig. 81). P.A.S. and Alcian blue (pH 2.5) method revealed the presence of neutral mucopolysaccharides and sulphur-containing proteins in the lamina epithelialis of isthmus region (Fig. 82). The proprial glands of the isthmus also showed positive reaction for P.A.S. and Alcian blue (pH 2.5) method with a peculiar pattern i.e., the glands positioned near the epithelium stained lightly and those situated more deeply were observed to stain darker (Fig. 82). Immature ducks, however, showed a very weak reaction. The presence of neutral and acid mucopolysaccharides was identified using P.A.S. and Alcian blue (pH 2.5) method in the epithelium of isthmus region; while in magnum, only the acid mucopolysaccharides were seen (Fig. 81). Vaginal region gave a negative reaction for neutral as well as acid mucopolysaccharides.

4.3.2 Lipids

Considerable amount of lipid was identified in the lining epithelium of sperm storage tubules and transitional glands.

4.3.3 Glycogen

Amongst various segments of the oviduct in Kuttanad duck, the presence of glycogen was observed only in the epithelial lining and tubular glands of isthmus region and was more pronounced in adult egg laying ducks wherein, actively secreting glands showed more intense reaction (Figs. 83 and 84).

4.3.4 Alkaline Phosphatase (ALP)

Activity of the enzyme was appreciable in tubular glands of the various regions of the oviduct. Alkaline phosphatase activity was seen in the uterus, isthmus and magnum (Fig. 85). The infundibular and vaginal epithelium on the other hand, showed only a mild positive reaction for alkaline phosphatase. The lining epithelium of sperm storage tubules also showed alkaline phosphatase activity.

4.3.5 Acid Phosphatase (ACP)

The ACP activity was observed in the epithelium and tubular glands of isthmus, shell gland and vagina.

Tables

Table. 2 Body weight and weight, length of oviduct at different ages (Mean ± S.E.)

Age	Body weight (g)	Oviduct weight (g)	Length of oviduct (cm)
Day-old	38.64 ± 0.34	0.05 ± 0.00	2.07 ± 0.03
2 weeks	253.33 ± 7.70	0.34 ± 0.01	5.72 ± 0.17
4 weeks	740.00 ± 13.33	0.61 ± 0.02	10.98 ± 0.13
6 weeks	990.00 ± 26.87	1.29 ± 0.01	11.40 ± 0.09
8 weeks	1256.67 ± 20.77	2.14 ± 0.01	11.70 ± 0.09
10 weeks	1386.67 ± 60.85	2.30 ± 0.01	12.90 ± 0.44
12 weeks	1433.33 ± 41.54	5.59 ± 0.02	17.69 ± 0.10
14 weeks	1540.00 ± 35.27	6.28 ± 0.02	20.06 ± 0.16
16 weeks	1656.67 ± 82.77	6.83 ± 0.03	24.83 ± 0.18
18 weeks	1783.33 ± 89.85	12.91 ± 0.02	29.23 ± 0.28
20 weeks	1706.67 ± 81.45	46.66 ± 0.23	54.98 ± 0.59
22 weeks	1690.00 ± 85.97	50.16 ± 0.04	58.85 ± 0.28
24 weeks	1476.67 ± 28.05	51.28 ± 0.05	63.03 ± 0.24

Table. 3 Age related changes in the parameters of funnel region of infundibulum (Mean ± S.E.)

Age	Weight of Funnel (g)	Length of Funnel (cm)	Width of Funnel (cm)
12 weeks	0.03 ± 0.00	0.70 ± 0.02	7.18 ± 0.05
14 weeks	0.08 ± 0.00	0.70 ± 0.02	8.40 ± 0.12
16 weeks	0.12 ± 0.00	0.80 ± 0.03	10.38 ± 0.14
18 weeks	0.69 ± 0.00	0.80 ± 0.02	11.15 ± 0.04
20 weeks	1.01 ± 0.02	3.10 ± 0.04	11.88 ± 0.08
22 weeks	1.35 ± 0.02	3.30 ± 0.09	12.16 ± 0.02
24 weeks	1.49 ± 0.00	3.40 ± 0.02	12.88 ± 0.10

Table. 4 Age related changes in the parameters of neck region of infundibulum (Mean ± S.E.)

Age	Weight of Neck (g)	Length of Neck (cm)	Width of Neck (cm)
12 weeks	0.02 ± 0.00	0.70 ± 0.03	0.40 ± 0.03
14 weeks	0.04 ± 0.00	0.80 ± 0.03	0.58 ± 0.02
16 weeks	0.05 ± 0.00	1.00 ± 0.02	0.68 ± 0.02
18 weeks	0.06 ± 0.00	1.20 ± 0.05	0.70 ± 0.01
20 weeks	0.60 ± 0.00	3.70 ± 0.05	0.74 ± 0.01
22 weeks	1.06 ± 0.00	3.80 ± 0.02	0.90 ± 0.17
24 weeks	1.16 ± 0.00	3.80 ± 0.04	1.27 ± 0.03

Table. 5 Age related changes in the parameters of magnum (Mean ± S.E.)

Age	Weight of Magnum (g)	Length of Magnum (cm)	Width of Magnum (cm)
12 weeks	0.53 ± 0.01	4.80 ± 0.07	0.50 ± 0.01
14 weeks	0.59 ± 0.00	5.50 ± 0.03	0.60 ± 0.00
16 weeks	0.79 ± 0.00	7.90 ± 0.07	0.70 ± 0.00
18 weeks	3.39 ± 0.00	8.90 ± 0.06	0.80 ± 0.01
20 weeks	18.31 ± 0.10	21.50 ± 0.19	1.90 ± 0.01
22 weeks	18.88 ± 0.03	23.60 ± 0.19	2.10 ± 0.04
24 weeks	19.18 ± 0.00	24.30 ± 0.07	2.30 ± 0.01

Table. 6 Age related changes in the parameters of isthmus (Mean ± S.E.)

Age	Weight of Isthmus (g)	Length of Isthmus (cm)	Width of Isthmus (cm)
12 weeks	0.20 ± 0.03	3.81 ± 0.03	0.42 ± 0.01
14 weeks	0.29 ± 0.05	4.38 ± 0.09	0.48 ± 0.01
16 weeks	0.38 ± 0.03	5.03 ± 0.04	0.59 ± 0.01
18 weeks	1.12 ± 0.07	6.53 ± 0.15	0.63 ± 0.01
20 weeks	4.03 ± 0.14	9.88 ± 0.23	1.11 ± 0.06
22 weeks	4.22 ± 0.03	10.70 ± 0.07	1.42 ± 0.04
24 weeks	4.42 ± 0.05	12.31 ± 0.17	1.49 ± 0.01

Table. 7 Age related changes in the parameters of uterus (Mean ± S.E.)

Age	Weight of Uterus (g)	Length of Uterus (cm)	Width of Uterus (cm)
Day-old	0.01 ± 0.00	0.40 ± 0.01	0.42 ± 0.01
2 weeks	0.12 ± 0.01	1.04 ± 0.02	0.49 ± 0.00
4 weeks	0.24 ± 0.00	1.75 ± 0.06	0.62 ± 0.01
6 weeks	0.30 ± 0.00	2.13 ± 0.01	0.89 ± 0.00
8 weeks	0.34 ± 0.01	2.20 ± 0.03	0.98 ± 0.01
10 weeks	0.40 ± 0.01	2.58 ± 0.07	1.19 ± 0.00
12 weeks	0.53 ± 0.00	3.85 ± 0.05	1.29 ± 0.01
14 weeks	0.59 ± 0.01	3.98 ± 0.03	1.33 ± 0.01
16 weeks	0.79 ± 0.00	4.22 ± 0.08	1.38 ± 0.01
18 weeks	2.60 ± 0.00	5.63 ± 0.07	1.48 ± 0.01
20 weeks	15.67 ± 0.07	8.08 ± 0.10	3.41 ± 0.34
22 weeks	15.87 ± 0.00	8.47 ± 0.11	4.71 ± 0.13
24 weeks	15.94 ± 0.00	9.12 ± 0.02	4.98 ± 0.05

Table. 8 Age related changes in the parameters of vagina (Mean ± S.E.)

Age	Weight of Vagina (g)	Length of Vagina (cm)	Width of Vagina (cm)
Day-old	0.02 ± 0.00	0.47 ± 0.02	0.51 ± 0.00
2 weeks	0.13 ± 0.00	1.36 ± 0.03	0.64 ± 0.00
4 weeks	0.27 ± 0.00	2.36 ± 0.03	0.92 ± 0.01
6 weeks	0.62 ± 0.01	2.36 ± 0.01	1.00 ± 0.01
8 weeks	0.78 ± 0.01	2.58 ± 0.05	1.11 ± 0.01
10 weeks	0.89 ± 0.01	2.70 ± 0.05	1.31 ± 0.01
12 weeks	4.27 ± 0.01	3.85 ± 0.02	1.41 ± 0.03
14 weeks	4.68 ± 0.01	4.67 ± 0.05	1.62 ± 0.03
16 weeks	4.70 ± 0.03	5.80 ± 0.05	1.67 ± 0.01
18 weeks	5.06 ± 0.01	6.17 ± 0.08	1.79 ± 0.02
20 weeks	7.12 ± 0.00	8.72 ± 0.09	2.02 ± 0.03
22 weeks	8.79 ± 0.01	9.05 ± 0.04	2.06 ± 0.05
24 weeks	9.10 ± 0.01	10.08 ± 0.07	2.19 ± 0.01

Table. 9 Correlation coefficients (r) of oviductal parameters on age and body weight

Parameters	Age	Body weight
Body weight	0.873**	-
Weight of Oviduct (g)	0.832**	0.517$^{N.S.}$
Weight of Funnel of Infundibulum (g)	0.970**	0.225 $^{N.S.}$
Weight of Neck of Infundibulum (g)	0.908**	0.038 $^{N.S.}$
Weight of Magnum (g)	0.904**	0.145 $^{N.S.}$
Weight of Isthmus (g)	0.925**	0.165 $^{N.S.}$
Weight of Uterus (g)	0.778**	0.455 $^{N.S.}$
Weight of Vagina (g)	0.959**	0.735**
Length of Oviduct (cm)	0.920**	0.657*
Length of Funnel of Infundibulum (cm)	0.900**	0.084 $^{N.S.}$
Length of Neck of Infundibulum (cm)	0.906**	0.153 $^{N.S.}$
Length of Magnum (cm)	0.940**	0.169 $^{N.S.}$
Length of Isthmus (cm)	0.975**	0.163 $^{N.S.}$
Length of Uterus (cm)	0.964**	0.748**
Length of Vagina (cm)	0.973**	0.759**
Width of Funnel of Infundibulum (cm)	0.965**	0.483 $^{N.S.}$
Width of Neck of Infundibulum (cm)	0.930**	0.018 $^{N.S.}$
Width of Magnum (cm)	0.938**	0.103 $^{N.S.}$
Width of Isthmus (cm)	0.954**	0.102 $^{N.S.}$
Width of Uterus (cm)	0.860**	0.561*
Width of Vagina (cm)	0.996**	0.896**

** Correlation is significant at 1% level, * Correlation is significant at 5% level,
$^{N.S.}$ Correlation is non-significant.

Table. 10 Correlation coefficients (r) of oviductal parameters on weight and length of oviduct

Parameters	Weight of Oviduct (g)	Length of Oviduct (cm)
Weight of Funnel of Infundibulum (g)	0.953**	0.969**
Weight of Neck of Infundibulum (g)	0.955**	0.958**
Weight of Magnum (g)	0.999**	0.990**
Weight of Isthmus (g)	0.999**	0.995**
Weight of Uterus (g)	0.995**	0.958**
Weight of Vagina (g)	0.894**	0.948**
Length of Funnel of Infundibulum (cm)	0.997**	0.989**
Length of Neck of Infundibulum (cm)	0.998**	0.991**
Length of Magnum (cm)	0.995**	0.998**
Length of Isthmus (cm)	0.976**	0.992**
Length of Uterus (cm)	0.942**	0.987**
Length of Vagina (cm)	0.924**	0.981**
Width of Funnel of Infundibulum (cm)	0.829*	0.884**
Width of Neck of Infundibulum (cm)	0.787*	0.844*
Width of Magnum (cm)	0.994**	0.996**
Width of Isthmus (cm)	0.976**	0.987**
Width of Uterus (cm)	0.978**	0.948**
Width of Vagina (cm)	0.814*	0.908**
Length of Oviduct (cm)	0.979**	-

** Correlation is significant at 1% level.

* Correlation is significant at 5% level.

Figures

1. Oviduct
2. Kidney
3. Ureter
4. Ovary

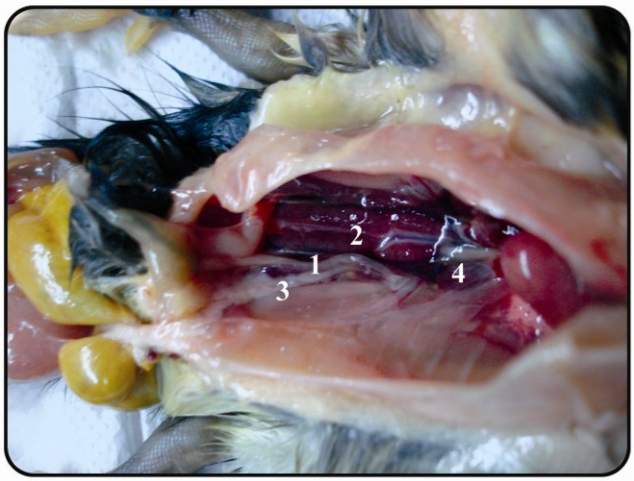

Fig. 1 In situ position of oviduct in day-old duckling

1. Heart
2. Lung
3. Ovary
4. Left lumbar lymph node
5. Aorta
6. Oviduct
7. Kidney
8. Bursa of fabricius
9. Uterus
10. Cloaca
11. Vent

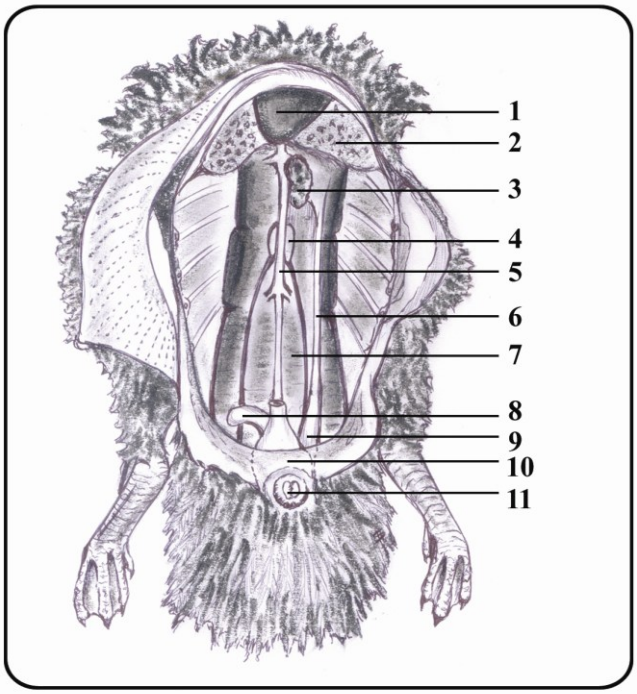

Fig. 2 Schematic drawing of in situ position of oviduct in day-old duckling

1. Ovary
2. Oviduct
3. Magnum
4. Uterus
5. Ventral ligament
6. Vagina

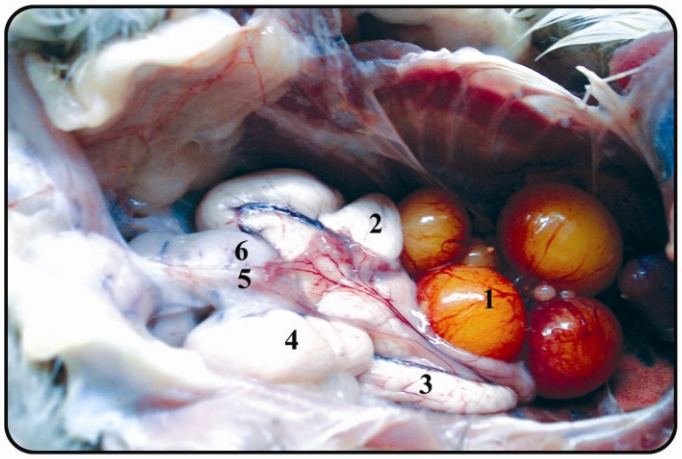

Fig. 3 In situ position of oviduct without an egg (20 weeks)

1. Ovary
2. Oviduct
3. Uterus with an egg
4. Vagina
5. Colorectum
6. Cloaca

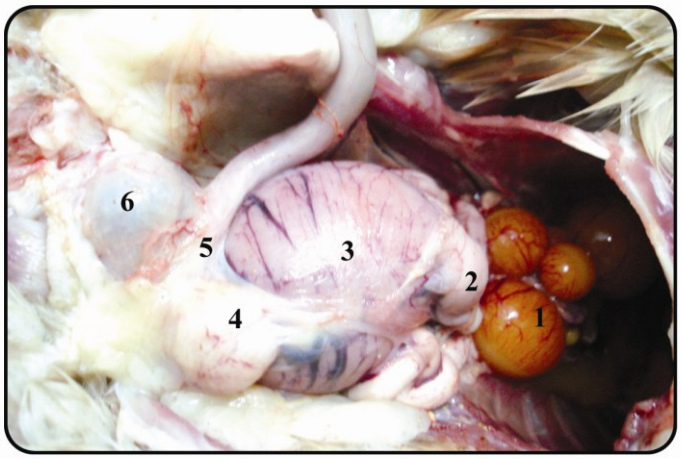

Fig. 4 In situ position of oviduct with an egg in uterus (20 weeks)

1. Right oviduct
2. Colorectum
3. Vagina
4. Cloaca

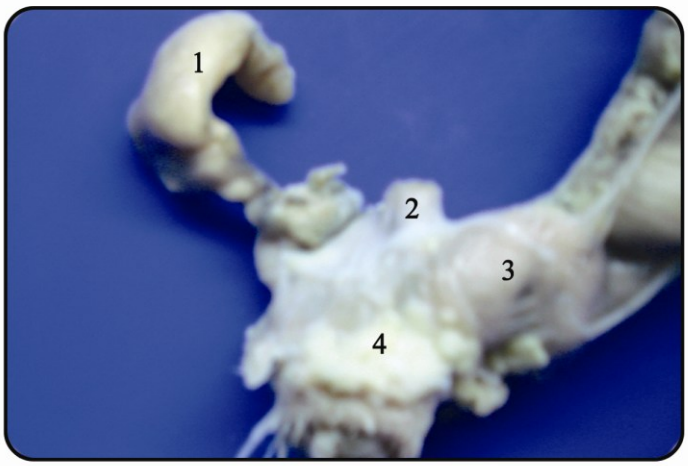

Fig. 5 Persistant right oviduct (18 weeks)

1. Magnum
2. Isthmus
3. Uterus
4. Ventral ligament
5. Bursa of fabricius
6. Colorectum

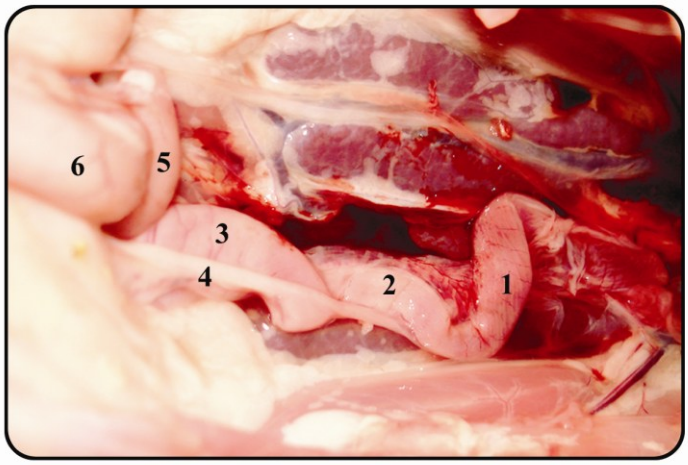

Fig. 6 In situ position of oviduct (12 weeks)

1. Ovary
2. Funnel of infundibulum
3. Neck of infundibulum
4. Dorsal ligament
5. Ventral ligament
6. Magnum
7. Magnum-isthmus junction
8. Isthmus
9. Uterus with an egg
10. Vagina
11. Cloaca.

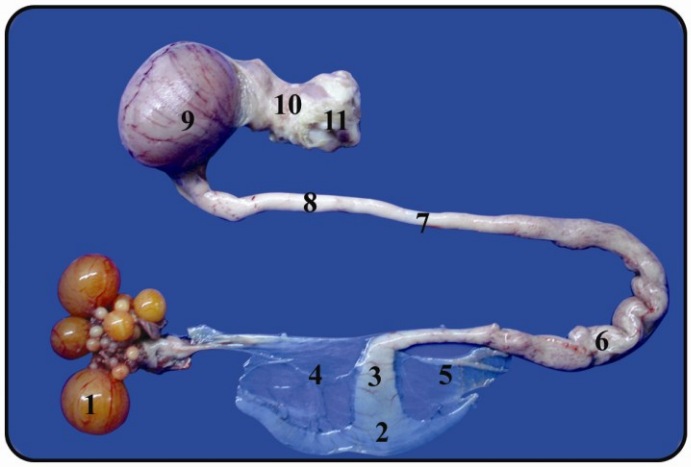

Fig. 7 Segments of the oviduct (20 weeks)

1. Ovary
2. Oviduct showing slight coiling
3. Left kidney
4. Bursa of fabricius

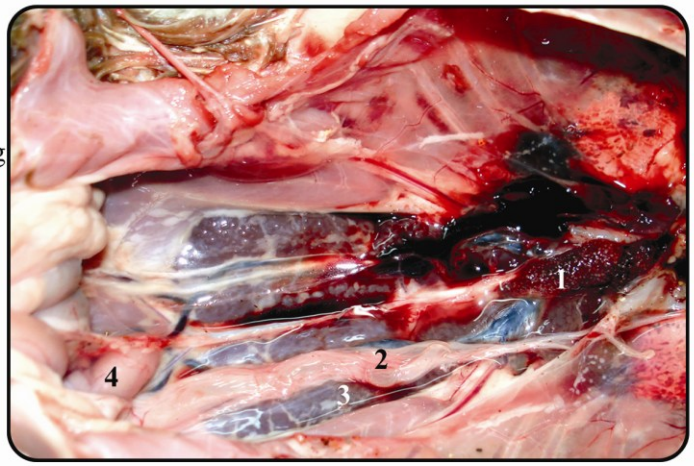

Fig. 8 Beginning of coiling of the oviduct (10 weeks)

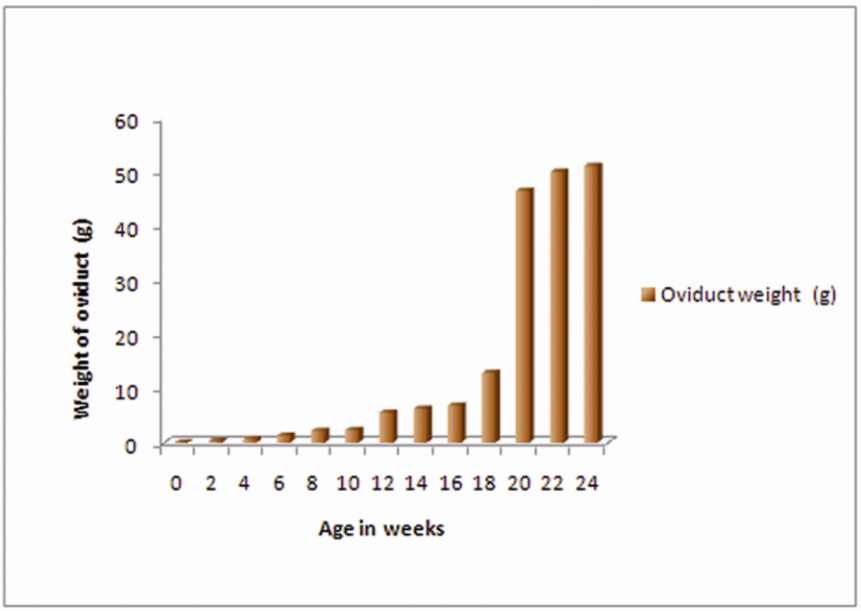

Fig. 9 Relationship between age and weight of oviduct in Kuttanad ducks

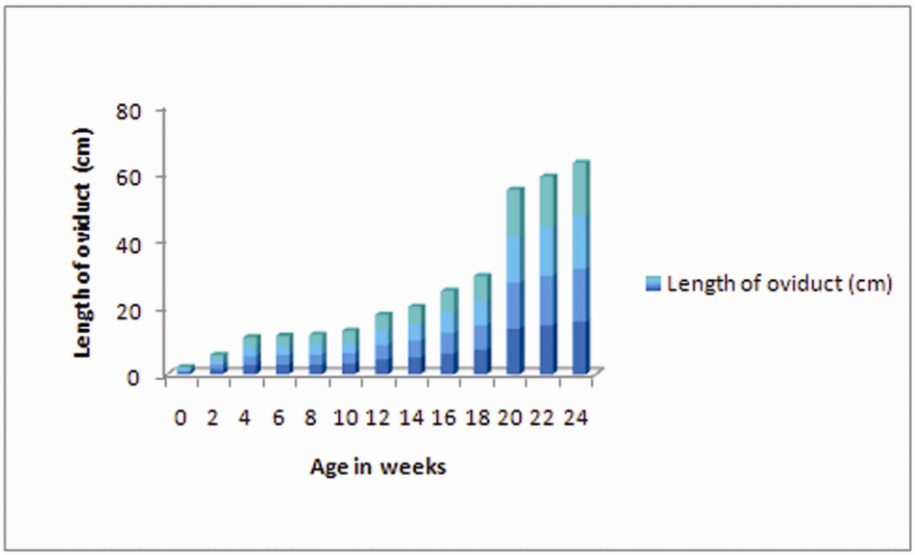

Fig. 10 Relationship between age and length of oviduct in Kuttanad ducks

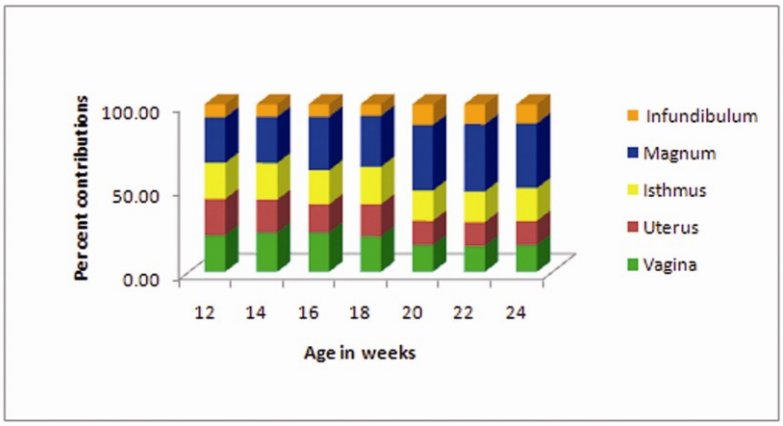

Fig. 11 Percentage contribution of segments of oviduct to the total length at different ages

1. Uterus
2. Vagina with annular rings
3. Cut edge of Colorectum

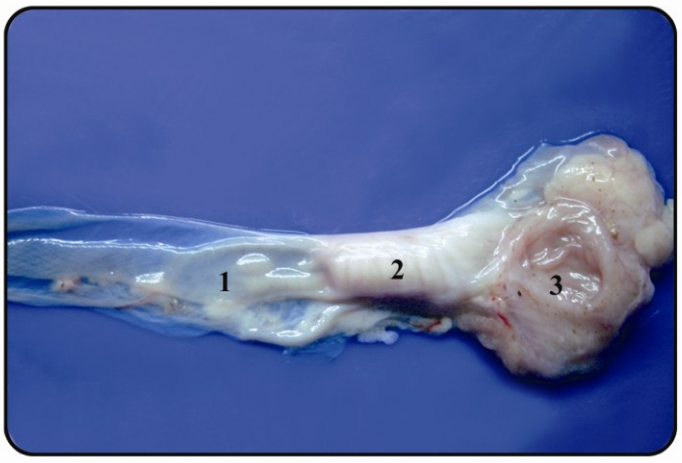

Fig. 12 Caudal portion of oviduct showing well developed vagina (10 weeks)

1. Epithelium
2. Sub-epithelial connective tissue
3. Mucosal fold

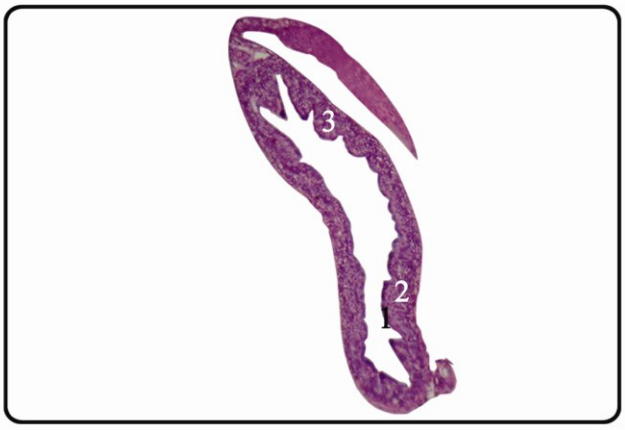

Fig. 13 C. S. of cranial region of the oviduct (day-old). H & E. x 100

1. Wall of oviduct
2. Ventral ligament with smooth muscles

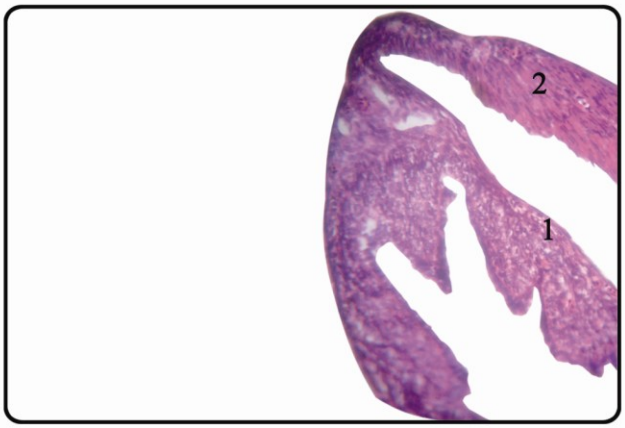

Fig.14 C. S. of oviduct with ventral ligament (day-old). H & E. x 400

1. Lamina epithelialis
2. Lamina propria without glands
3. Blood vessels
4. Tunica muscularis

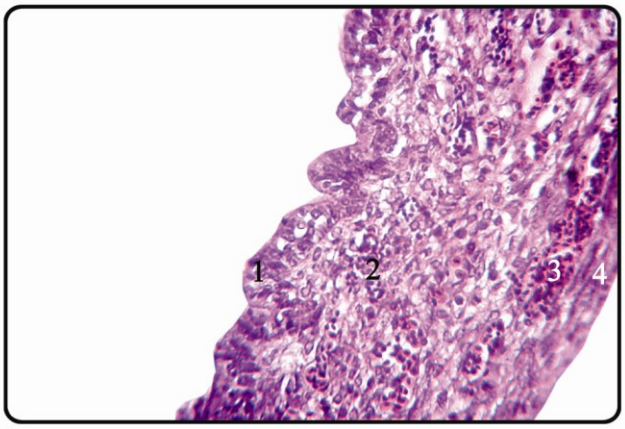

Fig. 15 C. S. of funnel of infundibulum showing low mucosal folds (12 weeks). H & E. x 400

1. Lamina epithelialis
2. Cilia
3. Vacuolated glands

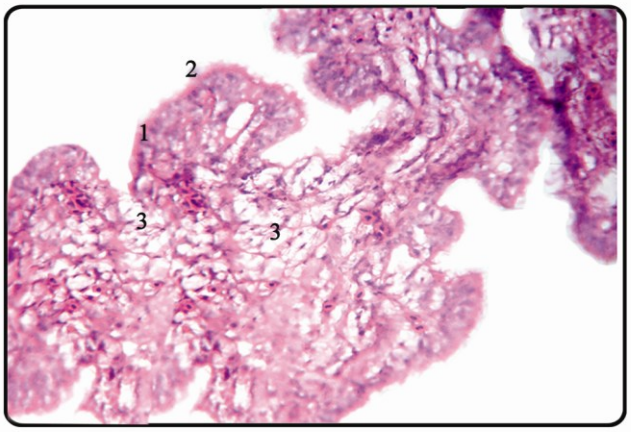

Fig. 16 C. S. of neck of infundibulum (14 weeks). H & E. x 400

1. Lamina epithelialis
2. Lamina propria with vacuolated glands
3. Core of mucosal fold

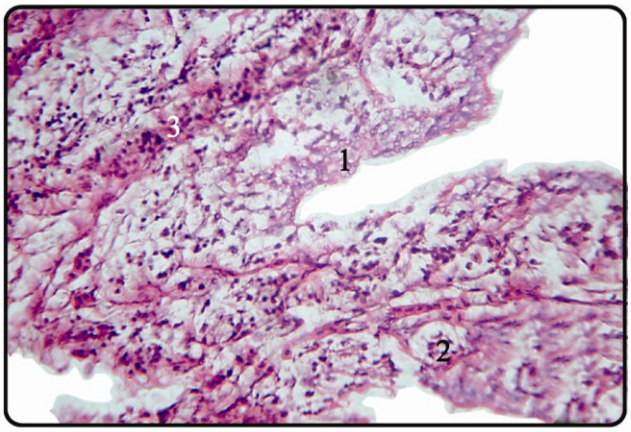

Fig. 17 C. S. of infundibular neck showing tubular glands (16 weeks). H & E. x 400

1. Lamina epithelialis
2. Cilia
3. Lamina propria
4. Lymphocytes

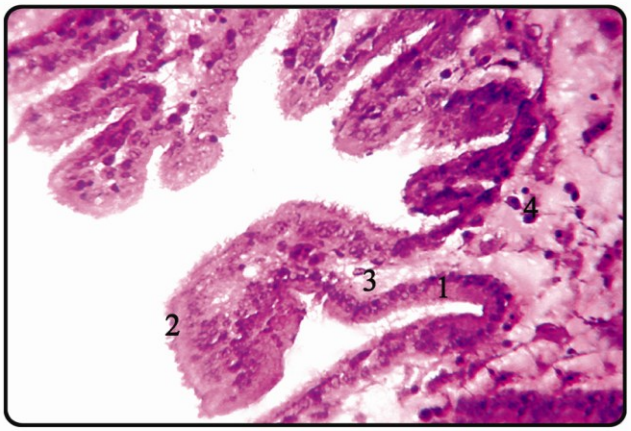

Fig. 18 C. S. of funnel of infundibulum showing mucosal folds (16 weeks). H & E. x 400

1. Lamina epithelialis
2. Lamina propria
3. Tunica muscularis
4. Tunica serosa

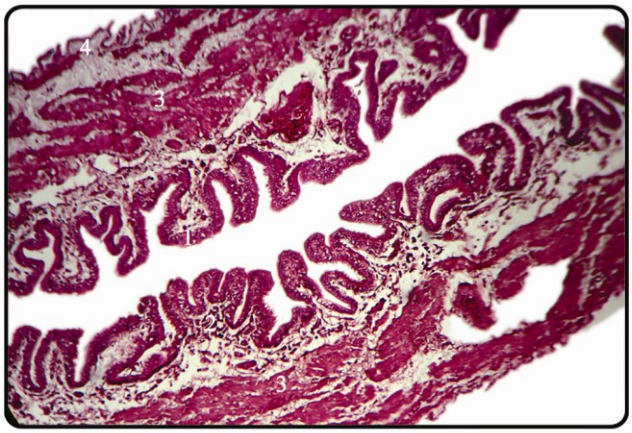

Fig. 19 C. S. of infundibular neck (20 weeks). H & E. x100

1. Lamina epithelialis
2. Glandular groove
3. Lamina propria
4. Tunica muscularis

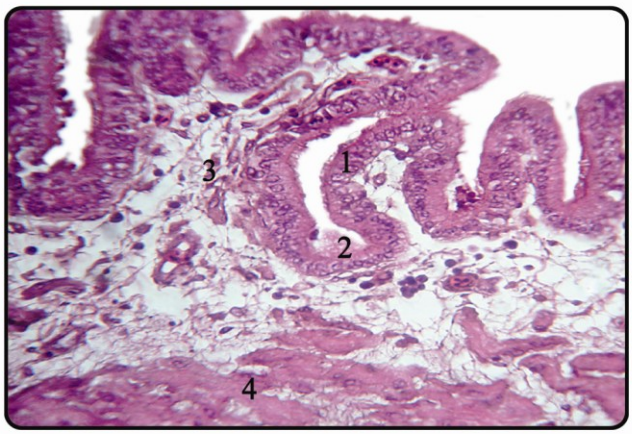

Fig. 20 C. S. of infundibular neck showing glandular groove (20 weeks). H & E. x 400

1. Lamina epithelialis with ciliated cells
2. Lamina propria
3. Tubular gland

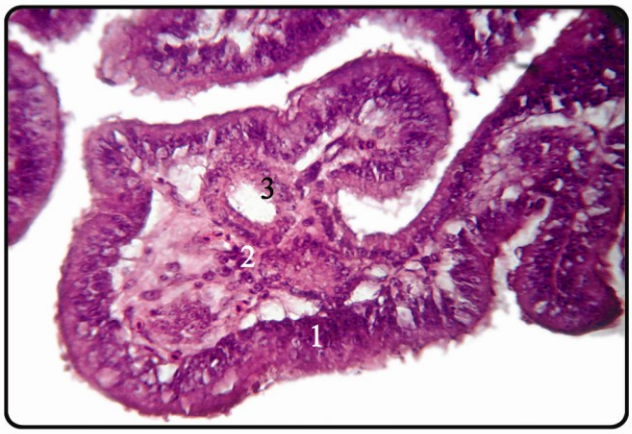

Fig. 21 C. S. of infundibular neck (22 weeks). H & E. x 400

1. Lamina epithelialis
2. Tubular glands in lamina propria
3. Goblet cells

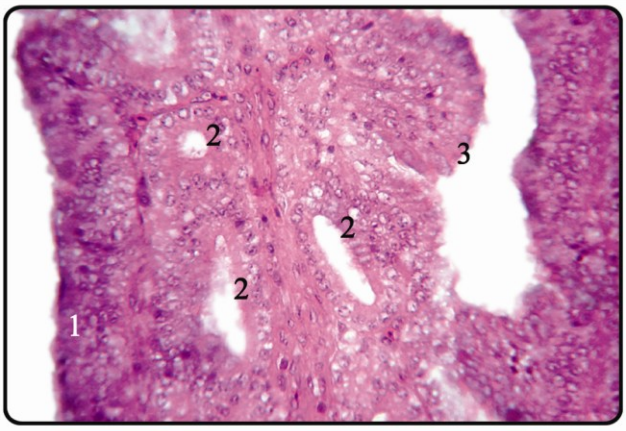

Fig.22 C. S. of caudal most region of infundibular neck (20 weeks). H & E. x 100

1. Tunica muscularis
2. Serosa

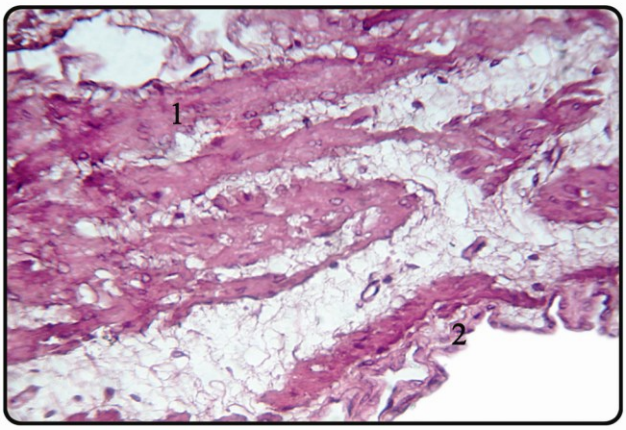

Fig. 23 C. S. of infundibular neck showing outer layers (20 weeks). H & E. x 400

1. Lamina epithelialis
2. Lamina propria
3. Tunica muscularis
4. Serosa

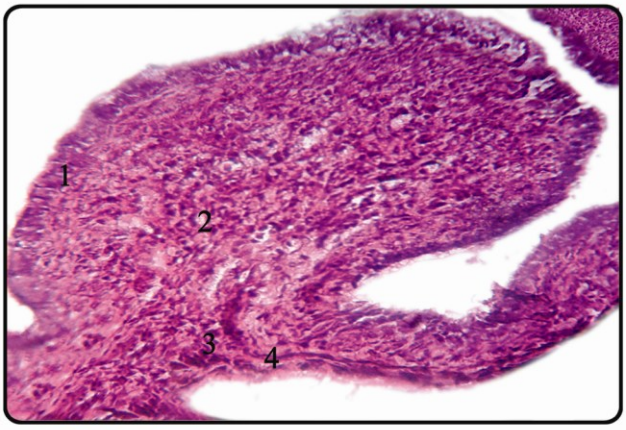

Fig.24 L. S. of infundibulum-magnum junction showing high mucosal folds (10 weeks). H & E. x 100

1. Infundibular glands with wider lumen
2. Glands of magnum
3 Lamina epithelialis
4. Core

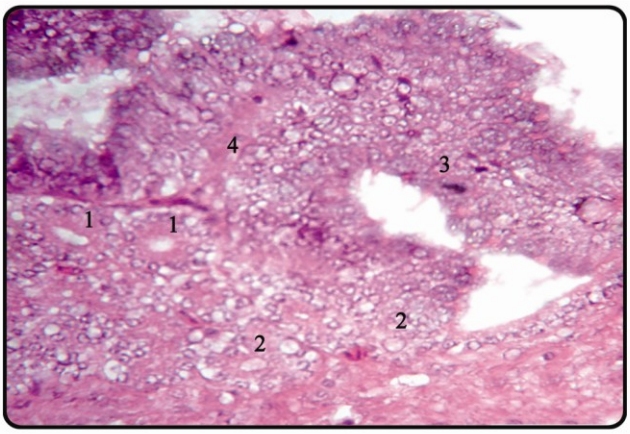

Fig. 25 L.S. of infundibulum-magnum junction showing two types of glands (20 weeks). H & E. x 400

1. Mucosal fold
2. Lamina epithelialis
3. Base of mucosal fold
4. Wall of magnum

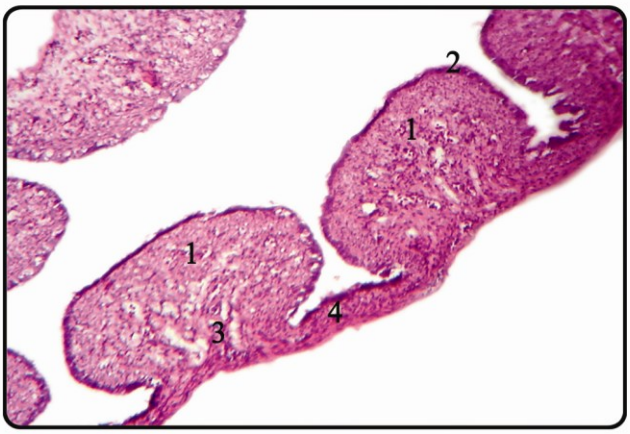

Fig. 26 C. S. of magnum (10 weeks). H & E. x 100

1. Mucosal fold
2. Lamina epithelialis
3. Glands in lamina propria
4. Tunica muscularis

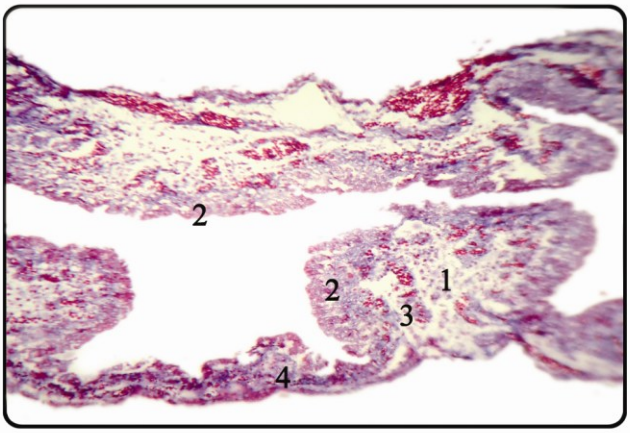

Fig. 27 C. S. of magnum (12 weeks) Gomori's one step trichrome method x 100

1. Mucosal fold
2. Lamina epithelialis
3. Glands in lamina propria
4. Tunica muscularis
5. Blood vessels

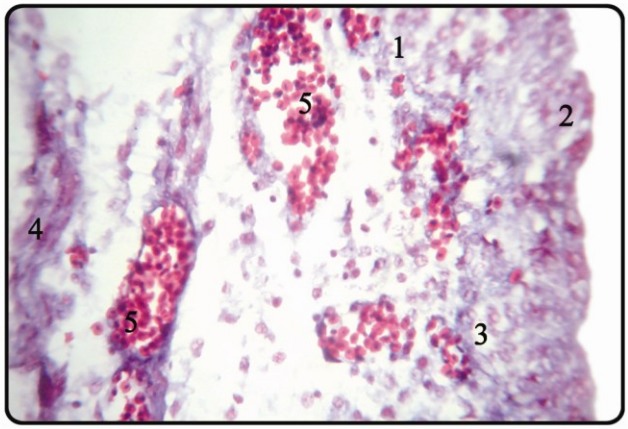

Fig. 28 C. S. of magnum (12 weeks) Gomori's one step trichrome method x 400

1. Lamina epithelialis
2. Radiating glands in lamina propria

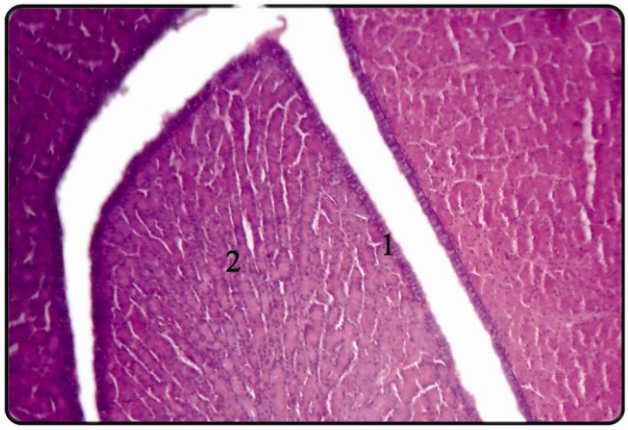

Fig. 29 C. S. of magnum showing mucosal folds (20 weeks). H & E. x 100

1. Ciliated columnar cells
2. Capillaries beneath lamina epithelialis
3. Tubular glands

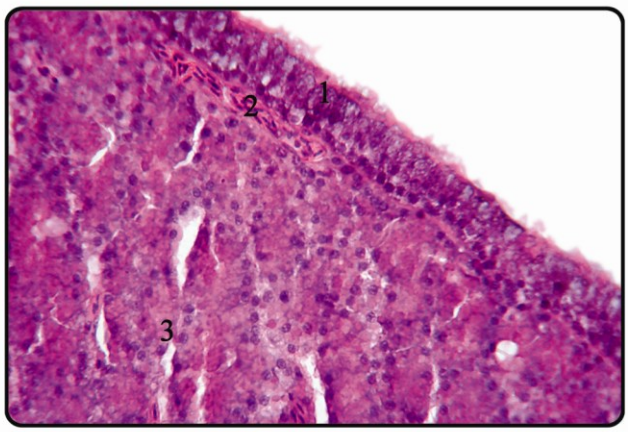

Fig. 30 C. S. of fold of magnum (20 weeks). H & E. x 400

1. Mucosal fold
2. Inner circular layer of tunica muscularis
3. Outer longitudinal layer of tunica muscularis

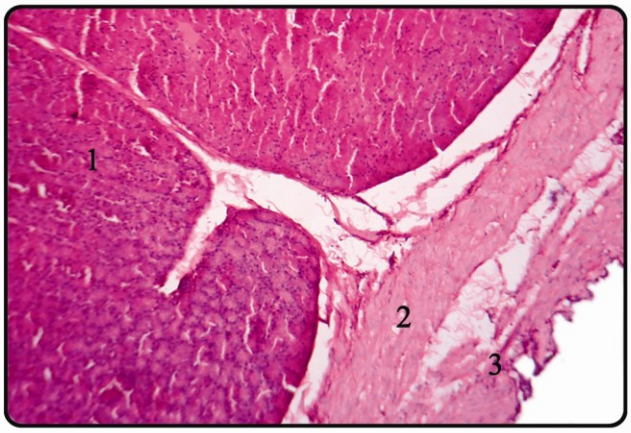

Fig. 31 C. S. of magnum (20 weeks). H & E. x 100

1. Goblet cells in lamina epithelialis
2. Tubular glands in lamina propria

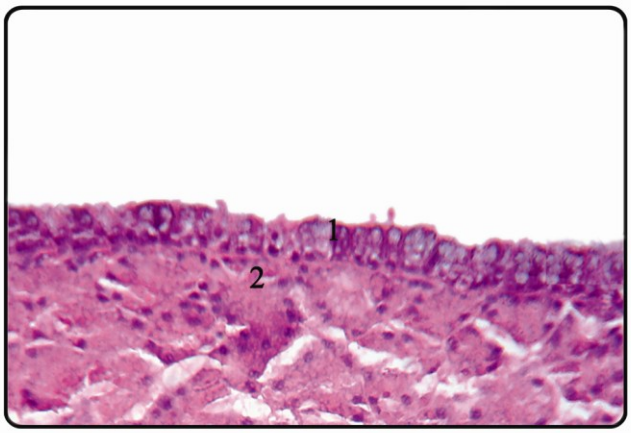

Fig. 32 C. S. of fold of magnum (20 weeks). H & E. x 400

1. Lamina epithelialis with tall cells
2. Lamina propria

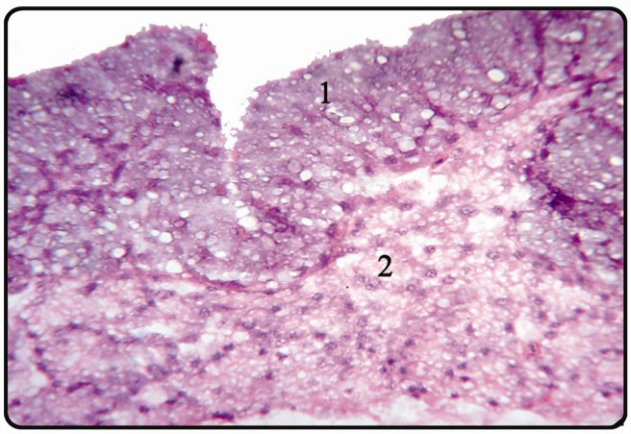

Fig. 33 L. S. of caudal region of magnum (20 weeks). H & E. x 400

1. Lamina epithelialis
2. Glands in lamina propria
3. Capillaries at the base of epithelium

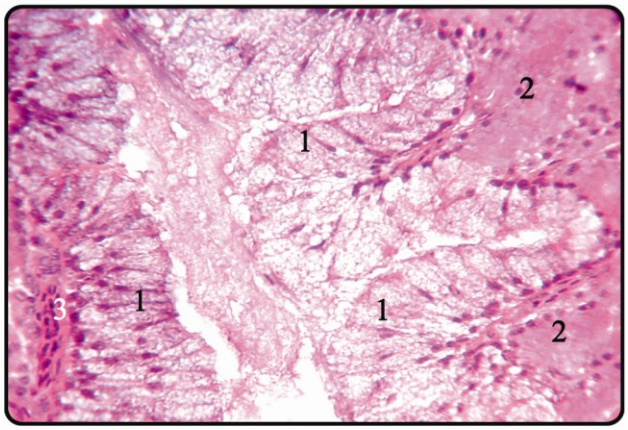

Fig. 34 C. S. of caudal portion of magnum showing the peculiar infolding. H & E. x 400

1. Lamina epithelialis
2. Lamina propria with glands
3. Mucosal folds devoid of glands
4. Tunica muscularis

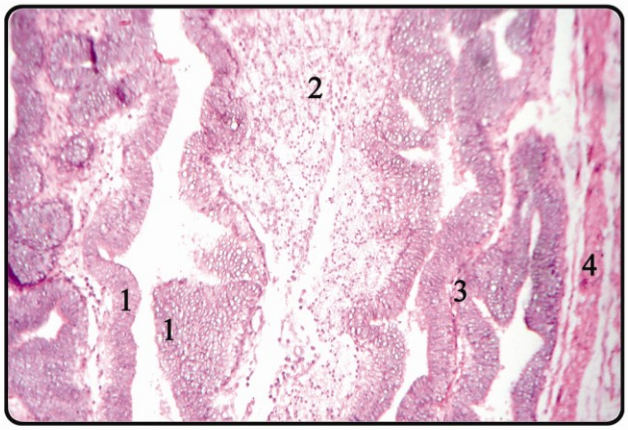

Fig. 35 L. S. of magnum-isthmus junction (20 weeks). H & E. x 400

1. Primary fold with angular shape
2. Secondary fold
3. Lamina propria

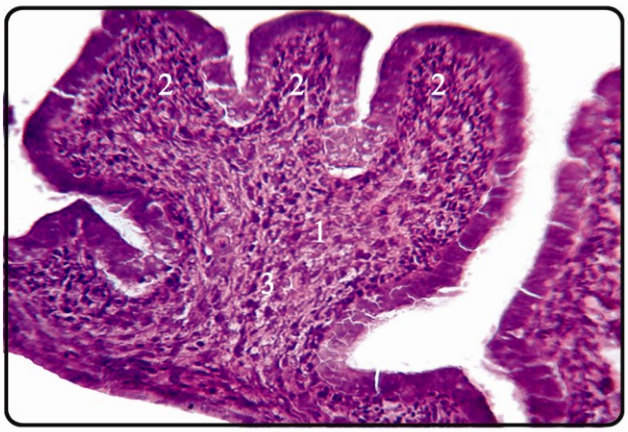

Fig. 36 C.S. of isthmus (4 weeks). H & E. x 400

1. Mucosal fold
2. Lamina propria with glands
3. Inner circular layer of tunica muscularis
4. Connective tissue containing blood vessels

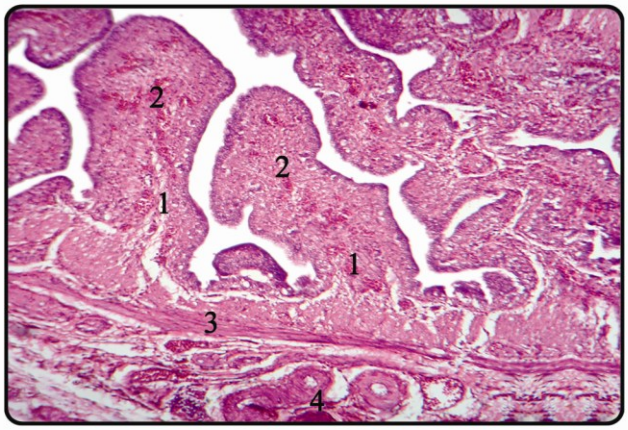

Fig. 37 L.S. of isthmus (12 weeks). H & E. x 100

1. Lamina epithelialis
2. Vacuolated glands in lamina propria
3. Capillaries

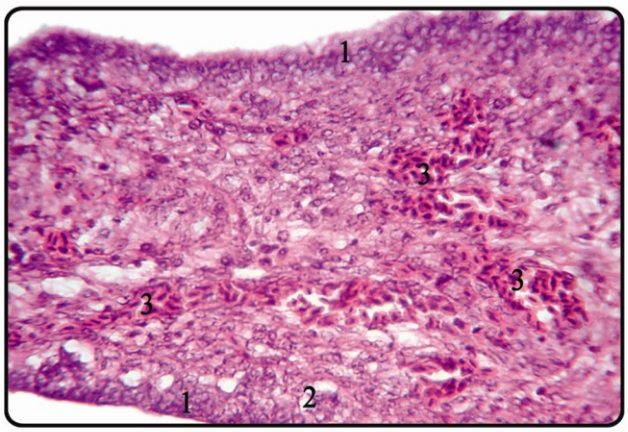

Fig. 38 L. S. of mucosal fold of isthmus (12 weeks). H & E. x 400

1. Lamina epithelialis
2. Lamina propria with loosely arranged glands
3. Central core

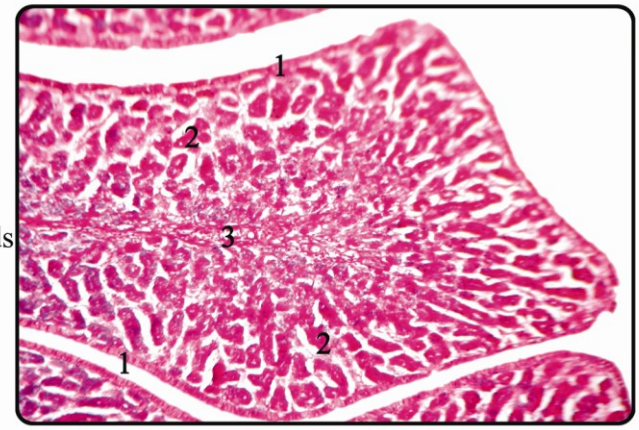

Fig. 39 C. S. of isthmus with angular mucosal fold (22 weeks). Gomori's one step trichrome method x 100

1.Lamina epithelialis
2. Ciliated cell with dark nucleus
3. Ciliated cell with light nucleus
4. Glands in non secretory phase
5. Glands in secretory phase

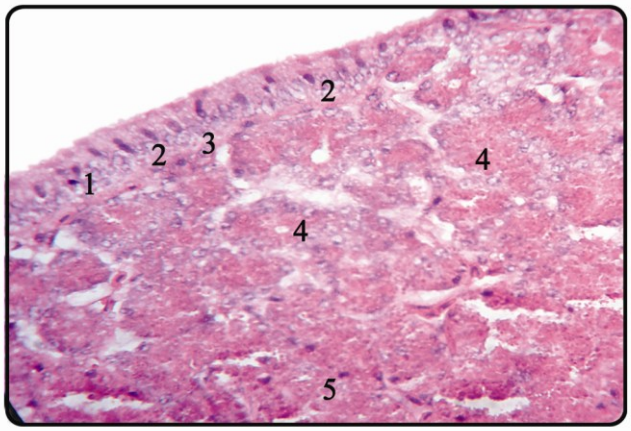

Fig. 40 C.S. of isthmus with angular mucosal fold (20 weeks). H & E. x 400

1. Glandular aperture
2. Lamina epithelialis
3. Secretory mass
4. Lumen

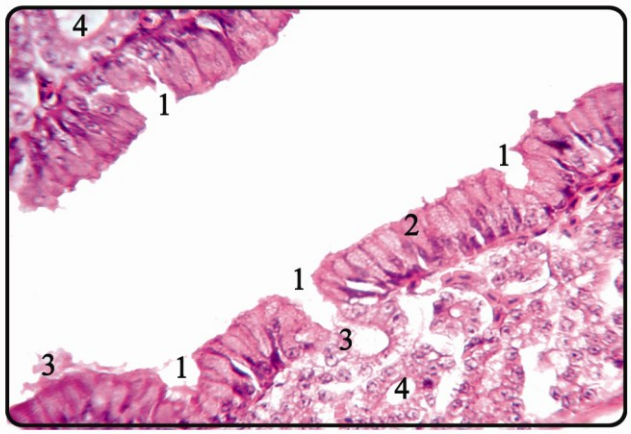

Fig. 41 C. S. of isthmus (22 weeks). H & E. x 400

1. Mucosal fold
2. Subepithelial connective tissue

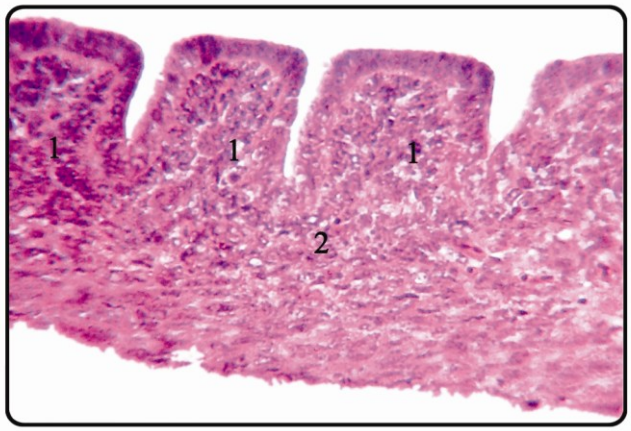

Fig. 42 C. S. of uterus (day-old). H & E. x 400

1. Lamina epithelialis
2. Lamina propria
3. Tunica muscularis
4. Serosa

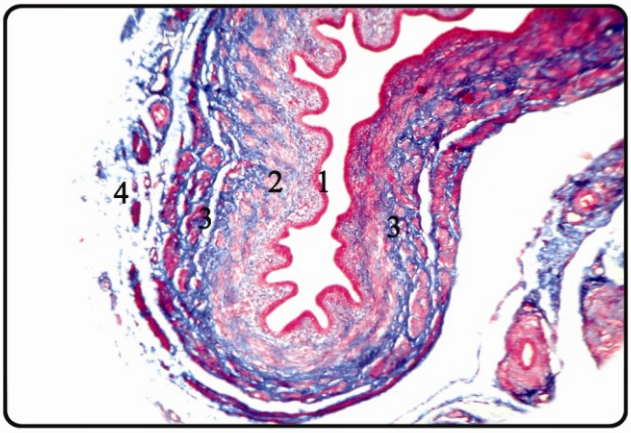

Fig. 43 C. S. of uterus (4 weeks). Gomori's one step trichrome method x 100

1. Lamina epithelialis
2. Lamina propria
3. Collagen fibres
4. Tunica muscularis
5. Serosa

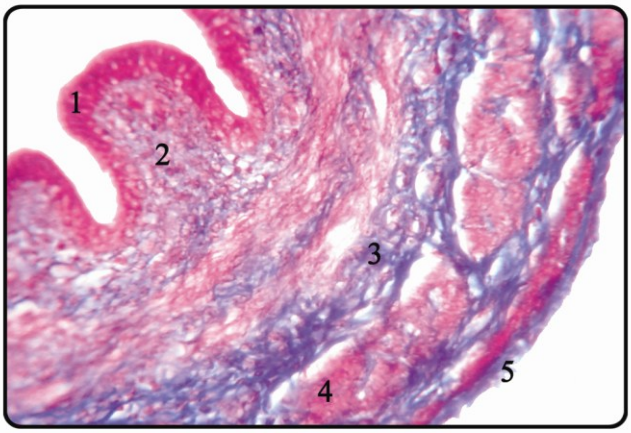

Fig. 44 C. S. of uterus (4 weeks). Gomori's one step trichrome method x 400

1. Uterus
2. Ventral ligament
3. Vagina
4. blood vessel
5. Ganglion

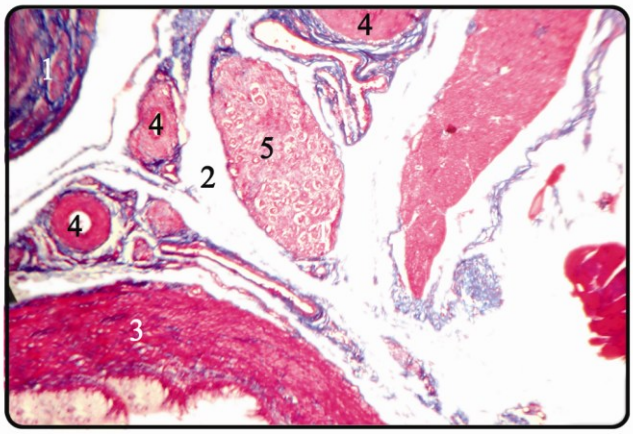

Fig. 45 Section of ventral ligament connecting uterus and vagina (4 weeks). Gomori's one step trichrome method x 100

1. Lamina epithelialis
2. Core
3. Lamina propria
4. Vacuolated glands
5. Tunica muscularis

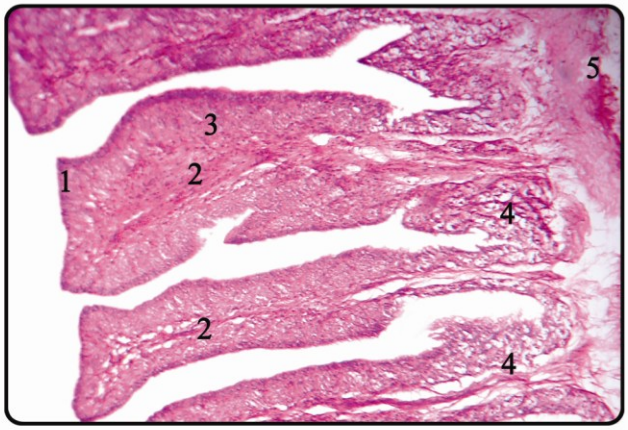

Fig. 46 C. S. of uterus (12 weeks). H & E. x 100

1. Lamina epithelialis with ciliated cells
2. Tubular glands in lamina propria

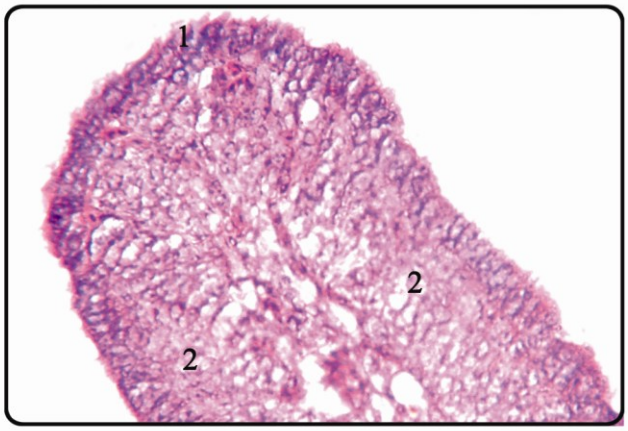

Fig. 47 C. S. of uterine mucosal fold (12 weeks). H & E. x 400

1. Lamina epithelialis
2. Invagination of surface epithelium to form glands
3. Vacuolation in glandular epithelium

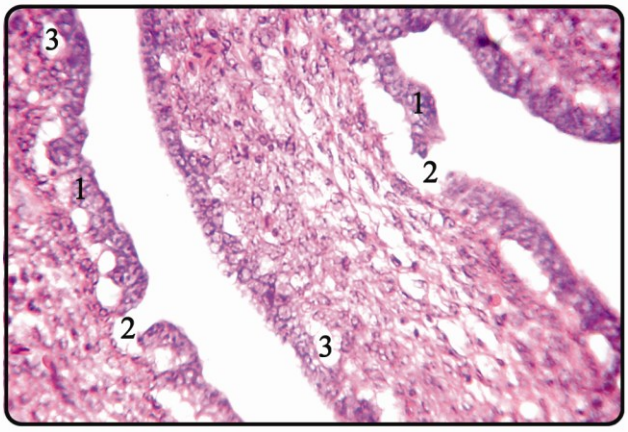

Fig. 48 C. S. of uterus (12 weeks). H & E. x 400

1. Lamina epithelialis with ciliated cells
2. Lamina propria

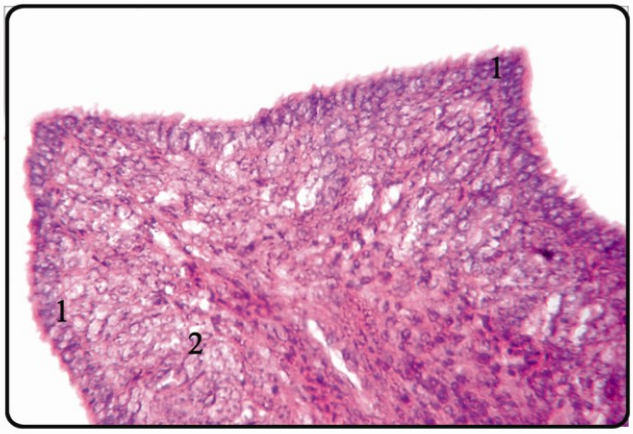

Fig. 49 C. S. of uterine fold showing apical cilia (14 weeks). H & E. x 100

1. Tubular glands with vacuolated cells
2. Core of mucosal fold

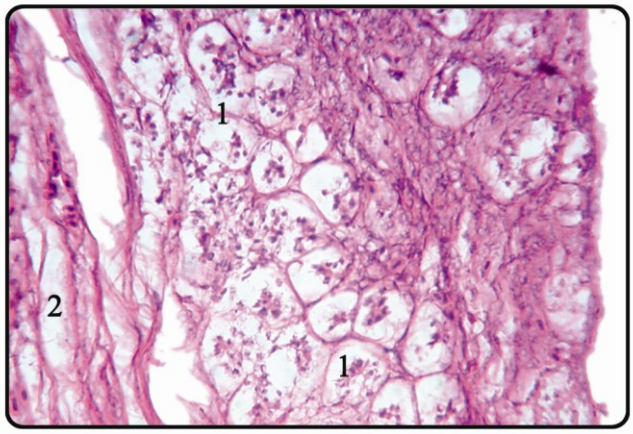

Fig. 50 C. S. of uterus showing base of mucosal fold (14 weeks). H & E. x 400

1. Lamina epithelialis
2. Lamina propria with glands

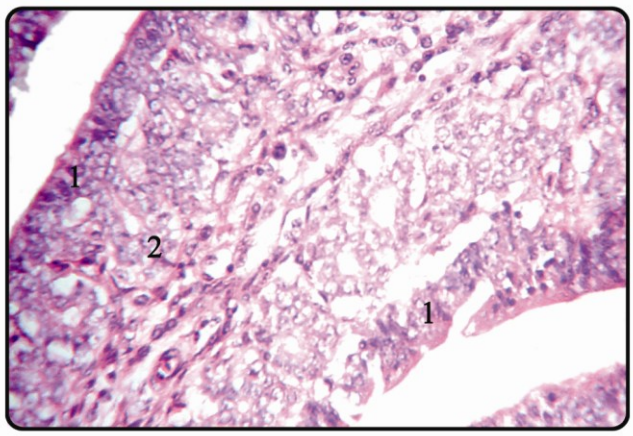

Fig. 51 C. S. of uterine fold showing mucosal glands (16 weeks). H & E. x 400

1. Mucosal fold
2. Lamina epithelialis
3. Lamina propria
4. Core of mucosa

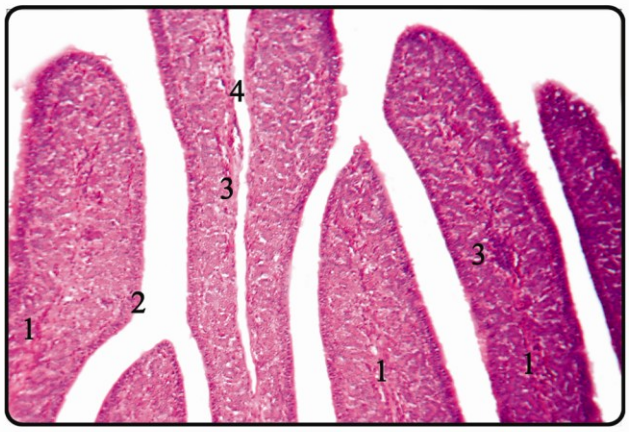

Fig. 52 C. S. of uterine fold showing leaf-like mucosal folds (16 weeks). H & E. x 100

1. Inner circular layer of tunica muscularis
2. Connective tissue
3. Outer longitudinal layer of tunica muscularis

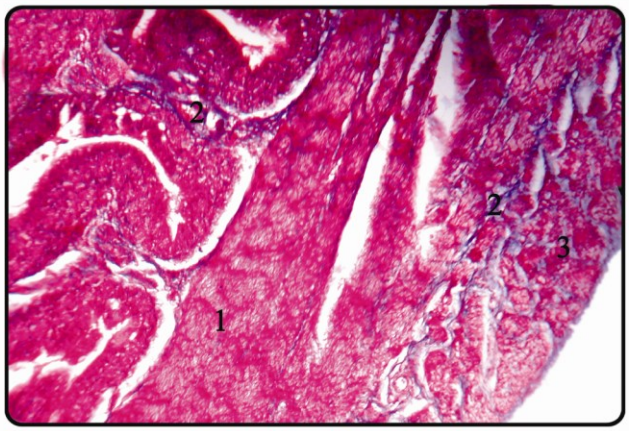

Fig. 53 C. S. of uterine fold showing tunica muscularis (22 weeks). Gomori's one step trichrome method x 100

1. Mucosal fold
2. Smooth muscles extending to the core
3. Tunica muscularis

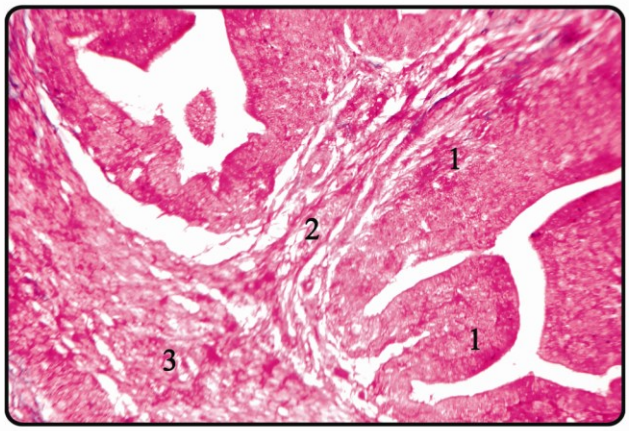

Fig. 54 C. S. of uterine fold showing tunica muscularis (22 weeks). Gomori's one step trichrome method x 100

1. Lamina epithelialis
2. Clear cell
3. Core

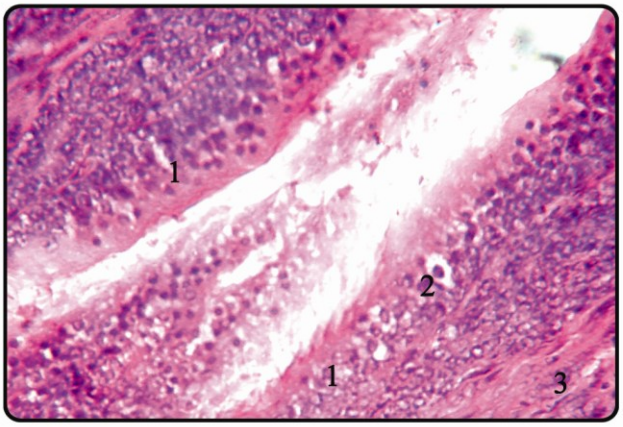

Fig.55 C. S. of uterine fold showing clear cells (22 weeks).
H & E. x 400

1. Basal cells
2. Apical cells
3. Lamina propria

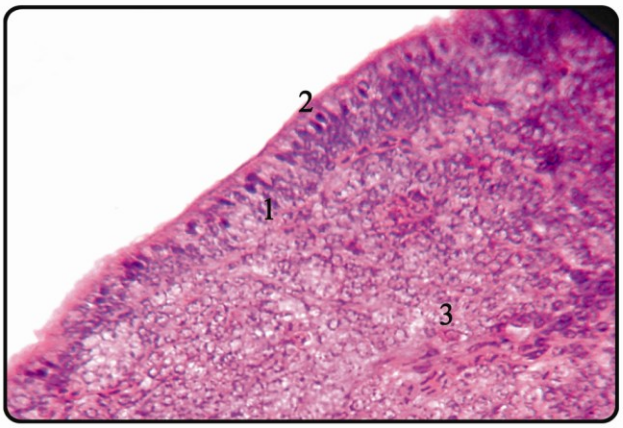

Fig. 56 C. S. of uterus showing the lining epithelium (22 weeks).
H & E. x 400

1. Mucosal fold
2. Lamina propria
3. Tunica muscularis

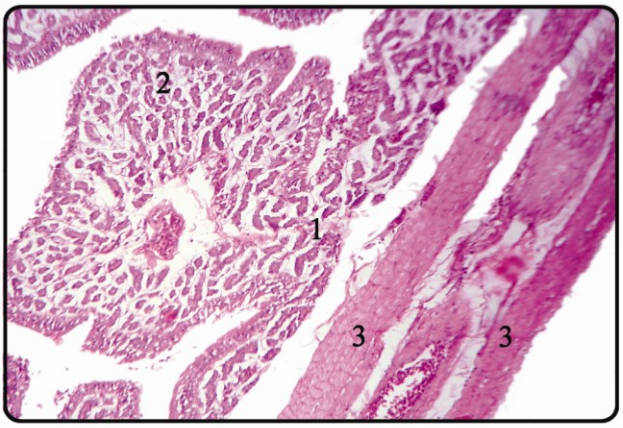

Fig. 57 L. S. of uterus showing flattened mucosal fold (22 weeks).
H & E. x 100

1. Lamina epithelialis showing denuded epithelium
2. Tubular glands in lamina propria

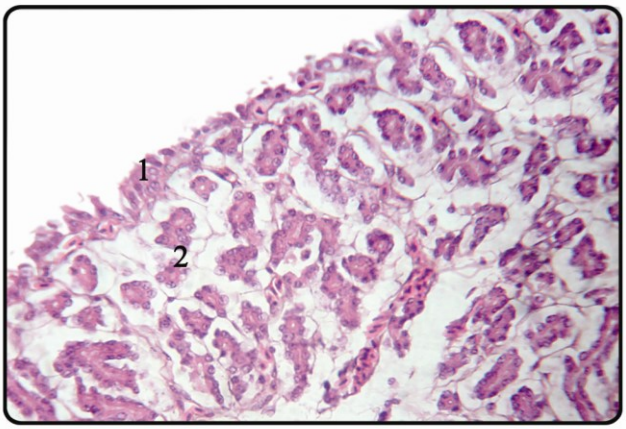

Fig. 58 L. S. of uterine mucosal fold (22 weeks). H & E. x 400

1. Lamina epithelialis
2. U-shaped tubular glands in lamina propria

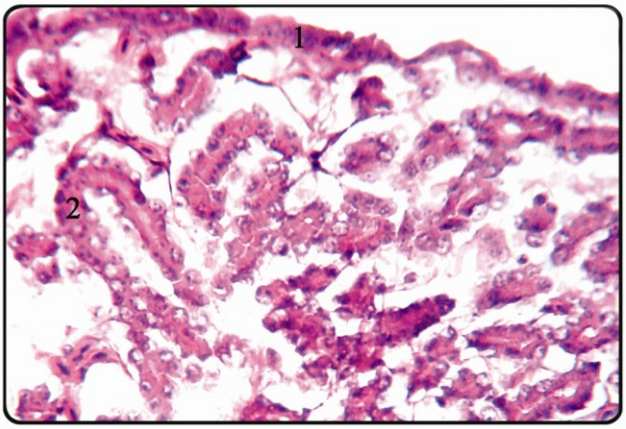

Fig. 59 L. S. of uterine mucosal fold (22 weeks). H & E. x 400

1. Lamina epithelialis
2. Lamina propria
3. Sperm storage tubules

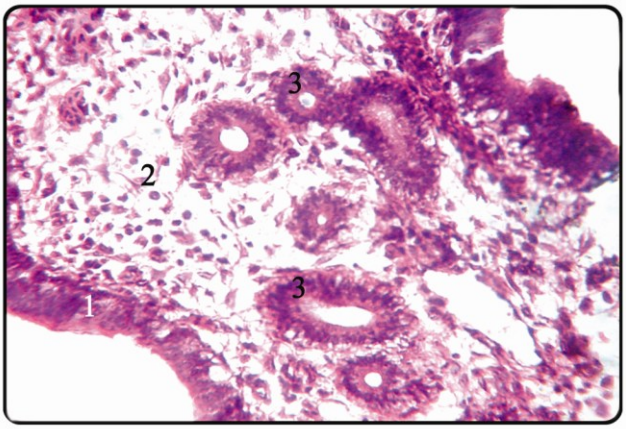

Fig. 60 C. S. of utero- vaginal junction (12 weeks). H & E. x 400

1. Lamina epithelialis
2. Lamina propria
3. Transitional glands

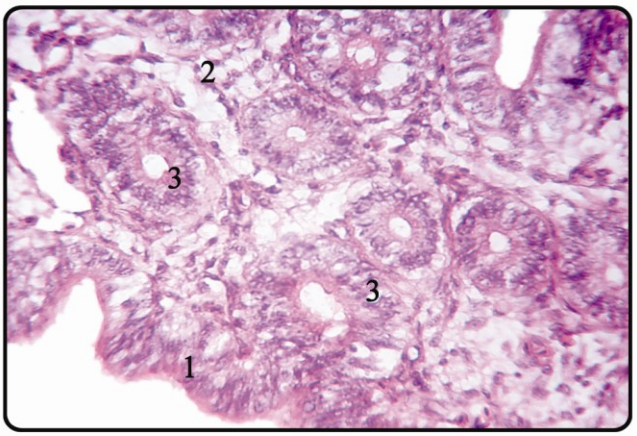

Fig. 61 C. S. of utero- vaginal junction (14 weeks). H & E. x 400

1. Lamina epithelialis
2. Lamina propria
3. Sperm storage tubules

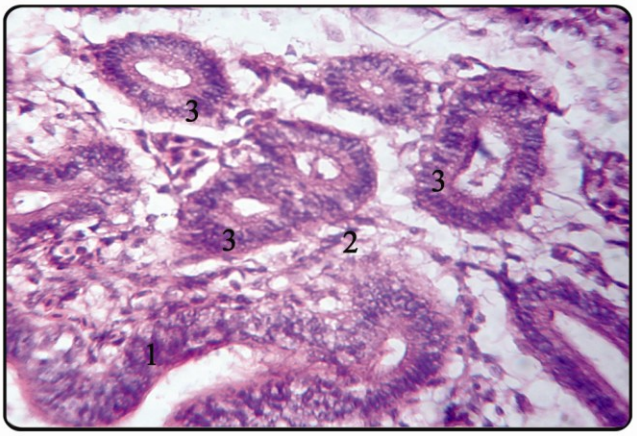

Fig. 62 C. S. of utero- vaginal junction (14 weeks). H & E. x 400

1. Lamina epithelialis
2. Lamina propria
3. Sperm storage tubules

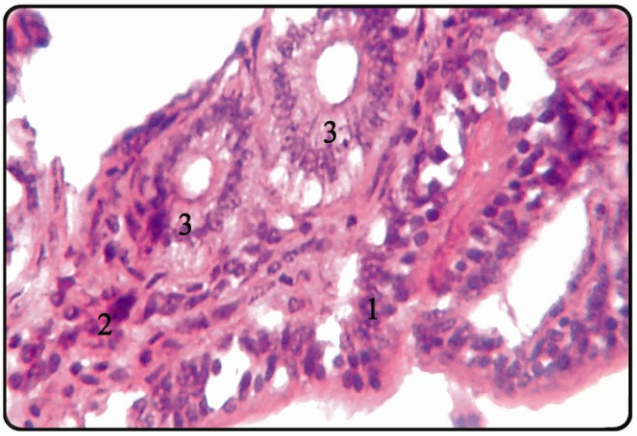

Fig. 63 C. S. of utero- vaginal junction (18 weeks). H & E. x 400

1. Lamina epithelialis
2. Lamina propria
3. Tunica muscularis
4. Serosa

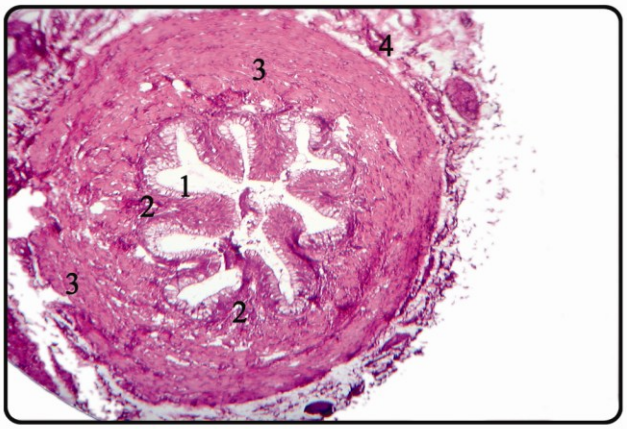

Fig. 64 C. S. of vagina (2 weeks). H & E. x 100

1. Lamina epithelialis
2. Lamina propria

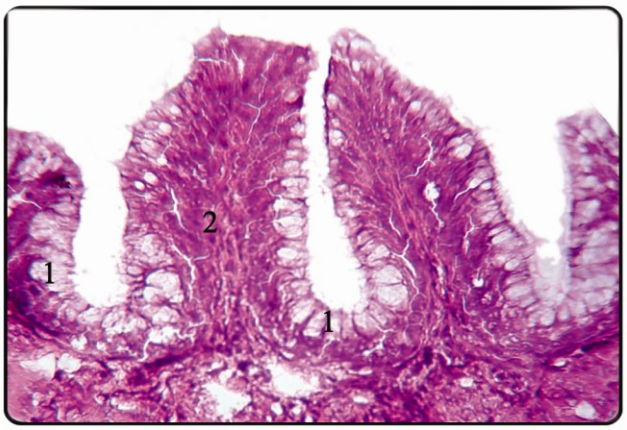

Fig. 65 C. S. of vagina (2 weeks). H & E. x 400

1. Mucosa
2. Tunica muscularis
3. Serosa
4. Ventral ligament
5. Blood vessel

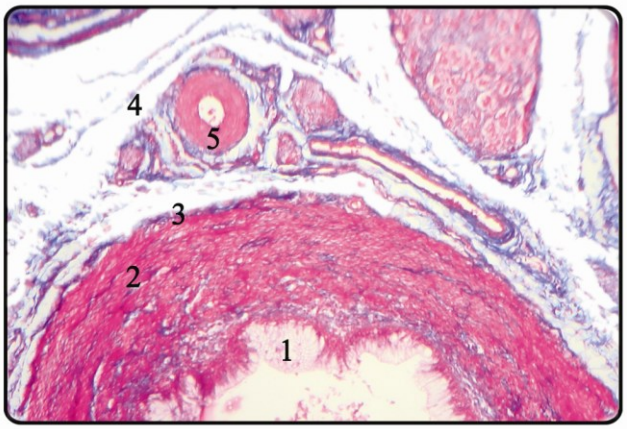

Fig. 66 C. S. of vagina (4 weeks). Gomori's one step trichrome method x 100

1. Mucosa
2. Tunica muscularis
3. Serosa
4. Blood vessel

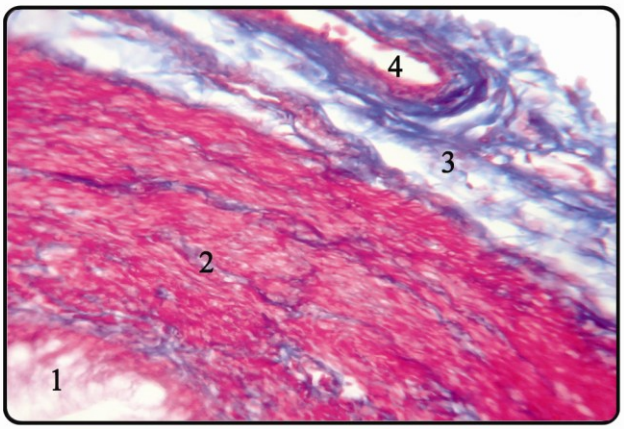

Fig. 67 C. S. of vagina (4 weeks). Gomori's one step trichrome method x 400

1.Mucosa
2. Tunica muscularis

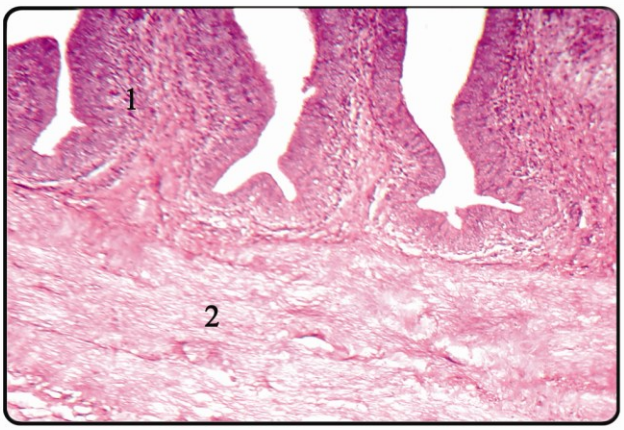

Fig. 68 C. S. of vagina (10 weeks). H & E. x 100

1. Epithelial cells showing cilia
2. Clear cell
3. Lamina propria without glands

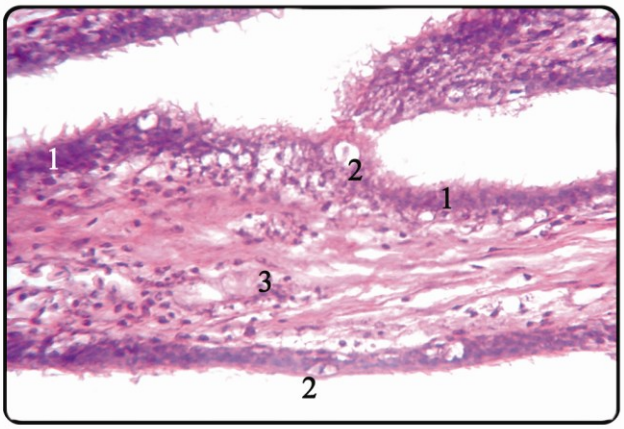

Fig. 69 C. S. of vaginal fold (22 weeks). H & E. x 400

1. Lamina epithelialis
2. Lamina propria
3. Core of mucosal fold with bundles of collagen fibres

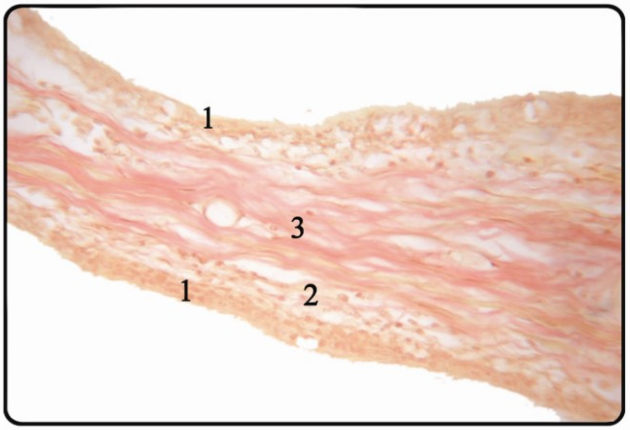

Fig. 70 C. S. of vaginal fold (22 weeks). Van Gieson's stain x 400

1. Epithelium with goblet cells
2. Lamina propria without glands

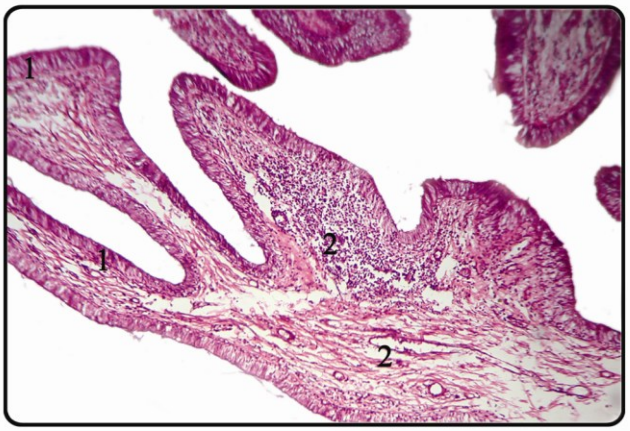

Fig. 71 C. S. of vaginal fold (22 weeks). H & E. x 400

1. Mucosal fold
2. Tunica muscularis extending into the core of mucosal fold

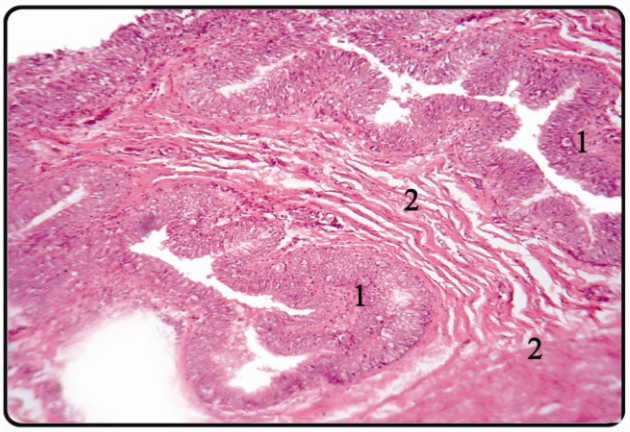

Fig. 72 C. S. of vaginal fold showing tunica muscularis (22 weeks). H & E. x 400

1. Low mucosal fold
2. Tunica muscularis extending into the core of mucosal fold

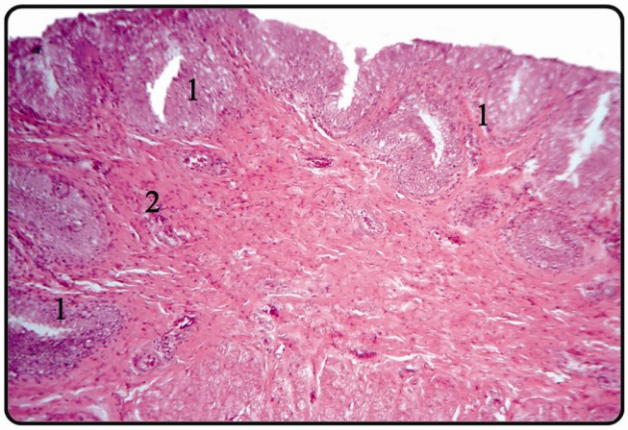

Fig. 73 C. S. of caudal most portion of vagina showing low mucosal folds (22 weeks). H & E. x 100

1. Lamina epithelialis
2. Lamina propria showing diffused blood cells
3. Tunica muscularis

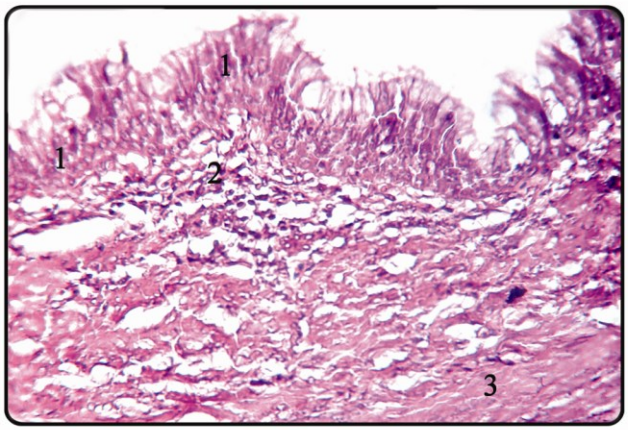

Fig. 74 C. S. of vagina showing diffused blood cells (4 weeks). H & E. x 400

1. Lamina epithelialis
2. Lamina propria showing diffused blood cells

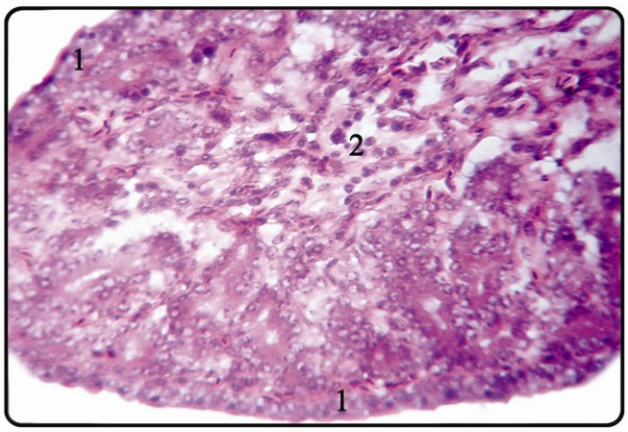

Fig. 75 C. S. of magnum showing diffused blood cells (18 weeks). H & E. x 400

1. Lamina epithelialis
2. Lamina propria showing diffused lymphocytes
3. Serosa

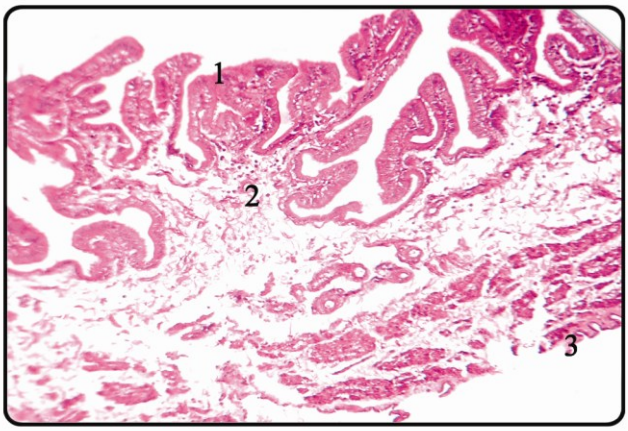

Fig. 76 L. S. of neck region of infundibulum showing lymphocytes (20 weeks). H & E. x 100

1. Lamina epithelialis
2. Lamina propria showing diffused lymphocytes

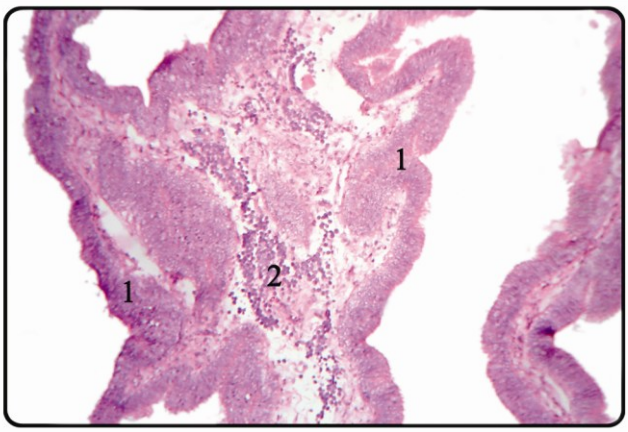

Fig. 77 L. S. of magnum-isthmus junction showing lymphocytes (20 weeks). H & E. x 100

1. Lamina epithelialis
2. Lamina propria showing aggregates of lymphocytes
3. Tubular glands

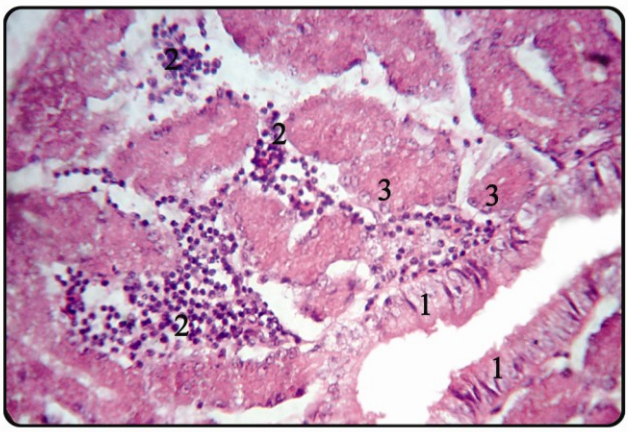

Fig. 78 C. S. of isthmus showing lymphocytes (22 weeks). H & E. x 400

1. Tunica muscularis
2. Diffused lymphocytes
3. Blood vessel

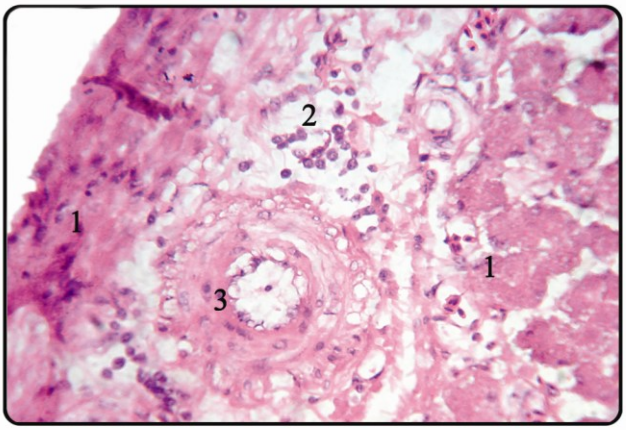

Fig. 79 C. S. of tunica muscularis of isthmus showing lymphocytes (22 weeks). H & E. x 400

1. Lamina epithelialis
2. Lamina propria showing aggregates of lymphocytes

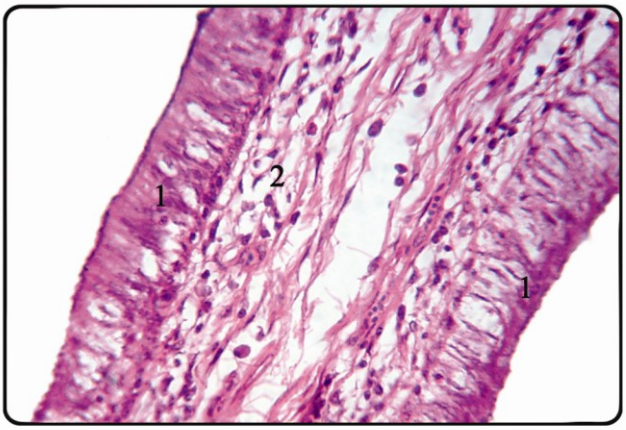

Fig. 80 C. S. of vaginal mucosal fold showing lymphocytes (22 weeks). H & E. x 400

1. Lamina epithelialis
2. Lamina propria with glands

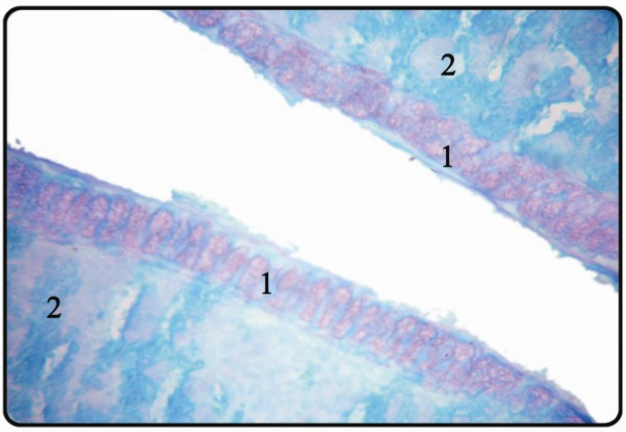

Fig. 81 C. S. of magnum showing goblet cells in mucous region (22 weeks). PAS and Alcian blue (pH 2.5) method x 400

1. Lamina epithelialis
2. Lamina propria
   with glands

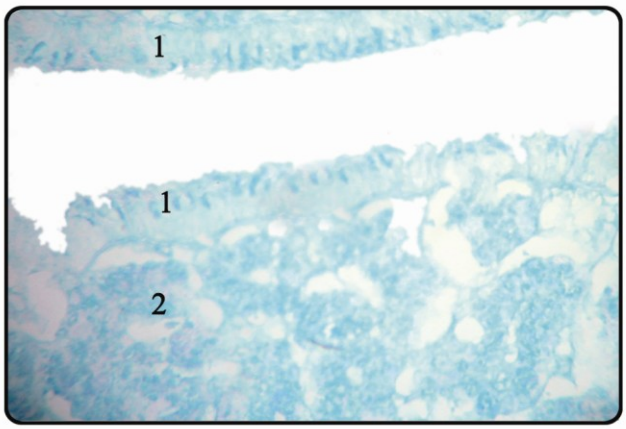

Fig. 82 C. S. of isthmus (22 weeks). PAS and Alcian blue (pH 2.5) method x 400

1. Lamina epithelialis
2. Lamina propria
   with glands

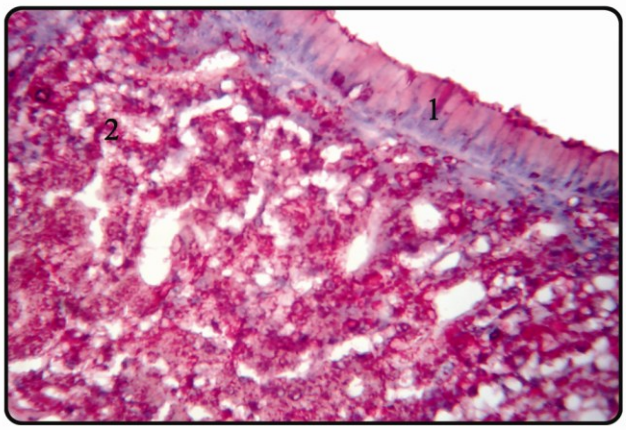

Fig. 83 C. S. of isthmus (22 weeks). Best's carmine method x 400

1. Lamina epithelialis
2. Proprial glands

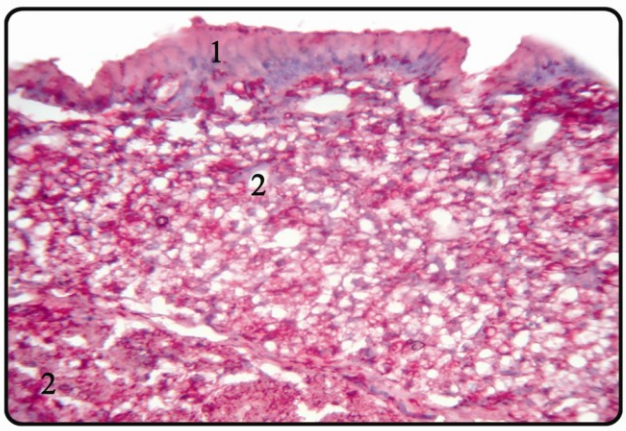

Fig. 84 C. S. of isthmus (22 weeks). Best's carmine method x 400

1. Lamina epithelialis
2. Lamina propria with glands

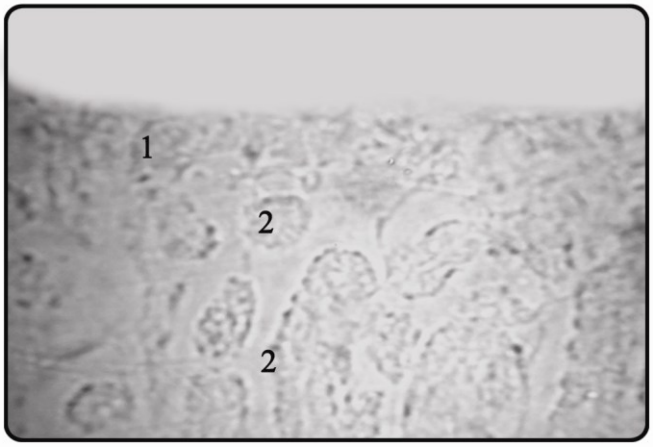

Fig. 85 C. S. of isthmus (22 weeks). Gomori's method for Alkaline Phosphatase x 400

Discussion

4. DISCUSSION

5.1 DEVELOPMENT AND GROSS MORPHOLOGY OF THE OVIDUCT

5.1.1 Topography of the Oviduct

In the day-old duckling, the left oviduct was thin, thread-like, translucent, straight, narrow tube which occupied left side of the coelomic cavity. It originated caudolateral to the left ovary and extended posteriorly along the ventral aspect of the left kidney and terminally joined the cloaca. At this age, it was poorly supplied by blood vessels as evident from its generalized pallor. Similar observations were also made by Lucy and Harshan (1999a) in non laying Japanese quails and Soley and Groenewald (1999) in young immature ostrich.

The oviduct was suspended from the left lateral body wall by the dorsal ligament, a thin fold of peritoneal membrane attached dorsally to the inner surface of abdominal cavity, running caudally from the region of the fourth thoracic rib to the cloaca. The dorsal ligament also carried left ureter dorsolateral to the oviduct and both the oviduct and ureter ran parallelly in the caudal direction. The ventral ligament was attached to the ventral surface of the oviduct and showed a gradually thickening muscular band along its lower free edge that fused posteriorly with the caudal most region of the oviduct, the vagina. These findings were in accordance with those recorded by Bradley and Grahame (1960) in domestic fowl.

Similar to in-lay hen (Surface, 1912), Japanese quail (Lucy and Harshan, 1999a) and ostrich (Soley and Groenewald, 1999), in the adult laying duck, the oviduct was observed to be highly convoluted tubular structure which occupied the left half of the coelom almost completely. However, the oviduct containing a developing egg extended towards the right side also and displaced the intestines in the right ventral direction.

Dorsal surface of the oviduct was related to the ventral surface of the left kidney and left lateral body wall. Ventrally on the left, it was related to the

proventriculus, gizzard and spleen towards the cranial aspect and intestines in the caudolateral part. Kern (1963) reported similar observations in the oviduct in domestic fowl.

5.1.2 Development of the Left and Right Paramesonephric Ducts

Kuttanad ducks, in general, followed the well accepted fact that, in most of the avian species, development of left paramesonephric duct predominates and showed much more developed left paramesonephric duct which was functionally advanced than the right one during the postnatal period. Although the occurrence of right oviduct was difficult to trace out during the period from day-old to eight weeks of age, from 10th week onwards, the presence of the much regressed right oviduct could be detected in two to three birds in each age group. Such rudimentary forms of the right oviduct were also recorded by Bradley and Grahame (1960), Aitken (1971), King and Mc Lelland (1975) and Paneerselvam *et al*. (1989) in the adult domestic hen, Kamar and Yamani (1962) in Pekin duck and Onyeanusi *et al*. (1986) in the guinea fowl (*Numida meleagris*). The observations made by Kinsky (1971) suggested that the reduction in the right oviduct of the birds might well have preceded the reduction of right ovary during their evolutionary history.

In present study, total number of birds with persistent right oviduct was recorded to be 20 and the average length of the rudimentary right oviduct ranged from 0.70 to 2.91 cm. Out of the twenty birds, an exceptional case was observed at 18th weeks of age, wherein, the right oviduct attained a maximum length of 13.80 cm, which was more than half the length of the left oviduct in the same age group. Right oviduct was a richly vascular blind sac-like tube with rounded tip and showed two to three convolutions along its length before it joined the cloaca exactly at the same level as that of the left functional oviduct. The width of right oviduct was maximum in the cranial region and minimum in the middle portion. The diameter slightly increased where it opened into the cloaca and this region was opaque white in colour and was the toughest region of the right oviduct.

While Kamar and Yamani (1962) in Pekin duck suggested a potent endocrine control over the occurrence of right oviduct, more recently, Wakamatsu *et al.* (2000) established that in birds, persistence of right oviduct was a hereditary phenomenon and attributed it to the expression of two pairs of autosomal recessive genes which were relatively stable in homozygous condition.

5.1.4 Structural Classification of Different Regions of the Oviduct

In the present study, five different segments of the oviduct namely, infundibulum, magnum, isthmus, uterus and vagina could be clearly distinguished from 12th week onwards. In the structurally and functionally differentiated oviduct of mature birds these five regions were described by Romanoff and Romanoff (1949), Hodges (1974), King (1975) and Gilbert (1979) in domestic fowl, Chakravorti and Sadhu (1961) in the laying kite, Fitzgerald (1969) and Lucy and Harshan (1999a) in the Japanese quail, Das and Biswal (1968) and Muwazi *et al.* (1982) in mature ostrich, Sharma and Duda (1986), Rao (1994) and Ozen *et al.* (2009) in the duck and Mohammadpour and Keshtmandi (2008) in turkey and pigeon.

Infundibulum was held in position by the dorsal and ventral ligaments and its funnel-like opening was stretched and flattened in the dorsoventral direction in longitudinal plane with its flared lips lying in close proximity of the ovary as observed by Hodges (1974) in domestic fowl. The infundibulum showed a distinct funnel and a tubular neck. This division was in accordance with the observations made by Aitken and Johnston (1963), Hodges (1974), King (1975) and Nickel *et al.* (1977) in domestic fowl. Walls of the funnel converged rapidly to form the infundibular neck, a narrow thin-walled tube which increased in size and thickness to form the magnum.

Romanoff and Romanoff (1949) found that the magnum region in fowl had the maximum number of coils and convolutions and Hodges (1974), King and Mc Lelland (1975), Solomon (1975) and many other workers found in domestic fowl and other birds that it was the longest segment in entire oviduct.

Accordingly, in Kuttanad duck also, during the postnatal period, the magnum was found to be by far the longest and most coiled segment of the oviduct and appeared darker than rest of the oviduct with a distinct brown to dark brown shade.

Hodges (1974), King and Mc Lelland (1975), Solomon (1975) and Gilbert (1979) described the macroscopic limits of the isthmus region in the domestic fowl similar to those observed in the present study and it was evident that a narrow translucent zone, the magnum-isthmus junction separated the magnum from the isthmus region and here the colour changed from brown to off-white. It was also noticed that the isthmus region was narrower than the magnum and diameter gradually increased at the isthmo-uterine junction.

The uterus or shell gland was subdivided into a short, comparatively narrow, cranial portion and a pouch-like caudal portion in which developing egg spent much of its time during the shell formation. The existence of this pouch region was independent of the presence of the egg, and, although it was much reduced in size, it could be distinguished in the oviduct of the non-laying duck. These results are in the total agreement with those of Johnston *et al.* (1963), Solomon (1975) and Gilbert (1979) in the uterus of the domestic fowl.

As observed by Bradley and Grahame (1960), Bobr *et al.* (1964), Hodges (1974) and King and Mc Lelland (1975) in domestic fowl and other birds, it was confirmed that, in Kuttanad duck also the last segment of the oviduct, the vagina, was a relatively short, much convoluted, S-shaped condensed tube that extended from a well developed muscular sphinctor at the posterior end of the uterus and it terminally opened into the urodeum of cloaca dorsomedial to the left ureter.

5.1.5 Postnatal Developmental Pattern of the Oviduct

5.1.4.1 Beginning of Coiling of the Oviduct

From day-old upto eight weeks of age, there were no signs of the coiling of the oviduct and oviduct remained as a straight tube with collapsed lumen. From 10th week onwards, slight coiling of the oviduct began. This feature may be due to absence of glandular elements, lesser vascularity, less developed

connective tissue and thinness of wall. By 14th week, the oviduct showed much coiling and exhibited turgidity instead of collapsed nature throughout its length. Similar investigations regarding the occurrence of signs of oviduct coiling were conducted by Rao (1994) in duck and Lucy and Harshan (1999a) in Japanese quail who found that the oviduct remained as a straight, pale and flattened tube before the beginning of its rapid development.

5.1.4.2 Phase of Partial Differentiation to Phase of Complete Differentiation of the Oviduct

In the day-old duckling itself, the caudal regions of the oviduct could be differentiated into dilated uterine region and a caudal opaque white vaginal region. However, the cranial segments like infundibulum, magnum and isthmus were difficult to differentiate from gross appearance. Thus in the day-old duckling the oviduct was observed to be partially differentiated. On similar lines in the domestic fowl also, development was more rapid in the caudal regions of the oviduct than in cranial regions as documented by Khokhlov and Kuznetcov (2007). Such phase of partial differentiation was also observed in Japanese quail by Lucy and Harshan (1999a).

The phase of partial differentiation persisted until 10th week of age. From 12th week onwards, all the segments of the oviduct were distinguished including the subdivisions of infundibulum. From 18th week onwards, the oviduct was morphologically and functionally differentiated at this stage as evident from concurrent development of shell gland pouch region and occurrence of developing egg within the oviduct of a bird in this age group. All the birds started laying by 20 weeks of age. These findings were in total agreement with those of Vernerova and Burda (1984) in ducks who found that the female genitalia showed maximum development between 130 and 160 days of age. Fitzgerald (1969) and Pageaux *et al.* (1984) indicated that in the coturnix quail due to short life cycle, the functional maturity of the oviduct was attained much early by about six to seven weeks of age.

5.1.4.3 Relationship between Age and Body Weight

The mean body weight of the day-old Kuttanad duckling was 38.64 ± 0.34 g. The body weight progressively increased upto 16th week of age, before the beginning of egg-laying and the maximum mean weight of 1783.33 ± 89.85 g was attained at 18th week, when egg laying started. The body weight increased by 46 times from the day-old to 18th week of age. From 20th week onwards, the body weight showed a decreasing trend and at 24th week of age, the mean body weight was 1476.67 ± 28.05 g. The reason for this cumulative increase in the body weight followed by a phase of rapid decrease was a result of the heavy deposition of lipids in pre-laying period followed by utilization of this stored lipids with concurrent egg formation as observed by Krapu (1981) in common mallard and Tome (1984) in migratory Ruddy ducks who observed increased body weights during arrival of birds to breeding site and pre-laying period followed by a drastic decline evident between the laying and early incubation phase. From day-old to 24th week of age, body weight exhibited highly significant correlation with the age at 1% level of significance.

5.1.4.4 Relationship between Age and Weight of the Oviduct

In the day-old duckling, the oviduct weighed 0.05 ± 0.00 g and showed more than six times increase within two weeks. From 3rd week onwards, weight of the oviduct doubled at fortnightly intervals and showed only a slight increase from 8th to 10th week. At 12th week of age, when all the segments were differentiated morphologically, the mean oviduct weight increased two and half times than that of the previous age group. From 12th week to 16th week, mean oviduct weight showed a gradual increase and almost doubled by 18 weeks of age. A spurt in growth was noticed from 18th to 20th week. By this age, all the birds started laying. From this age onwards the increase in weight of the oviduct was gradual and maximum mean weight of the oviduct recorded was 51.28 ± 0.05 g at the age of 24 weeks.

It was evident in the present study that weight of the oviduct increased along with the age during the postnatal period, and thus showed highly significant

correlation with the age. This was in accordance with the observations by Vernerova and Firmanova (1985) in turkeys and hens.

5.1.4.5 Relationship between Age and Length of the Oviduct

Length of the oviduct increased about 30 times from day-old to 24th week of age. The mean length of the oviduct increased about four times in four weeks and thereafter, the increase was gradual upto 10th week. From 10th week onwards, the lengthwise growth of the oviduct was rapid upto 18th week. This was accompanied by the morphological differentiation of the oviduct segments. A spurt in growth was recorded from 18th week to 20th week, when all the birds started laying. Thereafter, the increase was gradual and maximum mean length of the oviduct was recorded at 24th week. During postnatal period, length of the oviduct showed a highly significant correlation with the age which was more than that with the weight of the oviduct and age at 1% level of significance. Similar reports in the duck are not available for comparison.

5.1.4.6 Relationship between the Body Weight and Oviductal Parameters

Oviduct contributed 0.13% of the body weight in day-old ducklings and in adult birds (at 24th week) it contributed 3.47% of the body weight. In adult Japanese quails as recorded by Lucy and Harshan (1999a), the oviduct contributed 4.05% to body weight. Weight of the oviduct, however, during postnatal period showed no significant correlation with the body weight. On the other hand, the length of oviduct showed a significant correlation with the body weight.

5.1.4.7 Relationship between the Weight and Length of the Oviduct

During the postnatal period, the weight of the oviduct showed highly significant correlation with the length of oviduct. Similar findings were also recorded by Rao (1994) in duck.

5.1.5 Segmental Variation in the Developmental Pattern of the Oviduct

5.1.5.2 Infundibulum

From day-old to 10th week of age, infundibulum was not differentiated from the magnum and isthmus regions and morphological development was negligible during this period. From 12th week onwards, the five segments of the

oviduct including the infundibulum were differentiated and the infundibulum was morphologically divisible into cranial funnel and caudal neck regions. In Japanese quail different segments of the oviduct could be distinguished from 40 days of age (Lucy and Harshan, 1999a).

Weight of the funnel region of the infundibulum was greater than that of the neck. Length of the neck region was more than that of the funnel in all age groups. The funnel was much wider than the neck throughout the postnatal period. In adult birds (at 24th week), the infundibulum contributed 11.42% of the oviduct length. Thus Kuttanad duck showed a relatively short infundibulum similar to that of chicken and turkey, (Woodard and Mather, 1964). In adult Japanese quail, infundibulum was relatively longer and contributed 17.1% of the total oviduct length (Lucy and Harshan, 1999b). From 12th week to 24th week of age, the weight, length and width of the funnel region of the infundibulum showed highly significant correlation. But no significant correlation was noticed between funnel parameters and the body weight. Similarly, the weight, length and width of the neck region of the infundibulum also showed highly significant correlation with age but not with the body weight.

Weight and length of the funnel as well as the neck regions of the infundibulum showed highly significant positive correlation with the weight and length of the oviduct. In domestic fowl, similar findings were reported by Khokhlov (2008) who speculated that regular fluctuations in weight and length of the infundibulum with respect to total weight and length of the oviduct indicated the synchronization with the functional stage of the oviduct and it was observed that during the egg-laying period the weight-wise and length-wise contribution of infundibulum was found to be more than that in non-laying period.

5.1.5.3 Magnum

Magnum was not differentiated grossly from the infundibulum and isthmus upto 10th week. Magnum was the longest and the most coiled segment of the oviduct. Similar observations were also made by Lucy and Harshan (2000) in

Japanese quail. Contribution of magnum to the total length was 38.55% at 24th week which was greater than any other segment of the oviduct. However, in the case of domestic fowl (Rao Saheb and Iyengar, 1945 and Marshall, 1961) and Japanese quail (Lucy and Harshan, 2000) magnum contributed nearly 50% of the total length of the oviduct. The overall diameter of magnum was greater than that of the neck of infundibulum and this increase was mainly due to a marked increase in the thickness of walls. Various parameters of magnum showed maximum increase between 18 and 20 weeks of age, when the egg laying started in these birds. In differentiated oviduct, the weight, length and width of the magnum region showed highly significant positive correlation with the age and weight and length of oviduct but not significant with the body weight.

5.1.5.4 Isthmus

Differentiation of the isthmus region occurred at the same time as that of more cranial segments of the oviduct. Its overall diameter was lesser when compared to that of the magnum. Maximum growth rate in all the dimensions of the isthmus region was noted between 18 and 20 weeks of age. Isthmus contributed 8.61% of the total weight of oviduct. It was 12.31 ± 0.17 cm long and was about 19.53% of the total length. Thus, similar to Japanese quail (Lucy and Harshan, 1999c) and nothura spotted quail (Moraes *et al*., 2007) the isthmus was longer in Kuttanad duck as evident in present study which indicated the more time spent by the developing egg in this segment and hence thicker shell membrane when compared to that of chicken wherein, the isthmus region was observed to be relatively short by Woodard and Mather (1964). This partially explains the longer shelf life of duck egg when compared to chicken egg. From 12th week to 24th week of age, the dimensions of isthmus region showed highly significant correlation with the age and weight and length of oviduct but, no significant correlation was found with the body weight.

5.1.5.5 Uterus (Shell Gland)

In the day-old duckling itself, the uterus was a well differentiated segment of the oviduct as reported by Lucy and Harshan (1998) in Japanese quail. The cranial part was short and tube-shaped whereas, the caudal part was a sac-like pouch region, the shell gland proper. The wall was thin when compared to that of magnum and isthmus, but was more distensible to accommodate and pass the developing egg mass to the next segment, the vagina.

Uterine weight increased about three times from 16 weeks to 18 weeks of age and six times by 20 weeks. The shell gland contributed 31.08% of the total oviduct weight at 24th week. Contribution to the total length was 14.47%. Lucy and Harshan (1998) reported that the uterus in the Japanese quail contributed 11.4% of the total length. Proportionately longer uterus in Kuttanad duck may account for the thicker shell of the duck egg. During the postnatal period, the weight, length and width of the uterus region showed highly significant correlation with the age, length and weight of the oviduct. Weight of the uterus showed no significant correlation with the body weight, whereas, length and width of the uterus showed significant correlation with the body weight.

5.1.5.6 Vagina

In the day-old duckling itself, similar to the uterus, vagina was a well differentiated segment of the oviduct. The annular rings, nearly seven to eight in number, appeared over the external surface of the vagina at about 10th week of age and thereafter, these annular rings became nearly indistinct because of the more rapid development of the connective tissue which not only covered the external surface of the vagina but also bound the shell gland and cloaca with the vagina which resulted in condensed nature of the vagina in the adult Kuttanad duck. Similar observations were made in the vagina of Japanese quail by Lucy and Harshan (1999d).

Unlike in the case of other regions weight of vagina increased about five times from 10th week to 12th week, the period during which this region was morphologically differentiating. In the remaining age groups, the growth of vagina was gradual. Lack of spurt in growth rate between 18 and 20weeks of age, in the segment, unlike in cranial regions of the oviduct may be explained by the non glandular nature of the vagina. In the glandular segments, glandular development preceded the egg-laying. Vagina contributed 17.75% of the oviduct weight at 24 weeks of age and 15.99% of the total length of oviduct. In Japanese quail, vagina contributed 5.5% of the oviduct length (Lucy and Harshan, 1999d). More length of the vagina in the duck may be in relation to the copulatory organ in drake. From day-old to 24th week of age, the weight, length and width of the vagina showed significant positive correlation with the age, body weight and length and weight of oviduct.

5.2 SEGMENTAL VARIATION IN THE DEVELOPMENTAL HISTOMORPHOLOGY OF OVIDUCT

5.2.1 Infundibulum

In the day-old ducklings, the cranial end of the undifferentiated oviduct corresponded to the infundibulum and consisted of the innermost epithelium and subepithelial tissue. The oviduct wall was not differentiated into different tunics distinctly. The lumen was flanked by characteristic low mucosal folds which were lined by simple columnar epithelium. The total number of mucosal folds was in the range of 10 to12. Caudally number of folds increased upto 16 to 18. The sub-epithelial connective tissue was made up of densely packed cells with fine collagen fibres and was rich in capillaries. Both dorsal as well as ventral ligaments were attached to the oviduct and were abundantly supplied by blood vessels. Ventral ligament showed the presence of smooth muscle. These observations are in accordance with the observations made by Lucy and Harshan (1999b) in Japanese quail.

At two weeks of age, the secondary mucosal folds started appearing in the cranial end of the undifferentiated oviduct corresponding to the infundibulum. By 6th week, a very thin tunica muscularis appeared as a single layer. At 12th week of age, tubular glands first appeared in the lamina propria of the neck region. In Japanese quail, glands could be first located at the age of 40 days (Lucy and Harshan, 1999b). At 14th week, neck region of the infundibulum showed rapid development of the mucosal folds into primary, secondary and tertiary folds with consequent increase in their height and width. Total number of mucosal folds was about 15 to 16. The mucosal folds in the funnel were shorter than that of neck region. Ciliogenesis in the infundibulum began at this age and the lining epithelium also showed presence of few non-ciliated glandular cells and occasional vacuolation. Such extensive ciliation was also noticed by Mohammadpour and Keshtmandi (2008) who speculated that, the rhythmic beating of these cilia and the gross oblique arrangement of the mucosal folds created a vortex to pull and transport the egg. Tunica muscularis was thinner than more caudal segments of the oviduct.

In the adult Kuttanad duck (20 weeks of age), there were four types of epithelial cells in the mucosa of infundibulum namely non-secretory ciliated cells located primarily in the lining epithelium; non-ciliated mucous-secreting goblet cells found in between the ciliated cells; secretory cells other than goblet cells which were located in the glandular grooves and lining cells of the tubular glands of the caudal region of the infundibulum. These results are in total agreement with those of Aitken and Johnston (1963) in domestic fowl. Lamina propria of the funnel region of the infundibulum was devoid of any glands and contained collagen fibres along with fine reticular and a few elastic fibres as reported by Lucy and Harshan (1999b) in Japanese quail.

In differentiated oviduct, the lining epithelium at the opening of the lips of the funnel was low columnar ciliated type which rapidly changed into tall ciliated columnar in the distal funnel and neck of the infundibulum. Similar observations

were also made in pigeon by Dominic (1960) and Hodges (1974) in domestic fowl.

Ciliated cells possessed oval nuclei lying at or above the centre of the cell. Goblet cells showed a basal nucleus and apical granular cytoplasm. Similar findings were also documented by Hodges (1974) and Naragude *et al.* (1999) in domestic fowl, Lucy and Harshan (1999b) in Japanese quail and Moraes *et al.* (2007) in nothura spotted quail and Das and Biswal (1968), Rao (1994) and Ozen *et al.* (2009) in duck.

The formation of tubular glands was in accordance with observations made by Bradley and Grahame (1960) in domestic fowl. In the neck of the infundibulum the more complex mucosal folds with deeper glandular grooves eventually gave rise to small tubular glands from their corners. The deeper regions of the mucosal folds where the transformation of glandular grooves into tubular glands occurred, the lining cells lacked cilia and were cuboidal with eosinophilic supranuclear cytoplasm. The proprial glands were lined by cuboidal to columnar cells with indistinct boundaries, basally located light stained round nuclei, eosinophilic supranuclear cytoplasm and enclosed a large lumen. The proprial glands in the neck of the infundibulum were large in diameter, which increased in number as well as width gradually and attained maximum width at 24^{th} week of age. In the caudal most region of the neck of the infundibulum, goblet cells increased in number in the lining epithelium.

The funnel region showed mainly primary mucosal folds with some secondary folds. The depth of longitudinally oriented low mucosal folds appeared to increase as the funnel approached the neck region whereas within the neck of the infundibulum, the spirally oriented longitudinal folds increased in depth and gave rise to numerous secondary and tertiary folds. Among different segments of the oviduct, the infundibulum showed the least height and width of mucosal folds throughout the postnatal period. Similar observations were also made by Khokhlov and Kuznetcov (2007) in the domestic fowl.

The muscular tunic of the neck region was thicker than that of the funnel and was differentiated into ill defined inner circular and outer longitudinal muscle bundles. At the infundibulum-magnum junction, both the mucosal and muscular layers increased in thickness and structural complexity. These results are in total agreement with those of Hodges (1974) and Gilbert (1979) in domestic fowl and Lucy and Harshan (1999b) in Japanese quail. The collagenous serosal tunic was found to be thin with folds, blood vessels and nervous plexuses. Distinct serosal tunic was visible only in adult birds.

In adult birds, analysis of the wall of the infundibulum revealed that tunica mucosa occupied the maximum thickness (81%) followed by the tunica muscularis (16%) and tunica serosa (3%). In domestic fowl, as reported by Khokhlov (2008), similar relationship between percentage contribution of different tunics was observed except the fact that tunica mucosa was comparatively thinner (64%).

5.2.2 Infundibulum-Magnum Junction

Short mucosal folds which characterize the infundibulum increased in height and were more longitudinally oriented as they approached the magnum region. Similar observations were also made by Bakst and Howarth (1975) on the mucosal surface of the oviduct of *Gallus domesticus.*

In the adult Kuttanad duck, the branched tubular glands which were present in the tubular part of the infundibulum were more prominent in the region adjoining the magnum. At about 20 weeks of age, it was observed that, the eosinophilic supranuclear secretory granules of the infundibular glandular epithelium were invariably smaller, less numerous and more denser than those in the tubular glands of the magnum. At the infundibulum-magnum junction, group of infundibular glands intermingled with tubular glands of the magnum, but the epithelial lining of the two types of glands were always distinct. The presence of such admixture of both the type of glands at the infundibulum-magnum junction was also recorded by Aitken (1971) in domestic fowl.

5.2.3 Magnum

From day-old to four weeks of age, the magnum region was histologically similar to the infundibulum except in that the more number of mucosal folds were seen in magnum. At 4th week, secondary mucosal folds started appearing and total number of primary folds was in the range of 18 to 20. Thereafter the height and width of mucosal folds increased gradually. At 10th week (i.e. two weeks prior to external differentiation of oviduct into segments), each magnal fold was wide in nature with slightly narrow base lined by simple columnar epithelium. Proprial glands were not developed. At the region where magnum was supported by dorsal and ventral ligaments, the mucosal folds appeared higher comparatively. Similar reports are not available in the duck for comparison.

The most dramatic growth and differentiation of the magnum began at the age of 12 weeks when all the segments of the oviduct were differentiated morphologically. Lining epithelium of magnum started infolding and consequently the tubular glands appeared as invaginations of this surface epithelium. At this age, glands did not extend to the deeper parts of the lamina propria, which were made up of loosely arranged connective tissue with many blood vessels. At this age, the mucosal folds became broader and higher comparatively with the total number of folds ranging from 20 to 22 with lesser secondary folds. It was observed that, development of glandular layer although increased the size of the mucosal folds, tended to decrease the degree of secondary folding. These conclusions were in accordance with the findings made by Hodges (1974) in domestic fowl. In guinea fowl, however, the secondary mucosal folds were well developed forming many large duct-like structures in the lamina propria as reported by Yoshimura and Ogawa (1998). Ciliogenesis was observed almost at the same time of glandulogenesis. Similar phenomenon was documented in the morphogenesis of magnum by Pageaux *et al.* (1986) in Japanese quail. Tunica muscularis consisted of circularly arranged muscle fibres and was very thin. Tunica serosa was made up of loose connective tissue and was richly supplied by large blood vessels and nerves. Thereafter, the glandular

development continued and glands extended towards the core of the lamina propria.

At 18th week, with the beginning of egg-laying in a bird of this group, the blood capillary network which was limited to the core region of the lamina propria extended towards the margins of mucosal folds and was observed just beneath the basement membrane of the lining epithelium. Increased vascularity ensured the developmental requirements. These observations were in total agreement with those recorded by Rao (1994) in duck. At this age, secretory end piece of each tubular gland in the cross section was lined by 9 to 10 tall columnar cells with eosinophilic supranuclear cytoplasm.

In the adult Kuttanad duck, the overall diameter of the magnum was considerably greater than that of the infundibular neck. This was partly as a result of an increase in the development of muscle layers but was mainly due to the increased thickness of mucosa. Similar observations were reported by Hodges (1974) in the case of domestic fowl.

At 20th week, mucosal folds became wider and higher than the previous age groups and were lined by monolayered simple ciliated columnar epithelium with a few goblet cells. Even in H&E staining, the epithelium had a peculiar grayish, dark appearance unlike previous groups. The lamina propria was filled with tubular glands lined by cuboidal to columnar epithelium with deeply eosinophilic cytoplasm and the lumen of the glands was difficult to identify. Glands were arranged in radiating manner from the core to the periphery of the fold. Tunica muscularis was thin and made up of inner circular and outer very thin longitudinal muscle layers. The connective tissue core of each mucosal fold was also very thin from which connective tissue fibres were extending towards the periphery. In the caudal most region of the magnum, the surface epithelium became taller and contained very large number of goblet cells. Due to the abundance of goblet cells, this area was designated as ‘mucous region’ by Richardson (1935) in the last three to four centimetres of the magnum in the domestic fowl.

At 24th week, the lining epithelium, at and above the base of the mucosal fold showed peculiar invagination. Before the passage of yolk through the magnum the goblet cells predominated the surface epithelium, distended with secretory granules whereas, ciliated cells appeared as narrow structures; but once the secretory material was discharged, the goblet cells became slender and were compressed by the ciliated cells and thus, the surface epithelium exhibited extensive ciliation. Such ciliation was also noticed in domestic fowl by Hodges (1974).

5.2.4 Magnum-Isthmus Junction

At 12th week of age also, this region was distinctly identifiable. At the posterior end of the magnum, the longitudinal mucosal folds became reduced in height and each fold became thinner with the concurrent development of more secondary folds. Similar findings were also reported by Hodges (1974) in domestic fowl, Rao (1994) in duck, Lucy and Harshan (2000) in Japanese quail and Moraes *et al.* (2007) in nothura spotted quail. It was also observed that almost immediately, however, the mucosal folds increased in height again as they entered the isthmus proper but did not regain the height or width found in the magnum.

The most noteworthy feature of this region was the complete absence of tubular glands with subsequent occupation of the core of lamina propria by relatively dense connective tissue Lucy and Harshan (1999c) in Japanese quail. Thickness of tunica muscularis gradually increased from magnum to isthmus region with development of thicker inner circular layer.

5.2.5 Isthmus

The overall diameter of the isthmus was lesser than that of the magnum. Similar observation was also recorded by Romanoff and Romanoff (1949) in domestic fowl. In undifferentiated oviduct, the isthmus region was similar to the magnum without any secondary folds. Tunica muscularis was present as a

continuous layer at two weeks of age. At about 4th week, the primary folds attained angular shape characteristic to the isthmus and the secondary folds started appearing. At this age total number of mucosal folds was 9 to 10. Thereafter upto 8th week of age, all the tunics remained unchanged. At eight weeks of age, tunica muscularis was differentiated into inner circular and outer longitudinal layers. Similar findings regarding such differentiation of muscular tunic were also documented by Sharma and Duda (1992) in duck.

At 12th week, the glands started developing from the surface epithelium. The glands filled the lamina propria and compared to magnum glands, they extended to deeper regions of the lamina propria. The core of the mucosal fold showed numerous blood vessels. The lamina propria blended with the submucosa and no muscularis mucosa was present. Muscularis mucosa was absent throughout the oviduct as reported by Bradley and Grahame (1960) in domestic fowl. Tunica muscularis consisted of inner circular and outer longitudinal muscle layers separated by loose connective tissue rich in blood vessels. Tunica serosa was made up of loose connective tissue with blood vessels and nerves.

At 14th week, the glandular development continued and each mucosal fold was filled completely by tubular glands and was left with a very thin core of lamina propria. Glands were vacuolated. Cross sectional profile of tubular glands showed lining epithelium as cuboidal to columnar and enclosed a distinct lumen. Thereafter, in succeeding groups, the glandular development continued and simultaneously ciliogenesis followed. Vacuolation of the glandular epithelium decreased. Similar reports are not available in duck for comparison

In the adult Kuttanad duck, compared to all other segments of the oviduct, the glands in the isthmus were more loosely arranged. Each secretory end piece was lined by pyramidal cuboidal cells with a large round nucleus towards the base of the cell and supranuclear deeply eosinophilic cytoplasm. Cross section of each secretory end piece was lined by six to eight cells. In duck, similar observations were also made by Sharma and Duda (1992). The diameter of the tubular glands

gradually decreased in the caudal portion of the isthmus and possessed wider lumen.

Tubular glands within the mucosal folds were similar to those of the magnum, but were not so well developed. This was in total agreement with findings made by (Hodges, 1974) in domestic fowl. As observed by Mehta *et al.* (2005), in the present study as well, the secretory granules in the cells of isthmian glands were fine and smaller than magnal glands.

Each tubular gland showed transformation of cuboidal cells with centrally placed nuclei and sparse cytoplasm in non-secretory phase into pyriform cells with basal nuclei filled with coarse secretory eosinophilic granules in the secretory phase. In duck, such transformation of tubular gland epithelium was also observed by Sharma and Duda (1992).

Compared to cranial portion height of the epithelium gradually increased in the more caudal regions of the isthmus. Lining epithelium of the isthmus contained both ciliated and secretory non-ciliated columnar cells. Regular alteration of these cells was noticed and these cells were having approximately the same width. Such regular alterations of cells were also recorded in the domestic fowl by Hodges (1974).

There were two types of non-ciliated glandular cells in the surface epithelium along with ciliated cells. Some non-ciliated cells were having more eosinophilic cytoplasm and darkly stained nuclei while, the other type was lightly eosinophilic with large lightly stained nuclei. Occasionally certain surface epithelial cells of the isthmus showed vertically elongated darkly stained nuclei. The ciliated cells of the surface epithelium retained their columnar shape with an apical nucleus throughout the secretory activity, being unconstricted by the adjacent glandular cells. When the isthmus was secreting the shell membranes, the gland epithelium showed maximum activity and each cell became full of spherical, deeply eosinophilic granules. The secretory material in their lumina was either of similar granules or fused secretory masses which after passing out of the tubular glands

frequently took the form of threads or twisted strands. These are in accordance with the observations made by Hodges (1974) in domestic fowl.

The mucosal folds of the isthmus region were characteristically angular in the appearance and the apertures of the tubular glands were situated in the depressions of the surface epithelium, which were much more numerous than in any other part of the oviduct. Presence of angular mucosal folds, in the isthmus region of the Japanese quail was reported by Lucy and Harshan (1999c). During the development only the tunica serosa remained unchanged while the tunica mucosa and tunica muscularis exhibited significant increase in thickness.

5.2.6 Isthmus – Uterus Junction

In differentiated oviduct isthmus - uterine junction showed no abrupt change in gland architecture between the adjacent regions, but an area of intermingling of glands could be observed within the lamina propria. Hodges (1974) in domestic fowl and Rao (1994) in duck were able to locate similar area showing admixture of two types of proprial glands at isthmus - uterine junction.

In adult birds, the isthmus- uterine junction also showed some sparsely distributed glands which were different when compared to either the isthmus or the shell gland proper. These glands had much smaller and less numerous secretory granules than those of the isthmus and cytoplasm was frequently vacuolated. These are in accordance with the observations made by Richardson (1935) and Johnston *et al.* (1963) in domestic fowl.

5.2.7 Uterus or shell gland

In the day-old duckling itself, towards the caudal portion of the oviduct, shell gland region was observed as dilated portion. The mucosal folds were higher than more cranial regions of undifferentiated oviduct and were separated by deep furrows as observed by Lucy and Harshan (1998) in Japanese quail. Most of the mucosal folds were cuboidal with equal height and width. By two weeks, height of the mucosal folds increased and a few secondary folds also

started to develop. One of the noteworthy features was the appearance of smooth muscle towards the periphery of uterine wall indicating the developing tunica muscularis. The lining epithelium was simple columnar with darkly stained nuclei.

At 4th week of age, height of mucosal folds increased and more secondary folds started appearing. In terminal portion of uterus, height of the mucosal folds decreased and the wall became thicker with better developed tunica muscularis. Two types of cells, light cells and dark cells were visible in lamina epithelialis. These findings were in total agreement with observations made by Lucy and Harshan (1998) in Japanese quail. Lamina propria was made up of loose connective tissue and was devoid of any glands at this stage. Tunica muscularis was thin and a fine network of collagen fibres could be observed in between the bundles of muscle fibres. Ventral ligament was well developed with numerous blood vessels and nerve bundles and showed a continuous attachment of uterine region with the vagina indicating a close association between the two segments even at an early stage.

At 12th week, in the initial portions of the uterus, height of the mucosal folds was comparatively less. At this age, surface epithelium showed invagination to form tubular glands which started developing as a continuous layer just beneath the lining epithelium similar to the magnum and showed some degree of vacuolation. Lamina propria was similar to that of isthmus but glands did not extend to deeper portions of the lamina propria and were smaller than isthmian glands. The inner circular layer of tunica muscularis was thicker than outer longitudinal layer at this age. Similar reports are not available for comparison.

At 14th week, the folds increased in height and were comparatively thinner and were spatula shaped. Ciliogenesis was in progress and appearance of cilia was more evident in apical regions of the mucosal folds. Lamina propria was completely filled with tubular glands and central core showed numerous capillaries for meeting extra demands for glandulogenesis. Glands towards the

base of the mucosal folds showed vacuolated cytoplasm. However, no vacuolation was evident at the apical region or towards the central region of the mucosal folds. Each secretory end piece was lined by five to six cells with their nuclei arranged towards the apical portion and cytoplasm appeared eosinophilic. The basal portion of cells showed vacuolated appearance.

At 16th week, glandular development continued and towards the apex of uterine folds glandular end pieces were more distinct. Similar to previous group, basal portion of mucosal fold showed vacuolated glandular end pieces. Tubular glands were closely packed in the mucosal folds and were empty during non laying phase. But, prior to and during secretory phase, cells contained pale-staining granules and after the shell formation, the cytoplasm was found to be markedly vacuolated, with relatively large nucleus located towards the base of each cell.

By 18 weeks, mucosal folds acquired their characteristic leaf-like appearance and lamina propria was loosely filled with glands with loose arrangement at the central core. Similar observations were also documented by Hodges (1974) who observed that in the cross section, the mucosal folds were of lower height than those of the magnum and were less distended by the glandular layer such that some secondary folding was visible. Instead of the usual continuous mucosal folds, the shell gland mucosa was thrown into longitudinally numerous flat, discontinuous, leaf-shaped folds. These results are in accordance with those documented by Mohammadpour (2007) who found that height and number of the primary mucosal folds were greater in duck than in hen but secondary mucosal folds were larger in the case of hen. In the present study, towards the base of the fold, the vacuolated appearance of glands was reduced and glands had a uniform appearance throughout. In guinea fowl and domestic fowl, however, as reported by Yoshimura and Ogawa (1998), the distribution of the tubular glands in the lamina propria was not uniform and glands were arranged more densely at the bottom region of the mucosal folds than at the apical region. Uterine glands were similar to isthmian glands, but were more closely packed and the lining

epithelium was cuboidal to columnar with more closely packed cells having basally placed nuclei and eosinophilic cytoplasm.

In adult laying ducks, tunica muscularis was very thick with an inner circular and thicker outer longitudinal layers separated by loose connective tissue showing numerous vessels and nerves as noticed by Lucy and Harshan (1998) in Japanese quail. The most important feature was that at this age, smooth muscle fibres were observed to be extending to the core of the mucosal fold along with numerous collagen fibres. Clear cells were also identified in the lining epithelium.

The surface epithelium of the uterus showed a single layer of columnar cells with alternating apical and basal nuclei. Accordingly these were named as apical and basal cells, with the apical cells bearing cilia and the basal cells having restricted apical surface. Similar observations were also made by Madekurozwa (2007) in ostrich.

Secretory granules were a constant feature of the supranuclear cytoplasm of the apical cells. These granules were actively secreted during the earlier phases of shell formation and during the latter half of shell secretion many secretory granules migrated to supranuclear cytoplasm. Similar observations were also recorded by Breen and Bruyn (1969) and Hodges (1974) in fowl, who suggested that the secretory granules were subjected to a process of disintegration in supranuclear cytoplasm with concurrent formation of a large membraneless space, which was termed as vacuoloid, which subsequently regressed. Non-ciliated glandular cells of the shell gland contained more vacuoles which regressed during the onset of egg formation.

In the uterus containing the egg mass, the region opposing the surface of the developing egg showed flattened mucosal folds. In the surface epithelium, apical portion of the cells were denuded as part of secretory process and thus in some cells only basal half could be seen. Secretory granules towards the lumen could also be located in the lumen. Apart from usual round to elliptical secretory end pieces of the tubular glands, some uterine glands were also in the shape of ‘U’ or

'Y' and their nuclei were seen projecting from basal portion of cells. Similar reports are not available in duck for comparison.

5.2.8 Utero-vaginal junction

The microscopic structure of sperm storage tubules was similar to that described by several workers as like Fujii (1963), Bobr *et al.* (1964), Schindler *et al.* (1967), Vankrey *et al.* (1967), Gilbert *et al.* (1968), Burke (1972) and Tingari and Lake (1973) in domestic fowl, Vankrey *et al*. (1974), Schuppin *et al.* (1984), Bakst and Richards (1985), Bakst (1992), Bakst (1994), Bakst and Akuffo (2008), Bakst and Akuffo (2009) and Miranda *et al.* (2009) in the turkey, Bakst and Bird (1987) in American kestrel, Pal (1977) and Rao (1994) in the domestic duck and Renden *et al*. (1981) and Lucy and Harshan (1999e) in the Japanese quail.

At utero-vaginal junction the sperm storage tubules appeared at 12 weeks of age in between two muscular ridges, cranial and caudal utero-vaginal ridges. Similar observations were also made by Rao and Vijayaragavan (2000) in duck. The mucosal folds were low and lined by tall ciliated columnar cells with the apical nuclei alternating with mucous secreting goblet cells containing basal nuclei. The lamina propria of the mucosa housed numerous tubular glands called the sperm storage tubules which were responsible for sperm storage after copulation.

There was no evidence of smooth muscle fibres or other contractile elements associated with sperm host glands and these were surrounded by connective tissue elements only. These findings are in total agreement with those made by Tingari and Lake (1973).

At 12^{th} week and in all the succeeding age groups, mucosal folds towards the uterine side of the cranial utero-vaginal ridge contained transitional glands intermediate in appearance between sperm storage tubules and uterine glands as reported by Rao and Vijayaragavan (2000) in duck. The transitional glands were

more densely packed compared to sperm host glands and were lined by large cells with large oval nucleus at the base and enclosed a narrow lumen.

In the present study, as per the observations made by, Gilbert *et al.* (1968) the sperm storage tubules were lined by simple cuboidal to low columnar cells with centrally placed nuclei and at 14th week of age, these glands could be seen as small invaginations of surface epithelium lined by tall columnar cells with round nuclei placed in the basal halves of cells. These sperm storage tubules were more sparsely distributed and less convoluted than the uterine glands and transitional glands but had larger diameter than both the gland types. Similar observations were also recorded by Rao and VIjayaragavan (2000) in duck.

With the beginning of egg-laying phase, at about 18th week of age, sperm storage tubules also exhibited rapid phase of development and more number of cross sectional profiles of the glands were visible indicative of increased number of sperm storage tubules. The number of transitional glands was also increased. In adult laying Kuttanad ducks, the sperm storage tubules were lined by tall columnar cells with eosinophilic cytoplasm and basally placed nuclei. In the reproductive phase, the total number and inner and outer diameters of sperm storage tubules were increased when compared to pre-laying phase. In the turkey similar observations were recorded by Miranda *et al.* (2009).

5.2.9 Vagina

In the day-old duckling itself, the vagina, the caudal most portion of the oviduct was observed to be morphologically distinct. At two weeks of age, the vagina showed roughly triangular seven to eight primary folds lined by tall columnar cells interspersed with numerous goblet cells. Even at this age, tunica muscularis was well developed and was differentiated into moderately developed outer longitudinal layer and well developed inner circular layer. Vagina showed the thickest tunica muscularis among different regions of oviduct. These results are in total agreement with findings made by King and Mc Lelland (1975) in domestic fowl and Lucy and Harshan (1999d) in Japanese quail. Connective

tissue surrounding the vagina was very well developed and was responsible for highly condensed nature of vagina. In the domestic fowl also, this connective tissue was well developed as observed by Fujii (1963). Caudal portions of the vagina showed low mucosal folds compared to cranial region in four weeks-old birds. Tunica serosa was also well demarcated.

By 10 weeks of age, the number of goblet cells reduced and typical secondary mucosal folds started appearing. Cilia also appeared in the lining epithelium. At 12th week, folds were characteristically filiform or narrow and pointed in appearance with a few secondary folds. The lamina propria was devoid of any glands. Absence of glands in the vaginal region was as reported by Lucy and Harshan (1999d) in Japanese quail. At 18th week, mucosal folds were taller but the height of the epithelium reduced considerably and in between the goblet cells and ciliated cells, some clear cells were also identified. The core of mucosal fold consisted of bundles of collagen fibres with fibrocytes in between.

In adult laying birds, lining epithelium of vagina showed large number of goblet cells and the ciliated cells occurred only rarely. The height of the epithelium over the crest of the vaginal folds was greater than that of the shell gland as observed by Richardson (1935) in domestic fowl. However, in the depressions between the folds, the non-ciliated goblet cells showed predominance and were comparatively shorter. Similar observation was also documented by Fujii (1963) in *Gallus domesticus*.

The most peculiar feature at this age was extension of muscle fibres from inner circular layer into the core of the mucosal fold as in the case of uterine region.

5.2.10 The Immune Status of the Oviduct During Postnatal Development

As reported by Biswal (1954), Trautmann and Fiebiger (1957), Bradley and Grahame (1960) and Kimijima (1989) in domestic fowl, Rao (1994) in duck, Lucy and Harshan (1999d) and Parida *et al.* (2000) in the Japanese quail, Moraes *et al.*

(2007) in nothura spotted quail and Mohammadpour and Keshtmandi (2008) in turkey and pigeon, lymphocytes were identified in almost all the segments of the oviduct in present study either in diffused form or in aggregates along with many plasma cells. Contrary to this, Das and Biswal (1968) could not locate lymphocytes in the oviduct of duck.

Lamina propria of mucosal folds of the vagina showed large number of lymphocytes along with diffused blood cells from 4 weeks-old birds. At 16th week of age, core of the mucosal folds of uterus showed more eosinophilic areas along with blood cell infiltration. At 18th week of age, the connective tissue core of the magnum showed infiltration of blood cells including lymphocytes and heterophils. The uterine region showed large immature lymphocytes at the base of the mucosal folds. Lamina propria of the utero-vaginal junction also showed accumulation of lymphocytes. At 20th week, lamina propria of the neck region of the infundibulum and magnum-isthmus junction showed loose aggregates of lymphocytes. At 22nd week, loose aggregations of lymphocytes were also evident in the lamina propria of the funnel region of the infundibulum, infundibulum-magnum junction and isthmus region of the oviduct where lymphocytes were also observed in the tunica muscularis. Appearance of lymphatic tissue in the oviduct occurred in a sequential pattern from the caudal regions to the cranial segments indicating the pattern of exposure to antigens from the cloacal end to more anterior regions

The loose aggregations of lymphocytes in the lamina propria were a common feature in the vaginal region in adult egg-laying Kuttanad duck. The presence of these aggregates in lamina propria of vagina was beneficial for strengthening immunity against large array of antigens which are well expected because of the obligatory structural association of vagina with cloaca. The role of presence of such aggregates of lymphocytes may be supportive to physiologically hygienic process of egg laying with special reference to Kuttanad ducks with well known high immune status.

According to Khan *et al.* (1996) in domestic fowl, the vaginal part showed relatively higher frequency of T-cell subpopulations than in other parts of the oviduct. The lymphocytic aggregates which were identified in vaginal lamina propria throughout the postnatal period should be subjected to immunohistochemistry and other labeling techniques to ascertain their identity as either T or B lymphocytes.

5.2.11 General Histomorphology and Developmental Changes in the Oviduct

During the postnatal development of the oviduct it was observed that in all age groups the wall of the oviduct was made up of seven layers viz., tunica serosa an outer covering of peritoneum made of connective tissue and mesothelium, an outer longitudinal smooth muscle layer covering an inner circular smooth muscle layer with an intervening connective tissue layer rich blood vessels and nerves were present, a layer of connective tissue internal to the circular muscle layer followed by the mucosa consisting of a lamina propria containing glands in most regions of the oviduct and an inner epithelial lining. Similar pattern was also described by Hodges (1974) in the case of domestic fowl.

The oviduct of Kuttanad duck throughout the developmental period was devoid of any muscularis mucosa. Similar observation was also recorded by Bradley and Grahame (1960) in domestic fowl and Rao (1994) in duck.

The mucosal folds were seen more or less continuous throughout the oviduct, although they varied in height and thickness. Similar conclusions were also derived by King and Mc Lelland (1975) who ascribed the function of upstream transport of spermatozoa and downstream transport of the egg to the peristaltic activities of tunica muscularis in the wall of the oviduct.

Similar to adult Muscovy duck (Evencioneto *et al.,* 1997) in the adult Kuttanad duck, it was found that in the laying period, the mucosal folds and its lining epithelium were much more developed.

In the case of immature ducks, the ciliated and secretory cells lining the epithelium and the proprial glands were not fully developed and during this pre-laying period ciliated cells showed a great predominance. These observations are in accordance with those of Bakst and Howarth (1975) in domestic fowl. While, similar to adult Muscovy duck (Evencioneto *et al.,* 1997), the mucosal folds and its lining epithelium in the adult Kuttanad duck in the laying period, were much more developed, with a certain predominance of non-ciliated cells.

The cellular architecture of the monolayered ciliated and secretory cells which formed the lamina epithelialis, particularly in the regions of uterus and vagina was responsible for creating a false impression of cellular stratification or pseudostratification, because of the differential positioning of the nuclei of these cells.

The glandulogenesis started in the developing oviduct of Kuttanad duck at about 12^{th} week of age when the oviduct weighed around 5.59 ± 0.02 g. Glandulogenesis and ciliogenesis occurred almost at the same time in almost all the segments of the oviduct of Kuttanad duck during the postnatal period. Vagina had the thickest tunica muscularis while the infundibulum had the thinnest.

Bundles of collagen fibres were observed between the muscle cells in almost all regions. In the case of quail, however, Arjaama and Talo (1983) were able to observe bundles of collagen between the muscle cells in the magnum and to a lesser extent in the isthmus. Similar to adult Japanese quail (Geetha *et al.,* 1992) elastic and reticular fibres were also present in the lamina propria of all the regions of the adult Kuttanad duck oviduct.

5.3 HISTOCHEMISTRY

5.3.1 Mucopolysaccharides

The goblet cells found throughout the oviduct showed intense P.A.S. positive reaction. This is in total agreement with findings made by Aitken and Johnston, (1963) in fowl, Pal (1977) and Rao (1994) in duck, Renden *et al.* (1981)

and Lucy and Harshan (1998) in quail and Madekurozwa (2007) in immature ostriches. In the magnum, lining epithelium consisted of an acidic mucopolysaccharide. Similar observation was also made by Aitken (1971) and Draper *et al*. (1972).

P.A.S. and Alcian blue (pH 2.5) method revealed the presence of neutral mucopolysaccharides and sulphur-containing proteins in the lamina epithelialis of isthmus region. The proprial glands of the isthmus also showed positive reaction for P.A.S. and Alcian blue (pH 2.5) method with a peculiar pattern i.e., the glands positioned near the epithelium stained lightly and those situated more deeply were observed to stain darker. Immature ducks, however, showed a very weak reaction. The presence of neutral and acid mucopolysaccharides was identified using P.A.S. and Alcian blue (pH 2.5) method in the epithelium of isthmus region; while in magnum, only the acid mucopolysaccharides were seen. Vaginal region gave a negative reaction for neutral as well as acid mucopolysaccharides. These observations are in accordance with the observations made by Ozen *et al.* (2009) in Pekin ducks.

5.3.2 Lipids

Considerable amount of lipid was identified in the lining epithelium of sperm storage tubules and transitional glands. Similar observations are also recorded by Fujii, (1963), Gilbert *et al*. (1968) and Tingari and Lake (1973) in birds, Pal (1977) and Rao (1994) in ducks, Schuppin *et al*. (1984) in turkeys and Lucy and Harshan (1998) in Japanese quail. The significance of lipids in maintaining sperms was suggested by the absence of lipid containing vacuoles from the utero-vaginal gland cells of infertile turkeys (Gilbert *et al*., 1968).

5.3.3 Glycogen

Amongst various segments of the oviduct in Kuttanad duck, the presence of glycogen was observed only in the epithelial lining and tubular glands of isthmus region and was more pronounced in adult egg laying ducks wherein,

actively secreting glands showed more intense reaction. These are in accordance with observations made by Lucy and Harshan (1999b) and Ozen *et al.* (2009) in the oviduct of Japanese quail and Pekin ducks, respectively

5.3.4 Alkaline Phosphatase (ALP)

Activity of the enzyme was appreciable in tubular glands of the various regions of the oviduct. Marked alkaline phosphatase activity was seen in the uterus, isthmus and magnum. The infundibular and vaginal epithelium on the other hand, showed only a mild positive reaction for alkaline phosphatase. The lining epithelium of sperm storage tubules also showed alkaline phosphatase activity. Similar observations were also recorded by Lucy and Harshan (1999b) in Japanese quail and Bakst and Akuffo (2007) in turkey.

5.3.5 Acid Phosphatase (ACP)

The activity of Acid phosphatase was observed in the epithelium and tubular glands of isthmus, shell gland and vagina. These results are in total agreement with Fujii, (1963), Gilbert *et al*. (1968) and Tingari and Lake (1973) in birds, Pal (1977) and Rao (1994) in ducks, Schuppin *et al*. (1984) in turkeys and Lucy and Harshan (1998) in Japanese quail.

Summary

6. SUMMARY

Postnatal development of the oviduct in the Kuttanad duck was studied using 78 ducklings from day-old to 24 weeks of age. The material was collected from six birds in each group at fortnightly intervals. In the day-old duckling, the left oviduct could be seen as a thin, thread-like, translucent, straight tube towards the left side of the coelomic cavity supported by the dorsal and ventral ligaments. The infundibulum, magnum and isthmus regions were not differentiated, but the uterus and vagina were recognizable with an overall increase in the total diameter of uterine region. All the five segments of the oviduct namely, infundibulum, magnum, isthmus, uterus and vagina could be clearly distinguished from 12th week onwards. Among the 78 birds, persistent right oviduct could be detected in 20 birds.

Signs of coiling of the oviduct were evident from 10th week onwards. In the adult bird, the oviduct was highly convoluted and occupied the left half of the coelom almost completely. However, the oviduct containing a developing egg extended towards the right side also and displaced the intestines in right ventral direction.

The body weight of the birds increased progressively up to 16th week of age, before the beginning of egg-laying and the maximum weight was attained at 18th week, thereafter, the body weight showed a decreasing trend until 24th week. In the initial stages, the increase in weight, length and width of the oviduct was in accordance with the growth of the bird. At 12th week of age, when all the segments were differentiated morphologically, the mean oviduct weight increased two and half times than that of the previous group. Then it showed a gradual increase up to 16th week and the oviduct weight almost doubled by 18 weeks of age. A spurt in growth was noticed between 18 and 20 weeks when all the birds started laying. The contribution of oviduct to the body weight was 3.47% at 24th week of age. During postnatal period, correlation between the length of the oviduct and age was more significant than with the oviduct weight and age. Weight of the oviduct showed no significant correlation with the body weight. On

the other hand, the body weight showed a significant correlation with the length of oviduct.

At 12th week of age, the infundibulum was divided into funnel and neck regions. The thin walled funnel was flattened dorsoventrally and its flared lips were in close proximity to the ovary in adult birds. Magnum was the longest and the most coiled segment of the oviduct and was wider than the neck of infundibulum. Contribution of magnum to the total length of oviduct was 38.55% at 24th week. Junction between magnum and isthmus was marked by a narrow translucent zone. Isthmus was narrow and contributed 19.53% of the total length at 24th week. The cranial part of the uterus was short and tube-shaped whereas, the caudal part was a sac-like pouch region. Contribution of uterus to the total length was 14.47% at 24 weeks of age. Rapid increase in the parameters of infundibulum, magnum, isthmus and uterus was observed between 18 and 20 weeks of age, whereas weight of vagina increased about five times from 10th week to 12th week. Vagina contributed 15.99% of the total length of oviduct at 24th week and it opened posteriorly into the urodeum of cloaca.

In the day-old duckling, the cranial regions of the oviduct corresponding to the infundibulum, magnum and isthmus showed innermost simple columnar epithelium and subepithelial tissue. The oviduct wall was not differentiated into different tunics distinctly. The lumen was flanked by characteristic low mucosal folds. Both dorsal as well as ventral ligaments were attached to the oviduct and were abundantly supplied by blood vessels. At two weeks of age, the secondary mucosal folds started appearing in the future infundibular region. By 6th week, a very thin single layer of tunica muscularis appeared. At 12th week of age, tubular glands first appeared in the lamina propria of the neck region but were absent in the funnel portion. At 14th week, primary, secondary and tertiary folds developed in the neck region. The funnel region showed mainly low primary mucosal folds with a few secondary folds. In the adult birds, there were four types of epithelial cells in the mucosa namely non-secretory ciliated cells; non-ciliated mucous-secreting goblet cells; secretory cells other than goblet cells and lining cells of the

tubular glands. In the neck of the infundibulum, passing caudally, the mucosal folds became more complex with deeper glandular grooves which eventually gave rise to small tubular glands from their corners. In the caudal most region of the neck of the infundibulum, goblet cells increased in number in the lining epithelium. At the infundibulum-magnum junction, both the mucosal and muscular layers increased in thickness and structural complexity. Here groups of infundibular glands intermingled with tubular glands of the magnum, but the epithelial lining of the two types of glands were always identifiable. Distinct serosal tunic was visible only in adult birds.

From day-old to four weeks of age, the magnum region was histologically similar to the infundibulum except for the more number of mucosal folds. Thereafter, the height and width of mucosal folds increased gradually. At 10^{th} week, each magnal fold was wide with slightly narrow base. The most dramatic growth and differentiation of the magnum began at the age of 12 weeks when all the segments of the oviduct were differentiated morphologically. The tubular glands appeared as invaginations of surface epithelium but, did not extend to the deeper parts of the lamina propria and the mucosal folds became comparatively higher and broader. Thereafter, the glandular development continued and glands extended towards the core of the lamina propria.

At 18^{th} week, with the beginning of egg-laying in a few birds, the blood capillary network which was limited to the core region of the lamina propria extended towards the margins of mucosal folds and was observed just beneath the lining epithelium. At this age, secretory end piece of each tubular gland in the cross section was lined by nine to ten tall columnar cells with eosinophilic supranuclear cytoplasm. At 20^{th} week, mucosal folds became wider and higher than the previous age groups and were lined by monolayered simple columnar ciliated epithelium with a few goblet cells. The glands of lamina propria were arranged in a radiating manner from the core to the periphery. Thin tunica muscularis was made up of inner circular and outer very thin longitudinal muscle layers. In the caudal most region of the magnum, the surface epithelium became

taller and contained large number of goblet cells. Before the passage of yolk through the magnum the goblet cells distended with secretory granules dominated the surface epithelium whereas, ciliated cells appeared as narrow structures; but once the secretory material was discharged, the goblet cells appeared to be obscured by the ciliated cells and the surface showed ciliated appearance.

Magnum-isthmus junction was histologically distinct even at 12th week of age and was characterized by complete absence of tubular glands. In the undifferentiated oviduct, the isthmus region was similar to the magnum without any secondary folds. Tunica muscularis was present as a continuous layer at two weeks. At about 4th week, the primary folds attained characteristic angular shape and the secondary folds started appearing. Thereafter upto 8th week of age, all the tunics remained unchanged. At eight weeks of age, tunica muscularis was differentiated into inner circular and outer longitudinal layers. By 12 weeks, the glands appeared in the lamina propria and compared to magnum glands, they extended to deeper regions of the lamina propria. The core of the mucosal fold showed numerous blood vessels. The lamina propria blended with the submucosa and no muscularis mucosa was absent. At 14th week, the glandular development continued and each mucosal fold was filled completely by tubular glands and was left with a very thin core of lamina propria. In the adult birds, compared to all other segments of the oviduct, the glands in the isthmus were more loosely arranged. Each secretory end piece was lined by six to eight pyramidal cuboidal cells with a large round nucleus towards the base of the cell and supranuclear deeply eosinophilic cytoplasm. The secretory granules were fine and smaller than those of magnum. Regular alteration of both ciliated and secretory non-ciliated columnar cells was noticed in the lining epithelium. The apertures of the tubular glands were situated in the depressions of the surface epithelium, which were much more visible than in any other part of the oviduct. In differentiated oviduct, isthmus - uterine junction showed no abrupt change in gland architecture, but an area of intermingling of glands could be observed within the lamina propria.

In the day-old duckling itself, the shell gland region was observed as a dilated portion. The mucosal folds were higher, cuboidal and were separated by deep furrows. By two weeks, height of the mucosal folds increased and secondary folds started to develop. The lining epithelium was simple columnar with darkly stained nuclei. At 4th week of age, height of mucosal folds increased but in the terminal portion, height of the mucosal folds was less and tunica muscularis was well developed. Two types of cells, light cells and dark cells were visible in the lamina epithelialis. Lamina propria was made up of loose connective tissue and was devoid of any glands. At 12th week, tubular glands started developing as a continuous layer just beneath the lining epithelium similar to the magnum and showed some degree of vacuolation and were smaller than isthmian glands. At 14th week, the spatula shaped folds were higher and thinner with a few secondary mucosal folds. Ciliogenesis was more evident in apical regions of the mucosal folds. Glands towards the base of the mucosal folds showed vacuolated cytoplasm. Each secretory end piece was lined by five to six cells.

At 16th week, glandular development continued and closely packed tubular glands in the mucosal folds were empty during non laying phase. But, prior to and during secretory phase, cells contained pale staining granules and after the shell formation, the cytoplasm was markedly vacuolated, with relatively large basal nucleus. By 18 weeks, mucosal folds acquired their characteristic leaf-like appearance and lamina propria was filled with loosely arranged glands. Towards the base of the fold, the vacuolated appearance of glands was reduced and glands had a uniform appearance throughout.

In adult laying ducks, tunica muscularis was very thick with an inner circular and thicker outer longitudinal layers separated by loose connective tissue showing numerous vessels and nerves. The most important feature was that at this age, smooth muscle fibres were observed to be extending to the core of the mucosal fold along with collagen fibres. Clear cells were also identified in the lining epithelium. The surface epithelium of the uterus showed a single layer of columnar cells with alternating apical and basal nuclei. The apical cells carried

cilia and the basal cells had a restricted apical surface. Secretory granules were a constant feature of the supranuclear cytoplasm of the apical cells. Non-ciliated glandular cells of the lamina propria contained more vacuoles which regressed during the onset of egg formation.

At utero-vaginal junction the sperm storage tubules appeared at 12 weeks of age and were located in a thick ring of pushed-in mucosal folds found in the lumen with the vaginal folds on one side and uterine folds on the other. There was no evidence of smooth muscle fibres or other contractile elements associated with sperm storage tubules and these were surrounded by connective tissue elements only. Mucosal folds towards the uterine side of the cranial utero-vaginal ridge contained transitional glands intermediate in appearance between sperm storage tubules and uterine glands. These were more densely packed compared to sperm host glands and were lined by larger cells with oval nucleus at the base and enclosed a narrow lumen. The sperm storage tubules were lined by simple cuboidal to low columnar cells with centrally placed nuclei and at 14^{th} week of age, these glands appeared as small invaginations of surface epithelium lined by tall columnar cells with basal round nuclei. The sperm storage tubules were more sparsely distributed but had larger diameter than transitional and uterine glands. With the beginning of egg-laying phase, these glands exhibited rapid development.

In the day-old duckling itself, vagina, the caudal most portion of the oviduct was morphologically distinct. At two weeks of age, the vagina showed roughly triangular seven to eight primary folds lined by tall columnar cells interspersed with numerous goblet cells. Even at this age, tunica muscularis was well developed and differentiated into inner circular and outer longitudinal layers. Vagina showed the thickest tunica muscularis among different regions of oviduct. Caudal portions of the vagina showed low mucosal folds compared to cranial region in four weeks-old birds. Tunica serosa was also well demarcated. By ten weeks of age, the number of goblet cells reduced and typical secondary mucosal folds and cilia appeared. At 12^{th} week, folds were characteristically filiform in

appearance with secondary folds. The lamina propria was devoid of glands. At 18th week, mucosal folds were taller but the height of the epithelium reduced considerably and in between the goblet cells and ciliated cells, some clear cells were also identified. The core of mucosal fold consisted of bundles of collagen fibres with fibrocytes in between.

In adult laying birds, lining epithelium of vagina showed large number of goblet cells and the ciliated cells occurred only rarely. The height of the epithelium over the crest of the vaginal folds was greater than that of the shell gland. In the depressions between the folds, the shorter non-ciliated goblet cells predominated. At this age, inner circular muscle layer extended into the core of the mucosal fold as in the case of uterine region. Lamina propria of mucosal folds of the vagina showed large number of lymphocytes along with diffused blood cells from 4th week onwards. As age advanced, all regions of the oviduct showed lymphocytes in diffuse as well as aggregated form which was maximum in the vaginal region.

The goblet cells found throughout the oviduct showed intense P.A.S. positive reaction. The proprial glands of the isthmus showed positive reaction for P.A.S. and Alcian blue (pH 2.5) method and the glands positioned near the epithelium stained lightly than those situated more deeply. The presence of neutral and acid mucopolysaccharides was identified using P.A.S. and Alcian blue (pH 2.5) method in the epithelium of isthmus region; while in magnum, only the acid mucopolysaccharides were seen. Vaginal region gave a negative reaction for neutral as well as acid mucopolysaccharides. The lining epithelium of sperm storage tubules and transitional glands showed presence of lipid. The presence of glycogen was observed only in the epithelial lining and tubular glands of isthmus region and was more pronounced in adult egg laying ducks with actively secreting glands. Marked alkaline phosphatase activity was seen in the uterus, isthmus and magnum. The activity of Acid phosphatase was observed in the epithelium and tubular glands of isthmus, shell gland and vagina.

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POSTNATAL DEVELOPMENT OF THE OVIDUCT IN THE KUTTANAD DUCK (*Anas platyrhynchos domesticus*)

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ABSTRACT

The structure and postnatal development of the oviduct in the Kuttanad duck was studied using 78 ducklings from day-old to 24 weeks of age. The material was collected from six birds in each group at fortnightly intervals. In the day-old duckling, the left oviduct could be seen as a thin, thread-like, translucent, straight tube towards the left side of the coelomic cavity supported by the dorsal and ventral ligaments. Uterus and vagina were recognizable even at this age. Among the 78 birds, persistent right oviduct could be detected in 20 birds. Signs of coiling of the oviduct were evident from 10th week onwards. All the five segments of the oviduct namely, infundibulum, magnum, isthmus, uterus and vagina could be clearly distinguished from 12th week onwards. Rapid development of the organ occurred between 16 and 18 weeks at which stage a few birds started laying. A spurt in growth of the oviduct was noticed between 18 and 20 weeks when all the birds started laying. The contribution of oviduct to the body weight was 3.47% at 24th week of age. During postnatal period, correlation between age and the length of the oviduct was more significant than with the oviduct weight. Magnum was the longest and most coiled component. Isthmus, uterus and vagina were relatively longer.

In the day-old duckling, the oviduct wall was not differentiated into different tunics and showed innermost simple columnar epithelium and subepithelial tissue. The lumen was flanked by characteristic low mucosal folds. At two weeks of age, the secondary mucosal folds started appearing. By 6th week, a very thin single layer of tunica muscularis appeared. The most dramatic growth and differentiation began at the age of 12 weeks when all the segments of the oviduct were differentiated morphologically and histologically. The tubular glands appeared as invaginations of surface epithelium and the mucosal folds became comparatively higher and broader at this age. Glands were absent in the funnel portion of infundibulum, magnum isthmus junction and vagina. The core of the mucosal fold showed numerous blood vessels. The lamina propria blended with the submucosa and muscularis mucosa was absent. Tunica muscularis was

made up of inner circular and outer longitudinal smooth muscle layers and the thickness gradually increased from the cranial to the caudal direction. Distinct serosal tunic was visible in adult birds.

The funnel region of the infundibulum showed mainly low primary mucosal folds with a few secondary folds whereas in the neck region, numerous tertiary folds were also present. In the adult birds, there were four types of epithelial cells in the mucosa of neck namely non-secretory ciliated cells; non-ciliated mucous-secreting goblet cells; secretory cells other than goblet cells and lining cells of the tubular glands. Goblet cells increased in number caudally. At the infundibulum-magnum junction, groups of infundibular glands intermingled with tubular glands of the magnum.

In the magnum, mucosal folds were wider and higher and were lined by monolayered simple columnar ciliated epithelium with a few goblet cells. The glands of lamina propria were arranged in a radiating manner from the core to the periphery. Magnum-isthmus junction was characterized by complete absence of tubular glands. In the isthmus, by four weeks, the primary folds attained characteristic angular shape. In the adult birds, compared to all other segments of the oviduct, the glands in the isthmus were more loosely arranged. Regular alteration of both ciliated and secretory non-ciliated columnar cells was noticed in the lining epithelium. Isthmus - uterine junction showed an intermingling of glands within the lamina propria.

The mucosal folds of uterus were higher, leaf-shaped and were separated by deep furrows. Light cells and dark cells were visible in the lamina epithelialis. At 16^{th} week, glandular development continued and the closely packed tubular glands in the mucosal folds were empty during non laying phase. But, prior to and during secretory phase, cells contained pale staining granules and after the shell formation, the cytoplasm was markedly vacuolated, with relatively large basal nucleus. Tunica muscularis was very thick with an inner circular and thicker outer longitudinal layers separated by loose connective tissue showing

numerous vessels and nerves. At this age, smooth muscle fibres were observed to be extending to the core of the mucosal fold along with numerous collagen fibres.

At utero-vaginal junction the sperm storage tubules appeared at 12 weeks of age and were located in a thick ring of pushed-in mucosal folds found in the lumen with the vaginal folds on one side and uterine folds on the other. Mucosal folds towards the uterine side of the cranial utero-vaginal ridge contained transitional glands, which intermediate in appearance between sperm storage tubules and uterine glands. The sperm storage tubules were more sparsely distributed but had larger diameter than the transitional and uterine glands.

At two weeks of age, the vagina showed roughly triangular seven to eight primary folds lined by tall columnar cells interspersed with numerous goblet cells. Even at this age, tunica muscularis was well developed and differentiated into inner circular and outer longitudinal layers. By 12 weeks, the folds were characteristically filiform in appearance with secondary folds. The lamina propria was devoid of glands. Vagina showed the thickest tunica muscularis among different segments of the oviduct. Lamina propria of mucosal folds of the vagina showed large number of lymphocytes along with diffused blood cells from four weeks-old birds. As age advanced, all regions of the oviduct showed lymphocytes in diffuse as well as aggregated form which was maximum in the vaginal region.

The goblet cells found throughout the oviduct showed intense P.A.S. positive reaction. The presence of neutral and acid mucopolysaccharides was identified using P.A.S. and Alcian blue (pH 2.5) method in the epithelium of isthmus region; while in magnum, only the acid mucopolysaccharides were seen. The sperm storage tubules and transitional glands showed presence of lipid.

The presence of glycogen was observed only in the epithelial lining and tubular glands of isthmus region and was more pronounced in adult egg laying ducks with actively secreting glands. Marked alkaline phosphatase activity was seen in the uterus, isthmus and magnum. The activity of Acid phosphatase was observed in the epithelium and tubular glands of isthmus, shell gland and vagina.