HISTOMORPHOLOGICAL STUDIES OF REPRODUCTIVE SYSTEM IN LAYING KADAKNATH AND WHITE LEGHORN FOWL

THESIS

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Shashi Bharti
I.D. No. VP/JB/87/2010

Department of Veterinary Anatomy
College of Veterinary Science & Animal Husbandry,
Nanaji Deshmukh Veterinary Science University,
Jabalpur (M.P.)

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1. INTRODUCTION

The poultry industry has emerged as the fastest growing and dynamic segment of the livestock sector both globally and in India. As the human population increases, the poultry industry continues to grow to meet the demand for poultry products in the world markets (Augustine and Shukla, 2015). India is one of the world’s top producers of hen eggs, ranking 3rd after China (1st) and the U.S. (2nd). The growth rate of 7.30 per cent in Indian poultry sector has placed India at 5th position (APEDA, 2014).

In Indian poultry Kadaknath is an important breed which is well known as Kalamashi and is being reared by tribal, living in Dhar and Jhabua districts in western Madhya Pradesh. The Kadaknath breed has appreciable degree of resistance to diseases compared with other exotic breeds of fowl in its natural habitat. These birds are also resistant to extreme climatic conditions like summer heat and cold winter stress and thrive very well under adverse environment like poor housing, poor management and poor feeding (Thakur et al., 2006).

Meat of Kadaknath chicken is black in colour and has delicious flavour (Panda and Mohapatra, 1989) with 25.47 per cent of proteins (Mohan et al., 2008 and Rao and Thomas, 1984). In Kadaknath breed most of the internal organs exhibit intense black colouration which is due to the deposition of fibromelanin pigment in connective tissue of organs (Rao et al., 1980). Due to the meat’s alleged medicinal properties, high in iron, amino acids and low in fat; it is high in demand. Its consumption helps in increasing red blood cells and haemoglobin.

First egg produced by Kadaknath is at the age of 29 weeks (Parmar et al., 2003). Their egg production is less and ranges between 80 to 90 per year with egg weight of 49 g (Pathan et al., 2009).

White Leghorn mature early and start laying at about 19 weeks of age (Silversides et al., 2006). Birds peak lay period is from 25 to 39 weeks of age when they lay on average nearly one egg per day. White Leghorn are
the best known of the egg producing fowl averaging between 300 to 350 eggs per year with egg weight of 50 to 55 g. The Leghorn is a light breed that matures quickly; and is not considered a viable meat producer.

Since egg production in birds is regulated by several complex interaction in which avian reproduction play an important role, and studies have been conducted to understand this relationship in exotic breeds (Swain et al., 2000 and Surai, 2002). Comparative studies on developmental stages of chicken, guinea fowl, turkey, duck, japanese quail, goose were performed (Sellier et al., 2006).

Comparative study on ovaries of Assel and RIR fowl aging from 2 weeks to 13 months was conducted by Shyam et al. (2015). Comparative studies on prehatch development of Kadaknath and WLH was done in the department by Tekam (2015) and similar study in post hatch period up to 20 weeks of age was performed by Kumar (2016). However, in laying period such information is lacking. Therefore, the present study has been undertaken with the following objectives.

**OBJECTIVES**

1. To study the gross and histological features of female reproductive system of Kadaknath and White Leghorn fowl in different periods of laying.

2. To compare the different parameters of female reproductive system of Kadaknath fowl with the White Leghorn (WLH).
2. REVIEW OF LITERATURE

Female Reproductive system
Gross Anatomy

The female genalia of the fowl were comprised of left functional ovary and oviduct. The gross structure of female reproductive organs has been described by Sisson and Grossman (1956); Bradley and Grahame (1960); King (1975); Sturkie (1976); Nickel et al. (1977); Panda and Mohapatra (1989); McLelland (1991); Dyce et al. (1996); Johnson (2000); Singh (2001) and Ghosh (2003).

Ovary

In laying hens, the left ovary was in contact with the cranial division of left and right kidney, cranially related to caudal end of left lung, dorsally to the aorta, caudal venacava, left and right adrenals, ventrally to the left abdominal air sac, proventriculus and spleen. The base of ovary was attached with the dorsal wall of the coelom by mesovarium (Sisson and Grossman, 1956; King, 1975; Sturkie, 1976 and Nickel et al., 1977). The mature ovary resembles to a bunch of grapes (King, 1975; Nickel et al., 1977; Dyce et al., 1996 and Ghosh, 2003).

Gilbert (1970) and Banerjee et al. (2006) stated that the maximum growth of ovary occurred from 150 to 180 days of age in Rhode Island Red birds with an average weight of ovary at 10, 15, 18 and 20th week of age as 0.42±0.08 g, 0.481±0.01 g, 0.52±0.01 g and 18.49±1.07 g, respectively.

Waddington and Walker (1988) studied distribution of follicular growth, atresia and ovulation in the ovary of the domestic hen (Gallus domesticus) at different ages. Ovaries of laying hens of 14, 18, 30, 45 and 86 weeks of age were divided transversely into two or more distinct regions. They concluded that most of the eggs produced during the hen's laying year must be ovulated from the anterior part of the ovary.

Rahman et al. (1999) performed morphometric studies of the ovary and oviduct of high and low egg producing chicken in Bangladesh. They
found that at 36 weeks in ISA Brown, the mass of the ovary was 41.89 g whereas in indigenous hen it was 27.65 g. They also observed that the diameter and the number of various sized follicles were higher in ISA Brown than in indigenous chicken.

Lukanov and Genchev (2013) reported that fibromelanosis is a mutation in domestic chickens (Gallus gallus domesticus) emerging most probably in the first year AD in south eastern Asia. It is manifested with dark, bluish black coloration of the skin, eyes, serosa, muscles, nervous connective tissue sheaths, gonads, trachea and periosteum of birds due to abnormal accumulation of eumelanin in tissues. This unique mutation is known in more than 25 breeds, including Kadaknath in India, Silkie in Japan/ Europe, Silkie Bairiong in China etc.

Shyam et al. (2015) performed gross study on ovary of Aseel and Rhode Island Red of three different age groups- group I (2 weeks), group II (5 months) and group III (13 months) of Aseel and RIR breeds of poultry. They observed that right ovary was not present in any birds. In group II, it resembled to an irregular lump finely granular and lobulated and in group III it resembled to bunch of grapes. Its surface was smooth in group I and uneven in group II and III. The mean weight of ovary in group I, II and III was 35.5 mg in Aseel and 39 mg in RIR, 0.52 g in Aseel and 0.97 g in RIR and in group III 18.04 g in Aseel and 18.07 g in RIR, respectively.

**Oviduct**

Romanoff and Romanoff (1949) reported that resting adult oviduct range in length from 14-19 cm with a mean length of about 15 cm and a weight 5 g. In laying hens length range from 42-86 cm with a mean of about 65 cm and a weight of about 76 g. The length of the oviduct increased about four fold and the weight about 15-20 fold.

The oviduct of hen consists of five anatomically and functionally distinct segments, namely the infundibulum, magnum, isthmus, uterus, and vagina (Palmer and Guillette, 1988).

Shyam (2012) reported that the average length and weight of the oviduct was found as 57.20 cm and 28.41 g, respectively in Aseel and
63.26 cm and 41.64 g, respectively in RIR. The values obtained were significantly higher in RIR as compared to Aseel.

**Infundibulum**

In active stage, the length of infundibulum ranged from 2.00 to 11.00 cm with the diameter of 8.00 to 9.00 cm at the opening of the funnel (King, 1975 and Sturkie, 1976).

Naragude et al. (1999b) studied the infundibulum region in RIR birds at 20-24 weeks of age. The average length of infundibulum was 7.37±0.08 cm in RIR birds.

Sarma and Sarma (2001) studied on age related changes in the avian oviduct. They noticed length of infundibulum as 5.54±0.01 cm. However, Garg (2006) recorded it as 7.80±0.23 cm in Kadaknath. Mishra et al. (2014) measured the mean length and weight of infundibulum as 7.18±4.22 cm and 7.18±4.22 g, respectively in native chicken of Bangladesh.

**Magnum**

Garg (2006) reported length of magnum as 29.80 cm in Kadaknath. Shyam (2012) found that magnum was the longest segment of the oviduct and had an average length of 30.80 cm and width of 1.30 cm in Aseel and length of 32.64 cm and width of 1.53 cm in RIR birds.

Mishra et al. (2014) performed gross and histomorphological studies on the oviduct of native chicken of Bangladesh. The mean length of magnum at 8-11 months old chicken was 18.40±6.75 cm.

**Isthmus**

Bradley and Grahame (1960) and Sturkie (1976) observed that in laying hen, the length of isthmus ranged from 3.00 to 12.00 cm with the diameter from 0.90 to 1.00 cm. In inactive stage length was 2.20 cm and diameter 0.40 cm.

Sarma and Sarma (2001) studied on age related changes in the avian oviduct. They noticed length of isthmus as 7.68±0.03 cm in non-descript chicken. Garg (2006) recorded the length of isthmus as 5.42 cm in Kadaknath fowl.
Shyam (2012) reported that at 13 months of age the average length was 4.50 cm and width 0.76 cm in Aseel. In RIR, average length and width was 3.00 cm and 0.85 cm, respectively. Whereas, Mishra et al. (2014) measured the mean length of isthmus as 7.68±3.46 cm.

**Shell gland**

Hodges (1974) reported the length of uterus in fowl as 7.00 cm. Nickel et al. (1977) reported as 8.30 cm and Garg (2006) reported length of uterus as 9.04 cm in Kadaknath fowl at 26 weeks of age.

King (1975) stated absence of anatomical boundary between isthmus and shell gland. Shell gland is a sac like expanded region in laying hen, measured 4.00 cm to 12.00 cm in length and 2.90 cm to 3.00 cm in diameter, while in pullet length range from 3.00 to 6.00 cm and diameter were 2.40 cm to 1.20 cm, respectively.

Shyam (2012) observed the average length of isthmus as 6.35 cm with a range from 4.50 to 8.50 cm and width 2.51 cm in Aseel. In RIR birds, it was 7.60 cm long with a range from 5.00 to 9.00 cm and its average width was 3.19 cm.

Mishra et al. (2014) recorded the mean length and weight of uterus as 6.10±2.80 cm and 6.10±2.80 g in oviduct of native chicken of Bangladesh.

**Vagina**

Vagina is relatively short narrow muscular S- shaped tube joining the uterus to cloaca. Giersberg (1922) reported that in laying hen, the length of vagina was 4.00 to 12.00 cm.

Shyam (2012) reported that in Aseel the average length and width of vagina was 6.35 cm and 0.71 cm, respectively. In RIR birds, the mean length was 7.41 cm with a range from 6.00 to 9.00 cm and mean width as 0.76 cm. Mishra et al. (2014) reported that the mean length and weight of vagina was 6.83±2.82 cm and 6.83±2.82 g in native chicken of Bangladesh.
Histology

Ovary

Left ovary consists of two principal parts cortex and medulla (Bradley and Grahame, 1960; Das and Biswal, 1968; Hodges, 1974; Rao and Vijayaragvan, 1999a; Bacha and Bacha, 2000 and Aughey and Frye, 2001). The surface of ovary is covered by germinal epithelium of low columnar cells (Das and Biswal, 1968 and Hodges, 1974). Hodges (1974) in fowl and Rao and Vijayaragvan (1999a) in domestic duck reported the germinal epithelium consisted of simple cuboidal to squamous cells. The shape of the epithelial cells varies considerably depending upon the presence of follicles on the surface of ovary.

Aitken (1966) observed that by the second and third day after ovulation the shrunken empty follicle had been refilled with vacuolated cells derived from the granulosa and probably from the inner fibroblast layer and even from the outer vacuolated cell layer of the theca interna. There were also many eosinophils. He concluded that the yellow pigment of the cells which fill the post ovulatory follicle is hemosiderin.

Guzsal (1966) reported that immediately after ovulation the follicle shrinks to an empty thin walled sac about 10-12 mm long, its opening into the celom being about 8 mm in diameter; neither the opening nor the depth of the narrow cavity showed hemorrhage. There was no acceptable evidence for persistant post ovulatory corpora lutea in birds.

Hodges (1974) stated that distinction between theca interna and theca externa was possible when follicles attained the diameter of 2 mm. King (1975) reported that with the onset of sexual activity the distinction between cortex and medulla was virtually lost.

Rao and Vijayaragvan (1999a) observed that in the ovary of domestic duck primary follicles were covered by flattened granulosa cells. Secondary follicles were larger than primary and their cytoplasm was granular.
Plate 01: Photograph showing left ovary (Lo) and oviduct (Od) of Kadaknath and WLH fowl, group I (24 weeks)

Plate 02: Photograph showing left ovary (Lo) and oviduct (Od) of Kadaknath and WLH fowl, group II (32 weeks)

Plate 03: Photograph showing left ovary (Lo) and oviduct (Od) of Kadaknath and WLH fowl, group III (40 weeks)

Plate 04: Photograph showing left ovary (Lo) and oviduct (Od) of Kadaknath and WLH fowl, group IV (48 weeks)
Bacha and Bacha (2000) observed that mature follicles were surrounded by several layers from outside to in as relatively wider theca externa. Narrower theca interna composed of mainly collagen fibers and fibroblast like cells and about one quarter as thick as externa. It was formed of compact layer of spindle shaped cells, follicular epithelium or membrana granulosa consisting of single layer of cuboidal or pseudostratified cells.

Shyam (2012) observed the proliferation in the germinal epithelium at several places and cells were seen migrating from here into the cortical area. The cortex revealed ovarian follicles at different stages of development and were surrounded by cells of connective tissue and epithelial origin along with collagen and reticular fibers.

**Oviduct**

**Infundibulum**

Bakst and Howarth (1974) reported predominance of ciliated cells in the epithelium of infundibulum of hen. Naragude et al. (1999a) observed that the folds of infundibulum posses simple columnar epithelium equally populated with ciliated and non ciliated cells. They found goblet cells in the epithelium of infundibulum in RIR birds.

Ingole (1985) noticed that the oviduct comprised of luminal epithelium, glands, connective tissue, muscle layer and outer serosa. He reported infundibulum wall thickness as 273.60 µm and 279.30 µm in Narmada XI and normal WLH birds at 18 weeks of age. Islam et al. (2002) reported that the mucosal folds of laying hens were short and tongue shaped at one day old deshi chicken. With the aging, the infundibular folds were increased in height at 12 and 19 weeks old and became highly developed at 30 weeks of age.

Mehta et al. (2005) reported that the lamina epithelialis in funnel part was lined by simple columnar epithelium and tubular part by pseudostratified ciliated columnar epithelium with few goblet cells.

Shyam (2012) reported that the thickness of the infundibular wall at the age of 13 months ranged between 219.00 µm to 273.00 µm with an average of 243.00 µm in Aseel and these values were significantly higher in RIR birds with an average of 282.00 µm and ranged between 271.50 µm to
291.00 μm. The average height of an epithelium was similar in both breeds as 20.26 μm. Ciliated pseudostratified columnar type of epithelium was found.

**Magnum**

In this region the number, height and thickness of mucosal ridges with longitudinal and spiral orientation was found to be increased. Presence of the secondary and tertiary folds was not observed (Nickel *et al.*, 1977; King, 1975 and Gilbert, 1970).

Khan *et al.* (1999) observed albumin producing glands at 19 weeks old in White Leghorn chicken.

Islam *et al.* (2002) reported that the mucosal folds in the magnum were medium sized at 12 weeks and well developed at 19 and 30 weeks old deshi chicken. It was lined with columnar epithelium and consisted of huge number of glands. The tubular glands in the magnum were highly developed and open to the epithelial surface. The albumin producing glands in the magnum was observed at 30 weeks old.

Mehta *et al.* (2005) observed in oviduct of the developing hen (*Gallus domesticus*) that the mucosal folds were pyramidal in shape. Lamina epithelialis was consisted of pseudostratified columnar epithelium with goblet cells. Garg (2006) observed mainly sessile primary with few small secondary folds and ranged from 17.00 to 22.00 in number with an average of 18.40 folds.

Shyam (2012) reported the average maximum thickness of wall as 400.25 μm with range between 388.50 to 429.00 μm in Aseel. The values recorded were significantly higher in case of RIR with an average of 444.25 μm with a range between 418.50 to 465.00 μm. The number of mucosal folds in Aseel varied from 19.00 to 22.00 with an average of 20.56. In RIR, it varied between 20.00 to 23.00 folds with an average of 22.00.

Bharti and Gautam (2013) studied on gross and histomorphologic study of magnum in adult indigenous chicken (*Gallus domesticus*) of Assam. The mucosa of magnum was lined by simple columnar ciliated epithelium with primary and secondary folds. The propria submucosa was packed with long branched coiled tubular glands and it contained large amount of collagen fibers and fewer amounts of elastic fibers.
**Isthmus**

Hodges (1974) reported the epithelial height as 25.00 µm in adult fowl while Gopinath and Hafeezuddin (1980) recorded it as 11.70 µm. The smooth muscular layers of the wall were better developed and collagen fibers were observed between the two muscle layers.

Solomon (1975) noticed that in domestic fowl the surface epithelium of isthmus comprised of three cell types- ciliated, non-ciliated and mitochondrial of which only the non-ciliated cells contribute towards the carbohydrate moiety of the shell membranes.

Sharma and Duda (1992) reported the height of the epithelium as 19.20±1.80 µm in pre-laying phase and 28.50±2.00 µm in laying phase.

Naragude *et al.* (2000) observed that the lamina epithelialis mucosae comprised of simple columnar ciliated epithelium with equal number of mucus secreting goblet cells. They revealed that the submucosa consisted of loose connective tissue stroma with branched tubular submucosal glands and blood vessel. The length of primary mucosal fold was 190.33±3.40 µm and epithelial height was 3.64±0.50 µm. They studied the effect of age on the histomorphology of the isthmus of oviduct in RIR birds. The isthmal glands were tubular and less prominent at 20-24 weeks as compared to 16 weeks.

Garg (2006) reported an average of 24.40 longitudinally oriented folds. They were small initially and attained the height of 1 to 2 mm terminally. These folds were angular or flat leaf like. He reported that lamina propria was made up of loose connective tissue with collagen and reticular fibers, which consisted of loosely arranged simple and branched tubular glands.

Shyam (2012) reported that at 5 month of age the average wall thickness was 81.25 µm with a range between 78.00 to 84.00 µm. Significantly higher values were found in RIR bird as 115.25 µm with a range between 99.00 to 141.00 µm. The number of mucosal folds varied from 15.00 to 18.00 with an average of 16.50 and the height of mucosal folds ranged between 249.00 to 264.00 µm with an average of 251.75 µm in Aseel. In RIR, the number of folds was significantly higher than Aseel.
Shell gland

Bradley and Grahame (1960) and Nickel et al. (1977) observed flat, discontinuous, leaf shaped and longitudinally oriented mucosal folds in the shell gland.

Hodges (1974) stated that the mucosal folds were long, narrow and compact and spirally arranged. The lamina epithelialis mucosae were made up of intermittently ciliated pseudostratified columnar epithelium. The epithelial height was 11.70 to 30.00 µm.

Banks (1986) observed that coiled tubular glands of shell gland project into the underlying connective tissue.

Islam et al. (2002) reported that uterine mucosal folds were dome shaped at 12 weeks, elongated at 19 and 30 weeks old deshi chicken in the uterus and vagina. Mehta et al. (2005) observed branched and long pillar like folds in developing hen and Garg (2006) observed an average of 67.40 numbers of mural folds in Kadaknath.

Shyam (2012) reported the average thickness of the wall as 577.00 µm with range from 558.00 to 594.00 µm in Aseel and an average of 594.00 µm and a range between 573.00 to 633.00 µm in RIR, which was significantly higher as compared to Aseel. The number of mucosal folds varied from 76.00 to 108.00 with an average of 88.00 folds in Aseel and the value was significantly greater in RIR with an average of 99.16 folds with a range between 80.00 to 108.00 folds.

Vagina

Nickel et al. (1977) observed lamina epithelialis mucosae were lined by tall ciliated columnar epithelium with tall and narrow goblet cells at the tip of the folds.

Gopinath and Hafeezuddin (1980) noticed that the epithelial height of vagina was between 16.00 to 36.00 µm. In the tunica muscularis, outer longitudinal muscle layer was moderate with bundles of muscle fibers scattered in the connective tissue stroma whereas, inner circular muscle layer was developed as compared to the other regions.
Czareva (1990) observed that the vaginal wall was thicker than that of the other oviduct portions. The luminal surface of the vagina was composed of the thin longitudinally oriented mucosal folds. The lamina propria was formed of fibrous connective tissue with blood vessels and nerves along with aggregated lymph nodules in the submucosa.

Garg (2006) reported that lamina propria contained collagen fibers and blood vessels but was devoid of tubular glands. Ferdous et al. (2011) studied effects of age on growth and development of vagina in ISA Brown chickens and observed that rapid structural changes occurred at 23 weeks of age in the adult bird, mucosal folds were narrowed and tongue-shaped. Lamina propria was devoid of glands and contained lymphocytes. Tunica muscularis was well developed at 23 and 46 weeks.

**Histochemistry**

Mehta and Singh (2003) observed the strong PAS positive reaction in lamina epithelialis of tubular part of infundibulum, in tubular glands of developing birds and very strong affinity in laying birds.

Bharti and Gautam (2013) showed the intense PAS positive reaction in glands of magnum and moderate reaction in central part of mucosal folds.
3. MATERIAL AND METHODS

3.1 Location of work

The study was conducted in the Department of Veterinary Anatomy, College of Veterinary Science and Animal Husbandry, Nanaji Deshmukh Veterinary Science University, Jabalpur, Madhya Pradesh.

3.2 Collection of material

The experiment was conducted on gross, histomorphology and histochemistry of reproductive system of 48 female birds, comprised of 24 Kadaknath and 24 White Leghorn (WLH) breed. Birds were procured from All India Co-ordinated Research Project on Poultry, Livestock Farm, Krishinagar, Adhartal, Jabalpur (M.P.). Whereas, White Leghorn were collected from Phoenix poultry farm, Jabalpur. The birds of each breed were divided into 4 groups with 6 birds in each group as shown in the table below.

Table 01: Number of female Kadaknath and White Leghorn birds in each group with their age (weeks)

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Group</th>
<th>Age</th>
<th>Number of birds</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Kadaknath</td>
</tr>
<tr>
<td>1</td>
<td>I</td>
<td>24 weeks</td>
<td>6</td>
</tr>
<tr>
<td>2</td>
<td>II</td>
<td>32 weeks</td>
<td>6</td>
</tr>
<tr>
<td>3</td>
<td>III</td>
<td>40 weeks</td>
<td>6</td>
</tr>
<tr>
<td>4</td>
<td>IV</td>
<td>48 weeks</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>SUBTOTAL</td>
<td></td>
<td>24</td>
</tr>
<tr>
<td></td>
<td>TOTAL</td>
<td></td>
<td>48</td>
</tr>
</tbody>
</table>

3.3 Gross parameters

After procurement of birds on specified age their live weight (g or kg) was recorded, and then sacrificed by cervical fracture. The linear length of synsacrum was measured after dissection and ovaries and oviduct were collected and fixed in 10% buffered formalin. Following gross parameters were recorded.
- Linear length of synsacrum occupied by female reproductive system in-situ (cm)
- Length, width (cm) and weight (g) of left ovary
- Average size of follicle (mm)
- Number of coils of oviduct
- Length and width (cm) of left oviduct

3.4 Histological and Histochemical studies

The fixed tissue samples were collected from ovary and different regions of oviduct. Before processing for routine histological technique all the tissue samples were thoroughly washed under running tap water. Then after tissue samples processed in acetone - benzene sequence for preparation of paraffin blocks. Five to six micron (\(\mu\)m) thick sections were cut by using rotary microtome (Spencer) and stained with Haematoxylin and Eosin for general histomorphological observations (Drury and Wallington, 1980). The special staining methods were used to study the following parameters.

Table 02: Standard staining techniques for various histological and histochemical parameters

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Staining technique</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reticular fibers</td>
<td>Gomori’s silver impregnation method</td>
<td>Drury and Wallington (1980)</td>
</tr>
<tr>
<td>Elastic and collagen fibers</td>
<td>Weigert’s elastic stain with Van Gieson’s stain</td>
<td>Drury and Wallington (1980)</td>
</tr>
<tr>
<td>Polysaccharides</td>
<td>Periodic Acid Schiff’s stain</td>
<td>Pearse (1980)</td>
</tr>
<tr>
<td>Acid mucopolysaccharides</td>
<td>Mowry’s colloidal iron</td>
<td>Lillie and Fullmer (1976)</td>
</tr>
<tr>
<td>Lipid</td>
<td>Sudan black B</td>
<td>Pearse (1980)</td>
</tr>
<tr>
<td>Protein bound NH₂</td>
<td>Ninhydrin Schiff’s</td>
<td>Pearse (1980)</td>
</tr>
<tr>
<td>Calcium</td>
<td>Modified Von Kossa method</td>
<td>Drury and Wallington (1980)</td>
</tr>
</tbody>
</table>
3.5 Micrometry

Micrometry was done after calibration of ocular with stage micrometer scale (Drury and Wallington, 1980). The following parameters were recorded.

3.5.1 Ovary

- Number of follicles per field/ total number of follicles in the section
- Average diameter of follicles
- Thickness of follicular wall

3.5.2 Oviduct

Parameters of different segments of oviduct viz. (infundibulum, magnum, isthmus, shell gland and vagina) was measured.

- Lumen diameter and thickness of wall
- Number of primary and secondary mucosal folds
- Height of epithelium
- Size of epithelial nuclei
- Thickness of tunica mucosa
- Thickness of tunica muscularis
- Thickness of tunica serosa

3.6 Statistical analysis

Data gathered from the study was analysed, using standard statistical method as described by Snedecor and Cochran (1994).
4. RESULTS

4.1 Gross

4.1.1 Ovary

Gross observation revealed that left ovary was elongated triangular with base directed rostrally and conical apex caudally in group I of Kadaknath (Plate 01). From group II onwards the ovary became in the form of bunch of grapes (Plate 02). However, in WLH the ovary showed number of mature follicles in all the four groups (Plate 03 and 04).

The colour of the ovary in Kadaknath was grayish in all the groups. However, in WLH it was pinkish white to yellow in colour. In group I of Kadaknath fowl the left ovary was related to the cranial division of left kidney dorsally. However, in later groups of Kadaknath and all the groups of WLH was also related to the right kidney.

Mean weight of the ovary in group I was 3.51±0.12 g and 36.34±1.35 g in Kadaknath and WLH, respectively which increased to 28.23±1.46 g in group II of Kadaknath, whereas in WLH it slightly decreased to 36.07±1.57 g. In later group there was gradual decrease in the weight of ovary in Kadaknath. However, in WLH there was marked decrease of ovarian weight (8.10±0.98 g) in group IV. Per cent left ovarian weight to body weight was maximum in group II of Kadaknath fowl. Although it was maximum in group I of WLH (Table 03).

The length of the ovary was 3.10±0.15 cm in group I of Kadaknath which increased to 5.58±0.11 cm in group II and then gradually decreased and became 5.18±0.07 cm in group IV (Table 04). In WLH the ovary showed maximum length 6.45±0.08 cm in group I which decrease to 3.92±0.14 cm in group IV.

In Kadaknath breed of fowl there was gradual increase of width from group I to IV. However, in WLH there was decrease in width of ovary from group I to group IV (Table 04).
### Table 03: Range and mean of ovary weight (g), body weight (kg), length of synsacrum (cm) and per cent, left ovarian weight to body weight in Kadaknath and White Leghorn breeds of fowl

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Groups</th>
<th>Kadaknath</th>
<th>White Leghorn</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>I</td>
<td>II</td>
<td>III</td>
</tr>
<tr>
<td>Ovary weight (g)</td>
<td>Range</td>
<td>2.94-3.85</td>
<td>23.94-33.87</td>
</tr>
<tr>
<td></td>
<td>Mean±SE</td>
<td>3.51±0.12</td>
<td>28.23±1.46</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>Range</td>
<td>0.70-0.90</td>
<td>1.00-1.20</td>
</tr>
<tr>
<td></td>
<td>Mean±SE</td>
<td>0.78±0.03</td>
<td>1.12±0.03</td>
</tr>
<tr>
<td></td>
<td>Mean±SE</td>
<td>8.57±0.09</td>
<td>9.32±0.13</td>
</tr>
<tr>
<td>%, left ovarian weight to body weight</td>
<td>Range</td>
<td>0.34-0.45</td>
<td>2.18-2.82</td>
</tr>
<tr>
<td></td>
<td>Mean±SE</td>
<td>0.40±0.02</td>
<td>2.52±0.09</td>
</tr>
</tbody>
</table>

### Table 04: Range and mean of different parameters of left ovary and oviduct in Kadaknath and White Leghorn breeds of fowl
<table>
<thead>
<tr>
<th>Parameters</th>
<th>Kadaknath</th>
<th>White Leghorn</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Groups</td>
<td></td>
</tr>
<tr>
<td></td>
<td>I</td>
<td>II</td>
</tr>
<tr>
<td>Left Ovary</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Length (cm)</td>
<td>Range</td>
<td>2.70-3.60</td>
</tr>
<tr>
<td></td>
<td>Mean±SE</td>
<td>3.10±0.15</td>
</tr>
<tr>
<td>Width (cm)</td>
<td>Range</td>
<td>1.70-3.10</td>
</tr>
<tr>
<td></td>
<td>Mean±SE</td>
<td>2.62±0.21</td>
</tr>
<tr>
<td>Left Oviduct</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Length (cm)</td>
<td>Range</td>
<td>8.10-10.80</td>
</tr>
<tr>
<td></td>
<td>Mean±SE</td>
<td>9.43±0.37</td>
</tr>
<tr>
<td></td>
<td>Mean±SE</td>
<td>3.15±0.23</td>
</tr>
</tbody>
</table>
Table 05: Growth spurt (percentage increase) of length, width, and weight of left ovary in Kadaknath and White Leghorn breeds of fowl

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Kadaknath</th>
<th>White Leghorn</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>I-II</td>
<td>II-III</td>
</tr>
<tr>
<td>Length (cm)</td>
<td>80</td>
<td>-4.12</td>
</tr>
<tr>
<td>Width (cm)</td>
<td>70.61</td>
<td>6.26</td>
</tr>
<tr>
<td>Weight of left ovary (g)</td>
<td>704.27</td>
<td>-23.38</td>
</tr>
</tbody>
</table>

Growth spurt of ovary showed that the length was increased only in group I-II. However, the values decreased in all the groups. The decrease of length was maximum in group I-II of WLH. The width of the ovary showed increasing trend which was maximum (70.61%) from group I-II of Kadaknath. However, in WLH it showed decreasing trend. The maximum decrease of width was from group II-III. The maximum increase (704.27%) in weight of left ovary was from group I-II of Kadaknath and maximum decrease was noticed from group III-IV in WLH (Table 05).

4.1.2 Oviduct

The left oviduct extended caudally from the left ovary and opened in the urodeum of cloaca lateral to the left ureter. The coils of oviduct were not seen in group I of Kadaknath whereas, coiling was observed in rest of the groups of Kadaknath and all the groups of WLH (Plate 01 to 04).

The colour of the oviduct was grayish in all the groups of Kadaknath, however in group IV the colour intensity was reduced. In WLH, it was white in colour in all the groups.

Morphometrical data showed that there was five fold increase in length and ten fold increase in weight of the oviduct from group I to group II of Kadaknath. These values gradually reduced in group IV. However, in WLH the length and weight of oviduct gradually decreased from group I to group IV (Table 04).
Table 06: Growth spurt (percentage increase) of length and weight of oviduct in Kadaknath and White Leghorn breeds of fowl

<table>
<thead>
<tr>
<th>Parameters (cm)</th>
<th>Kadaknath</th>
<th>White Leghorn</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Groups</td>
<td></td>
</tr>
<tr>
<td></td>
<td>I-II</td>
<td>II-III</td>
</tr>
<tr>
<td>Length</td>
<td>391.19</td>
<td>-0.04</td>
</tr>
<tr>
<td>Weight</td>
<td>975.24</td>
<td>12.64</td>
</tr>
</tbody>
</table>

Growth spurt of length and weight of oviduct in Kadaknath and WLH revealed that there was tremendous increase of length (391.19%) and weight (975.24%) from group I-II Kadaknath breed of fowl. However, from group II-III the length was decreased (-0.04%) whereas weight was slightly increased (12.64%). In WLH both the parameters showed decreasing trend (Table 06).

The values of length and width of different parts of left oviduct namely infundibulum, magnum, isthmus, shell gland and vagina of Kadaknath and WLH breeds of fowl have been shown in table 07.

The mean length of the infundibulum was 1.92±0.09 cm in group I of Kadaknath which increased to 6.07±0.15 cm in group II. However, in WLH it was 9.22±0.63 cm in group I which decreased to 5.85±0.6 cm in group II. In group IV the length of infundibulum became 7.12±0.22 cm and 8.38±0.15 cm in Kadaknath and WLH, respectively. The width of infundibulum was greater in WLH in all the groups except in group II where it was greater (5.90±0.29 cm) in Kadaknath than WLH (5.30±0.64 cm) (Table 07).

The mean length of the magnum was 2.22±0.09 cm in group I of Kadaknath which suddenly increased to 23.70±0.94 cm in group II. However, in WLH the length was 34.38±1.07 cm in group I which gradually reduced and became 17.48±1.02 cm in group IV. There was no definite pattern of increase or decrease of width of magnum in both the groups.
Table 07: Mean regional length (cm) and width (cm) of left oviduct in Kadaknath and White Leghorn breeds of fowl

<table>
<thead>
<tr>
<th>Groups</th>
<th>Infundibulum</th>
<th>Magnum</th>
<th>Isthmus</th>
<th>Shell gland</th>
<th>Vagina</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Length</td>
<td>Width</td>
<td>Length</td>
<td>Width</td>
<td>Length</td>
</tr>
<tr>
<td>I</td>
<td>Kadaknath</td>
<td>1.92±0.09</td>
<td>1.01±0.63</td>
<td>2.22±0.09</td>
<td>0.30±0.12</td>
</tr>
<tr>
<td></td>
<td>WLH</td>
<td>9.22±0.63</td>
<td>7.72±0.47</td>
<td>34.38±1.07</td>
<td>1.73±0.07</td>
</tr>
<tr>
<td>II</td>
<td>Kadaknath</td>
<td>6.07±0.15</td>
<td>5.90±0.29</td>
<td>23.70±0.94</td>
<td>2.07±0.09</td>
</tr>
<tr>
<td></td>
<td>WLH</td>
<td>5.85±0.60</td>
<td>5.30±0.64</td>
<td>26.00±0.65</td>
<td>1.68±0.12</td>
</tr>
<tr>
<td>III</td>
<td>Kadaknath</td>
<td>4.53±0.30</td>
<td>5.33±0.26</td>
<td>27.00±1.15</td>
<td>1.75±0.14</td>
</tr>
<tr>
<td></td>
<td>WLH</td>
<td>8.50±0.57</td>
<td>7.05±0.26</td>
<td>22.57±2.07</td>
<td>1.52±0.10</td>
</tr>
<tr>
<td>IV</td>
<td>Kadaknath</td>
<td>7.12±0.22</td>
<td>5.73±0.19</td>
<td>23.25±0.77</td>
<td>1.95±0.05</td>
</tr>
<tr>
<td></td>
<td>WLH</td>
<td>8.38±0.15</td>
<td>7.52±0.12</td>
<td>17.48±1.02</td>
<td>1.78±0.16</td>
</tr>
</tbody>
</table>
Plate 05: Photomicrograph - Left ovary (group I, Kadaknath) showing germinal epithelium (Ge) and developing follicles (F). H&E X 400

Plate 06: Photomicrograph - Left ovary (group I, WLH) showing germinal epithelium (Ge) and developing follicles (F). H&E X 400

Plate 07: Photomicrograph - Left ovary (group I, Kadaknath) showing germinal epithelium (Ge) and primordial follicles (F). H&E X 400

Plate 08: Photomicrograph - Left ovary (group I, Kadaknath) showing primary follicles (F). H&E X 400
Plate 09: Photomicrograph - Left ovary (group I, Kadaknath) showing different stages of follicles (F), yolk (Y) and fibromelanin (Fm). H&E X 100

Plate 10: Photomicrograph - Left ovary (group I, WLH) showing scattered follicles (F). H&E X 40

Plate 11: Photomicrograph - Left ovary (group I, Kadaknath) showing cluster of developing follicles (F), yolk (Y) and fibromelanin (Fm). H&E X 400

Plate 12: Photomicrograph - Left ovary (group I, Kadaknath) showing secondary follicles (F), yolk (Y) and fibromelanin (Fm). H&E X 100
Plate 13: Photomicrograph - Left ovary (group I, Kadaknath) showing granulosa layer (Gr), theca interna (Ti), theca externa (Te) and yolk (Y) in a large follicle. H&E X 400

Plate 14: Photomicrograph - Left ovary (group I, WLH) showing granulosa layer (Gr), theca interna (Ti), theca externa (Te) and yolk (Y) in a large follicle. H&E X 400

Plate 15: Photomicrograph - Left ovary (group II, Kadaknath) showing large follicle (F), yolk (Y) and fibromelanin (Fm) in the medulla. H&E X 50

Plate 16: Photomicrograph - Left ovary (group IV, Kadaknath) showing developing follicles (F) and blood vessel (BV). H&E X 100
Plate 20: Photomicrograph - Left ovary (group I, WLH) showing post ovulatory follicle (Pf). H&E X 100

Plate 21: Photomicrograph - Left ovary (group I, WLH) showing post ovulatory follicle containing vacuolated cells (Vc) and fibrocytes (Fc). H&E X 400

Plate 22: Photomicrograph - Left ovary (group IV, WLH) showing post ovulatory follicle (Pf). H&E X 400

Plate 23: Photomicrograph - Left ovary (group I, WLH) showing hemosiderin pigment (H) in post ovulatory follicle. H&E X 400
Plate 17: Photomicrograph - Left ovary (group III, WLH) showing post ovulatory follicles (Pf). H&E X 100

Plate 18: Photomicrograph - Left ovary (group IV, WLH) showing post ovulatory follicles (Pf). H&E X 100

Plate 19: Photomicrograph - Left ovary (group IV, WLH) showing abundant fibrocytes (Fc) in between the post ovulatory follicles (Pf). H&E X 400
Plate 24: Photomicrograph - Cross section of infundibulum (group I, Kadaknath) showing small mural folds (Mf). H&E X 100

Plate 25: Photomicrograph - Cross section of infundibulum (group I, WLH) showing large mural folds (Mf), propria submucosa (Ps) and tunica serosa (S). H&E X 100

Plate 26: Photomicrograph - Cross section of infundibulum (group I, Kadaknath) showing epithelium (E). H&E X 400

Plate 27: Photomicrograph - Cross section of infundibulum (group I, WLH) showing mural folds (Mf), epithelium (E) and tubular glands (Tg). H&E X 400
Plate 28: Photomicrograph - Cross section of infundibulum (group II, Kadaknath) showing mural folds (Mf) and epithelium (E). H&E X 400

Plate 29: Photomicrograph - Cross section of infundibulum (group II, WLH) showing mural folds (Mf) and epithelium (E). H&E X 400

Plate 30: Photomicrograph - Cross section of infundibulum (group III, Kadaknath) showing epithelium (E) and propria submucosa (Ps). H&E X 400

Plate 31: Photomicrograph - Cross section of infundibulum (group III, WLH) showing epithelium (E) and propria submucosa (Ps). H&E X 400
Plate 32: Photomicrograph - Cross section of infundibulum (group IV, Kadaknath) showing mural folds (Mf). H&E X 100

Plate 33: Photomicrograph - Cross section of infundibulum (group IV, WLH) showing small mural folds (Mf). H&E X 100

Plate 34: Photomicrograph - Cross section of magnum (group I, Kadaknath) showing epithelium (E) and developing glands (G). H&E X 400

Plate 35: Photomicrograph - Cross section of magnum (group I, WLH) showing epithelium (E) and densely packed glands (Tg) in the propria submucosa. H&E X 400
Plate 36: Photomicrograph - Cross section of magnum (group II, Kadaknath) showing mural folds (Mf) and densely packed glands in the propria submucosa (Ps). H&E X 100

Plate 37: Photomicrograph - Cross section of magnum (group II, WLH) showing mural folds (Mf) and glands in the propria submucosa (Ps). H&E X 100

Plate 38: Photomicrograph - Cross section of magnum (group II, Kadaknath) showing epithelium (E) and densely packed tubular glands (Tg) in propria submucosa. H&E X 400

Plate 39: Photomicrograph - Cross section of magnum (group II, WLH) showing epithelium (E) and tubular glands (Tg) in propria submucosa. H&E X 400
Plate 40: Photomicrograph - Cross section of magnum (group III, Kadaknath) showing densely packed glands in the propria submucosa (Ps). H&E X 100

Plate 41: Photomicrograph - Cross section of magnum (group III, WLH) showing glands in the propria submucosa (Ps). H&E X 100

Plate 42: Photomicrograph - Cross section of magnum (group IV, Kadaknath) showing mural folds (Mf), tunica muscularis (Tm) and blood vessel (BV). H&E X 40

Plate 43: Photomicrograph - Cross section of magnum (group IV, WLH) showing mural folds (Mf) and tunica muscularis (Tm). H&E X 40
Plate 44: Photomicrograph - Cross section of isthmus (group I, Kadaknath) showing epithelium (E) and fibromelanin (Fm) in the core of folds. H&E X 400

Plate 45: Photomicrograph - Cross section of isthmus (group I, WLH) showing epithelium (E) and tubular glands (Tg) in propria submucosa. H&E X 400

Plate 46: Photomicrograph - Cross section of isthmus (group II, Kadaknath) showing mural folds (Mf) and densely packed glands in the propria submucosa (Ps). H&E X 100

Plate 47: Photomicrograph - Cross section of isthmus (group II, WLH) showing mural folds (Mf) and glands in propria submucosa (Ps). H&E X 100
Plate 48: Photomicrograph - Cross section of isthmus (group III, Kadaknath) showing mural folds (Mf) and tunica muscularis (Tm). H&E X 40

Plate 49: Photomicrograph - Cross section of isthmus (group III, WLH) showing mural folds (Mf) and tunica muscularis (Tm). H&E X 40

Plate 50: Photomicrograph - Cross section of isthmus (group III, Kadaknath) showing epithelium (E) and tubular glands (Tg) in propria submucosa (Ps). H&E X 400

Plate 51: Photomicrograph - Cross section of isthmus (group III, WLH) showing epithelium (E) and tubular glands (Tg) in propria submucosa (Ps). H&E X 400
Plate 52: Photomicrograph - Cross section of shell gland (group I, Kadaknath) showing mural folds (Mf) and tunica muscularis (Tm). H&E X 100

Plate 53: Photomicrograph - Cross section of shell gland (group I, WLH) showing mural folds (Mf) and glands in propria submucosa (Ps). H&E X 100

Plate 54: Photomicrograph - Cross section of shell gland (group I, Kadaknath) showing epithelium (E) and fibromelanin (Fm) in the core of the mural fold. H&E X 400

Plate 55: Photomicrograph - Cross section of shell gland (group I, WLH) showing epithelium (E) and tubular glands (Tg) in the propria submucosa. H&E X 400
Plate 56: Photomicrograph - Cross section of shell gland (group II, Kadaknath) showing mural folds (Mf), fibromelanin (Fm) and tunica muscularis (Tm). H&E X 100

Plate 57: Photomicrograph - Cross section of shell gland (group II, WLH) showing mural folds (Mf) and tunica muscularis (Tm). H&E X 100

Plate 58: Photomicrograph - Cross section of shell gland (group II, Kadaknath) showing epithelium (E), tubular glands (Tg) in the propria submucosa and fibromelanin (Fm) in the core of the mural fold. H&E X 400

Plate 59: Photomicrograph - Cross section of shell gland (group II, WLH) showing epithelium (E) and tubular glands (Tg) in the propria submucosa. H&E X 400
Plate 60: Photomicrograph - Cross section of shell gland (group III, Kadaknath) showing epithelium (E) and tubular glands (Tg) in the propria submucosa. H&E X 400

Plate 61: Photomicrograph - Cross section of shell gland (group III, WLH) showing epithelium (E) and tubular glands (Tg) in the propria submucosa. H&E X 400

Plate 62: Photomicrograph - Cross section of shell gland (group IV, Kadaknath) showing epithelium (E), tubular glands (Tg) and collagen fibers ( yellow arrow) in the propria submucosa. H&E X 400

Plate 63: Photomicrograph - Cross section of shell gland (group IV, WLH) showing epithelium (E), tubular glands (Tg) and collagen fibers ( yellow arrow) in the propria submucosa. H&E X 400
Plate 64: Photomicrograph - Cross section of vagina (group I, Kadaknath) showing mural folds (Mf), fibromelanin (Fm) and tunica muscularis (Tm). H&E X 100

Plate 65: Photomicrograph - Cross section of vagina (group I, WLH) showing mural folds (Mf) and tunica muscularis (Tm). H&E X 100

Plate 66: Photomicrograph - Cross section of vagina (group II, Kadaknath) showing long mural folds (Mf). H&E X 40

Plate 67: Photomicrograph - Cross section of vagina (group II, WLH) showing mural folds (Mf) and tunica muscularis (Tm). H&E X 40
Plate 68: Photomicrograph - Cross section of vagina (group III, Kadaknath) showing mural folds (Mf). H&E X 100

Plate 69: Photomicrograph - Cross section of vagina (group III, WLH) showing mural folds (Mf), tunica muscularis (Tm) and collagen fibers in the core of folds. H&E X 100

Plate 70: Photomicrograph - Cross section of vagina (group IV, Kadaknath) showing mural folds (Mf) and collagen fibers in the core of mural folds. H&E X 100

Plate 71: Photomicrograph - Cross section of vagina (group IV, WLH) showing mural folds (Mf) and collagen fibers in the core of mural folds. H&E X 100
Plate 72: Photomicrograph - Cross section of vagina (group II, Kadaknath) showing collagen fibers (↑). Van Gieson’s stain X 100

Plate 73: Photomicrograph - Cross section of infundibulum (group IV, Kadaknath) showing collagen fibers (↑). Van Gieson’s stain X 100

Plate 74: Photomicrograph - Cross section of magnum (group III, Kadaknath) showing collagen fibers (↑). Van Gieson’s stain X 40

Plate 75: Photomicrograph - Cross section of shell gland (group II, WLH) showing collagen fibers (↑). Van Gieson's stain X 100
Plate 78: Photomicrograph - Cross section of magnum (group II, WLH) showing moderate positive reaction for Acid mucopolysaccharides (↑). Mowry’s colloidal iron stain X 100

Plate 79: Photomicrograph - Cross section of vagina (group II, Kadaknath) showing moderate positive reaction for Acid mucopolysaccharides (↑). Mowry’s colloidal iron stain X 100
Plate 80: Photomicrograph - Cross section of magnum (group IV, Kadaknath) showing intense positive reaction (↑) in epithelium and moderate in glands. PAS stain X 100

Plate 81: Photomicrograph - Cross section of magnum (group I, WLH) showing intense positive reaction (↑) in epithelium and moderate in glands. PAS stain X 400

Plate 82: Photomicrograph - Cross section of magnum (group II, Kadaknath) showing moderate positive reaction (↑) in the luminal content. Ninhydrin Schiff’s X 400

Plate 83: Photomicrograph - Cross section of isthmus (group II, WLH) showing mild positive reaction (↑) in the glandular epithelium. Ninhydrin Schiff’s X 100
Plate 84: Photomicrograph - Cross section of shell gland (group III, Kadaknath) showing positive reaction (↑) for calcium in epithelium. Von Kossa X 100

Plate 85: Photomicrograph - Cross section of shell gland (group II, Kadaknath) showing positive reaction (↑) for calcium in epithelium. Von Kossa X 100
The mean length of the isthmus was greater in WLH than Kadaknath in all the groups. It was approximately ten times (0.93±0.06 cm in Kadaknath and 10.53±0.88 cm in WLH) greater in WLH in group I than Kadaknath. This difference was greatly reduced in group II (6.40±0.50 cm in Kadaknath and 8.60±0.17 cm in WLH).

Mean length of shell gland was approximately four times in WLH as compared to Kadaknath. However, in later groups the length of shell gland became approximately equal in both the groups (Table 07).

The mean length of the vagina was 2.48±0.07 cm in group I of Kadaknath which increased to 4.77±0.22 cm in group IV. In WLH the mean length of vagina was 5.08±0.07 cm in group I which decreased to 4.25±0.19 cm in group II. In later group there was gradual increase of mean length of vagina up to group IV and became 4.77±0.22 cm (Table 07).

4.2 Histology

4.2.1 Ovary

Histological observation of the ovary revealed that the division of the ovary into cortex and medulla was evident in group I of Kadaknath. However, in later groups of Kadaknath and all the groups of WLH the division between cortex and medulla was not seen.

The surface epithelium was made up of single layer of cuboidal cells with spherical to oval nuclei. However, squamous epithelium was also noticed at some places. Tunica albugenia was inconspicuous. Developing follicles were seen in the outer cortical zone just beneath the germinal epithelium in group I of both the breeds. Proliferation was noticed in germinal epithelium at several places and cells were seen migrating from here into the cortical area (Plate 05 and 07).

The follicles were categorized into four groups as per their size 0-50 μm, 51-100 μm, 101-200 μm and above 200 μm. In group I the follicles below 50 μm diameter were 21.24 and 3.10 per cent in Kadaknath and WLH,
respectively. The follicles above 200 µm diameter were normal and post ovulatory follicles.

The follicles below 50 µm diameter (primordial follicle) were lined by single layer of flattened cells with centrally placed primordial germ cells. There was no yolk present in the follicle. These follicles were observed just below the germinal epithelium in the outer cortical zone. These follicles were scattered in the cortex (Plate 07).

In group I the follicles were seen in clusters in Kadaknath. However, in WLH these were scattered with presence of abundant stroma/ loose connective tissue in between the follicles. Number of follicles was observed in the ovary at varying stages of development (Plate 09 and 10).

Occurrence of follicles between 51-100 µm (primary follicles) were maximum (37.93%) in group III of Kadaknath. However, in WLH it was maximum in group I (7.14%). In group I of Kadaknath these follicles were present in clusters in the outer (cortical) zone as well as in the deeper part of the ovary. Fibromelanin was seen in between the follicles (Plate 09 and 10). These follicles were lined by single layer of cuboidal epithelium. The nuclei were oval or round shaped. In some of the cells more than one nucleoli was visible. This layer is surrounded by flattened/ stromal cells and these cells formed almost continuous layers (Plate 08). Yolk filled in the lumen of follicle gives the vacuolated appearance due to dissolution of the yolk during the tissue processing.

The follicles ranging between 101-200 µm were lined by polyhedral/ cuboidal/ columnar granulosa cells. Nuclei of the cells were spherical, ovoid to elongated. At some places mitotic figures were also seen in the epithelium. This layer was surrounded by loosely arranged connective tissue layer with different types of oval, elongated and spindle shaped cells. In the peripheral region small blood vessels were present. Yolk was present in the follicle. Occurrence of these follicles was maximum in group I of Kadaknath and group II of WLH (Table 08).
In group I of Kadaknath no follicle was seen above 200 µm diameter. However, in WLH 75.57 per cent follicles were present above 200 µm diameter (Table 08). The average thickness of follicular epithelium above 200 µm diameter was more in WLH in all the groups except in group IV in which wall thickness was almost equal (25.36 µm in Kadaknath and 25.35 µm in WLH.

The normal follicles were lined with large granulosa cell layer having single layer of cuboidal cells but in some places pseudostratified epithelium was also seen having large oval to round nuclei. Cytoplasm was granular. The basement membrane of granulosa cells was well marked in these follicles (Plate 12). In some cells more than one nucleoli were present. This layer was surrounded by theca externa which was comprised of spindle/round/oval shaped cells. Theca externa was loosely packed layer surrounded by the theca interna (Plate 12). This layer has abundant blood capillaries. Theca externa was wider than theca interna. The narrower theca interna was composed of collagen fibers and fibroblast like cells formed of compact layer of spindle shaped cells.

In general the large follicles were round to oval shaped in Kadaknath with loosely arranged thecal layer. However, in WLH the follicles were irregular in outline with compact thecal layer (Plate 13 and 14).

In Kadaknath up to group IV developing and large follicles were seen (Plate 15 and 16). However, in WLH the post ovulatory follicles were seen in all the groups which were predominantly present from group III onwards. In group IV of WLH abundant connective tissue/fibrocytes occupied the spaces between the post ovulatory follicles (Plate 17, 18 and 19).

The post ovulatory follicles were filled with vacuolated cells and stromal cells which were considered to be derive from the thecal layer. The yellow pigment hemosiderin was also seen in some of the post ovulatory follicles (Plate 20, 21, 22 and 23).
4.2.2 Oviduct

The left oviduct consisted of five regions namely infundibulum, magnum, isthmus, shell gland and vagina. These divisions have structural differences. The different parts of the left oviduct showed mural folds. The folds varied in length as per breed and group.

Infundibulum

In group I the infundibulum had dorso-ventrally elongated lumen with average diameter of 896.43±1.54 µm and 1604±1.67 µm, respectively in Kadaknath and WLH. The wall thickness was less in all the groups of WLH and Kadaknath in comparison to other parts of oviduct. The mural folds were less developed in group I of Kadaknath fowl however, in WLH these folds were well developed, long with primary, secondary and tertiary folds (Plate 24 and 25).

The epithelium was pseudostratified comprised of single layer of ciliated columnar epithelium with goblet cells which was more in height and darkly stained in Kadaknath in comparison to WLH. The cilia were well developed and long in WLH. However, in Kadaknath ill developed cilia were observed (Plate 26).

In group I the propria submucosa was dense in Kadaknath in comparison to WLH with abundant adipocytes whereas, in WLH it was comprised of areolar connective tissue and collagen fibers with diffused lymphatic tissue.

In group II these folds became well defined with secondary and tertiary folds in Kadaknath. The height of epithelium was 14.69±0.45 µm in group I of Kadaknath which gradually increased and measured 19.71±0.45 µm in group IV.

In group II of Kadaknath the epithelium of infundibulum became taller with long well developed cilia and the propria submucosa composed of areolar tissue with fibroblasts, plasma cells and blood vessels. Glands
appeared in the propria submucosa in group II of Kadaknath however, in WLH these were well developed in group I. The glandular tissue increased up to group IV in Kadaknath whereas in WLH in the last group the signs of regression were observed (Plate 32 and 33). Collagen fibers were observed in propria submucosa which extends into the core of the mural folds in all the groups (Plate 73).

Muscle bundles were composed of scattered bundles of longitudinal smooth muscles which became pronounced in later groups of both the breeds except in group IV of WLH where it was decreased (Plate 33). Thickness of tunica muscularis was also greater in Kadaknath in comparison to WLH in all the groups. In Kadaknath it increased from group I to group IV. However, in WLH it was 121.83±1.35 µm in group I which increased to 147.33±2.26 µm in group III then decreased to 134.58±1.00 µm in group IV.

**Magnum**

The lumen of the magnum was elongated in group I of Kadaknath which became irregular star shaped in later groups and all the groups of WLH. The wall of the magnum presented tall, thick longitudinal primary and secondary folds in all the groups of WLH and Kadaknath except in group I of Kadaknath where these folds were short and blunt with few secondary folds. The number of primary folds in group I were 08 and 12 in Kadaknath and WLH, respectively which increased to 15 and 17 in later groups.

The wall thickness of magnum was 315.92±1.79 µm in group I which increased to 685.67±1.71 µm in group IV of Kadaknath fowl. The wall thickness was greater in WLH in comparison to Kadaknath except in group IV where total wall thickness in Kadaknath (685.67±1.71 µm) was greater than WLH (448.54±3.02 µm).

The epithelium was ciliated simple columnar with goblet cells. The height of epithelium was 15.05±0.46 µm and 14.69±0.45 µm in group I of Kadaknath and WLH, respectively. In Kadaknath it increased to 17.56±0.45 µm in group IV. However, it increased up to group III in WLH (23.29±0.45 µm) and then decreased to 14.69±0.45 µm in group IV (Table 10).
Developing glands were seen in the propria submucosa of group I of Kadaknath (Plate 34). However, in WLH well developed long, branched, coiled tubular glands were observed in the propria submucosa which completely filled the propria submucosa with intense staining affinity (Plate 35). In group II highly developed long, branched, coiled tubular glands were observed which completely packed the propria submucosa of the magnum. The cells of the gland were pyramidal and contained basophilic granules (Plate 36 and 38). The density of glands decreased from group III onwards in Kadaknath and group II onwards in WLH (Plate 37, 39, 40, 41, 42 and 43).

Collagen fibers were seen in the propria submucosa and extend into the core of folds. Collagen fibers bundles was seen in the tunica serosa, surrounding the blood vessels and adjacent to the tunica muscularis (Plate 74).

Fibromelanin was seen in the form of thick thread like structure in tunica serosa and propria submucosa of the oviduct in all the groups of Kadaknath.

**Isthmus**

In group I of Kadaknath the lumen of the isthmus was elongated with 06 primary folds whereas secondary folds were not pronounced. However, in WLH 20 longitudinally oriented flat leaf like or angular folds were present with 06 secondary folds in all the groups (Plate 49). From group II onwards the folds became well defined angular with secondary folds were seen in Kadaknath (Plate 46 and 48).

The epithelium was simple columnar ciliated and goblet cells were seen in the epithelium (Plate 44, 45, 50 and 51). The height of epithelium was 14.33±0.43 μm in group I of Kadaknath which increased to 20.43±0.40 μm in group IV of Kadaknath. However, in WLH epithelial height increased to 25.44±0.25 μm in group III which decreased to 18.99±0.45 μm in last group (Table 11).
In group I of Kadaknath developing glands were seen in the propria submucosa of core of the folds. However, in WLH well developed branched, tubular glands were observed which completely filled the propria submucosa. These glands completely fill the propria submucosa in group II and III of Kadaknath. Glands were less pronounced in all the groups with progression of age in WLH (Plate 47 and 51).

Shell gland

The thickness of shell gland wall was 296.08±1.79 μm in group I of Kadaknath which increased to 636.08±1.79 μm in group IV. However, the thickness of shell gland was 497.25±1.57 μm in group I of WLH which was maximum in group III (600.67±1.71 μm) thereafter, it decreased to 531.25±4.71 μm in group IV.

The height of epithelium was 19.35±0.46 μm and 24.37±0.43 μm in group I of Kadaknath and WLH, respectively. In Kadaknath it increased to 25.08±0.34 μm in group IV. The height of epithelium was maximum in group II of WLH (28.31±0.45 μm) thereafter, it decreased to 19.35±0.46 μm in group IV.

The thickness of tunica muscularis was maximum in group IV of Kadaknath (196.92±1.79 μm) as compared to WLH in which it was maximum in group III (162.92±1.79 μm) (Table 12).

In group I of Kadaknath the shell gland had elongated lumen and the wall presented 08 numbers of wide, short and discoid primary folds with occasional secondary folds (Plate 52). From group II onwards the folds became extensively longer, narrower and pedunculated with variable number of secondary folds. In WLH these folds were in the form of elongated triangular elevations. Secondary folds were also observed in group I (Plate 53).

The epithelium was ciliated pseudostratified columnar in all the groups. In group I of Kadaknath propria submucosa composed of loose connective tissue with predominantly cellular population and few developing glands were seen in an outer zone of propria submucosa (Plate 54).
In the later groups well developed branched, coiled, tubular glands were observed which completely filled the propria submucosa. In group II of WLH the glands in lamina propria were loosely packed in comparison to group I of WLH and group II of Kadaknath (Plate 55, 58 and 59). In group III of WLH the glands became more scattered than previous group of WLH. Tunica muscularis was comprised of inner circular and outer longitudinal smooth muscle bundles. In between these two stratum vasculare is observed with number of thick walled blood vessels. Collagen fibers were present in the form of bundles in sub-epithelial region and in middle of the core of folds (Plate 62 and 63).

**Vagina**

The thickness of vaginal wall was greater in all the groups of Kadaknath fowl in comparison to WLH. The thickness of vaginal wall was maximum in group IV of Kadaknath whereas in WLH, it was maximum in group III (Table 13).

The height of epithelium was increased with progression of age in all the groups of Kadaknath. However, in WLH it increased up to group III and sharply decreased in group IV (Table 13). The epithelium of the vagina was ciliated pseudostratified columnar with few goblet cells.

In group I of Kadaknath propria submucosa showed intense staining affinity with high cellular content. In later group the propria submucosa became less cellular and more fibrous with abundant fibroblast and fibrocytes (Plate 64, 68 and 70).

The thickness of tunica muscularis was greater in vagina in comparison to other segments of the oviduct in both the breeds. It was $178.50\pm1.81 \mu m$ and $226.67\pm1.71 \mu m$ in group I of Kadaknath and WLH, respectively. It reached to maximum in group IV ($687.08\pm1.79 \mu m$) in Kadaknath and group III ($277.67\pm1.71 \mu m$) in WLH (Table 13).

The mural folds of the vagina showed irregular pattern composed of short, blunt folds and long folds with secondary folds in group I of Kadaknath (Plate 64). From group II onwards these folds became taller and narrower with secondary folds extending into the lumen of vagina (Plate 66,
Average number of primary folds were 10 in WLH, well developed extensive narrow mural folds were noticed having secondary and tertiary folds in all the groups (Plate 65, 67, 69 and 71). Number of primary, secondary and tertiary folds was 26, 7 and 2.

**Histochemistry**

In the oviduct all the three types of fibers were noticed, amongst them collagen fibers were short present in the propria submucosa and extending into the core of mucosal folds (Plate 72 and 76). It was also observed in between the muscle bundles and in tunica serosa of the oviduct (Plate 73 and 74).

Network of fine reticular fibers were seen in the central part of the core of the folds of oviduct and in the basement membrane of the glands in both the breeds (Plate 77). Elastic fibers were very less and noticed in the propria submucosa, extending in to the core of folds and in tunica serosa (Plate 78).

Mowry’s colloidal iron method used for demonstration of acid mucopolysaccharides in left ovary showed the positive reaction in the connective stroma in between the follicles. Moderate positive reaction was seen in the propria submucosa of all the parts of oviduct (Plate 78 and 79).

Mild to moderate PAS positive reaction was noticed in the germinal epithelium and granulosa cell layer of the large follicles. In the oviduct the apical part of the epithelium of magnum and isthmus showed moderate PAS positive reaction. Mild to moderate PAS reaction was seen in the epithelium of the glands of magnum and isthmus (Plate 80 and 81).

The luminal content of the magnum showed mild to moderate reaction for the Ninhydrin Schiff’s reaction and glandular epithelium of isthmus showed mild reaction (Plate 82 and 83).

Epithelium of shell gland showed positive reaction for calcium in all the groups of Kadaknath and WLH, except group I of Kadaknath (Plate 84 and 85). Sudan black- B reaction showed the intense positive reaction in the follicular content of the large follicle and moderate reaction in the follicular epithelium.
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Table 09: Range and mean (µm) of different parameters of infundibulum in group I to IV of Kadaknath and White Leghorn breeds of fowl

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Table 10: Range and mean (µm) of different parameters of magnum in group I to IV of Kadaknath and White Leghorn breeds of fowl

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Table 11: Range and mean (μm) of different parameters of isthmus in group I to IV of Kadaknath and White Leghorn breeds of fowl

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Table 13: Range and mean (µm) of different parameters of vagina in group I to IV of Kadaknath and White Leghorn breeds of fowl

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5. DISCUSSION

Gross

Ovary

The colour of the ovary in Kadaknath was grayish in all the groups is indicative of presence of fibromelanin in gonads. This finding confirms the presence of fibromelanin in gonads. The present observation supports the reports of Lukanov and Genchev (2013). They reported the presence of the fibromelanosis in some of the breeds of fowl of south eastern Asia. These breeds are Kadaknath in India, Silkie in Japan/ Europe and Silkie Bairiong in China.

From group II onward in Kadaknath and all the groups of WLH the left ovary was also related with the right kidney is in agreement with the study of King (1975). Mean ovarian weight was 28.23±1.46 g in Kadaknath and 36.07±1.57 g in WLH is in line with the study of Rahman et al. (1999). They reported the mean weight of ISA Brown as 41.89 g and in indigenous deshi hen as 27.65 g.

The shape of the ovary was elongated triangular at 24 weeks of Kadaknath and in the form of bunch of grapes in later groups is indicative of that till date laying was not started in Kadaknath. However, in the same age group in WLH it was in the form of bunch of grapes showed the active laying period in WLH. The present investigation is confirmed by the report of Silversides et al. (2006). They reported that WLH starts laying at 19 weeks of age.

Oviduct

The mean length of infundibulum was 7.12±0.22 cm in group IV of Kadaknath is in agreement with the study of Garg (2006) and Mishra et al. (2014). They reported the mean length in Kadaknath and native chicken as 7.80±0.23 cm and 7.18±4.22 cm, respectively.

The mean length of infundibulum was 8.38±0.15 cm in group IV of WLH which is in accordance with the Sturkie (1976) and Naragude et al. (1999a). They measured the length of infundibulum as 2 to 11 cm and 7.37±0.08 cm in RIR birds respectively.
The length of the magnum was 27.00±1.15 cm in group III of Kadaknath which is in accordance with the Garg (2006). He reported the length of magnum as 29.80 cm in Kadaknath. However, Mishra et al. (2014) reported mean length of magnum in native chicken of Bangladesh 18.40±6.75 cm which is less than the length of magnum of Kadaknath and WLH.

The length of the isthmus was 10.53±0.88 cm in group I of WLH which is in the agreement with the Bradley and Grahame (1960) and Sturkie (1976). They reported the mean length of isthmus as 3.00 to 12.00 cm in laying hens. The length of the isthmus was 6.40±0.50 cm in group II of Kadaknath. This finding is in line with the Garg (2006) and Mishra et al. (2014). They recorded the length of isthmus as 5.42 cm in Kadaknath fowl and 7.68±3.46 cm in native chicken of Bangladesh, respectively.

The length of the shell gland was 5.82±0.45 cm in group II of Kadaknath. This is in accordance with the King (1975) and Mishra et al. (2014) who reported the length as 4.00-12.00 cm and 6.10±2.80 cm respectively. However, Garg (2006) reported the mean length of Kadaknath fowl as 9 cm in 26 weeks.

The length of the vagina was 4.33±0.13 cm and 4.25±0.19 cm in group II of Kadaknath and WLH. This result is in line with King (1975) and Giersberg (1922). They reported the length of vagina as 4.00-12.00 cm and Mishra et al. (2014) reported it as 6.83±2.82 cm.

The coils of the oviduct were not seen in group I of Kadaknath whereas 4-5 coils were observed in rest groups of Kadaknath and all the groups of WLH. This observation supports the finding of Ingole (1985) who reported the presence of 04 coils in dwarf birds and 04 in normal birds at 18 weeks of age. In Kadaknath absence of coiling might be due to the reason that till date laying was not started.

Morphometrical data showed that there was five fold increase in length and ten fold increase in weight of the oviduct from group I to group II of Kadaknath. This observation slightly differ from the findings of the Romanoff and Romanoff (1949) who reported that the increase in length from resting to laying period was four fold and increase in weight was 15 to 20 times.
**Histology**

**Ovary**

Distinction between the cortex and medulla was present only in group I of Kadaknath is in line with the finding of (Bradley and Grahame, 1960; Das and Biswal, 1968; Hodges, 1974; Rao and Vijayaragvan, 1999a; Bacha and Bacha, 2000 and Aughey and Frye, 2001). In rest of the groups of Kadaknath and all the groups of WLH the distinction between cortex and medulla was lost or not evident is in accordance with the findings of (Rao and Vijayaragvan, 1999a and King, 1975) who reported that with the onset of sexual maturity the distinction between cortex and medulla was virtually lost.

The surface epithelium was made up of single layer of cuboidal cells however squamous epithelium was also noticed at some places. This observation is in line with the reports of Das and Biswal (1968); Hodges (1974) and Rao and Vijayaragvan (1999a). The follicles were categorized in to four groups.

The surface epithelium was made up of single layer of cuboidal cells with spherical to oval nuclei, squamous epithelium was also noticed at some places. This finding corroborates with the findings of Das and Biswal, 1968; Hodges (1974) and Rao and Vijayaragvan (1999a) reported the simple squamous to cuboidal epithelium.

Accumulation of small sized follicles was the characteristic feature of the Kadaknath is in line with the observation of Shyam et al., 2015. They observed similar finding in Aseel.

Small follicles (51- 100 µm) diameter was lined with single layer of cuboidal epithelium. The nuclei were oval to round. This layer was surrounded by flattened/ stromal cells which formed the continuous layer is in agreement with the findings of Shyam et al. (2015).

The large sized follicles (above 200 µm diameter) had distinct large granulose cells, theca interna and theca externa. Theca externa was wider than the theca interna. Theca externa was composed collagen fibers and fibroblast like cells. Membrana granulosa was in the form of single layer
of cuboidal or pseudostratified cells. Similar finding was reported by Bacha and Bacha (2000). Some of the post ovulatory follicles were filled with vacuolated cells and others with yellow pigmented cells supports the observation of Aitken (1966).

**Oviduct**

The wall of oviduct was comprised of tunica mucosa, tunica submucosa, tunica muscularis and tunica serosa from within outwards, which corroborates with the reports of Banerjee *et al.* (2006), Garg (2006) Shyam (2012). Oviduct comprised of luminal epithelium, glands, connective tissue, muscle layer and outer serosa which is in accordance with the King (1975), Gopinath and Hafeezuddin (1980) and Ingole (1985).

**Infundibulum**

In 24 weeks of Kadaknath mural folds were less developed but became well developed at 32 weeks of age might be due to that at 24 weeks laying was not started in Kadaknath which was further supported by Parmar *et al.* (2003) that laying started at 29 weeks of age in Kadaknath. The epithelium of infundibulum was pseudostratified ciliated columnar epithelium with goblet cells. This result is in line with the findings of Bakst and Howarth (1974); Gopinath and Hafeezuddin (1980); Naragude *et al.* (1999a) and Mehta *et al.* (2005).

The thickness of infundibulum wall was 262.08±1.79 µm and 185.58±1.79 µm at 24 weeks of Kadaknath and WLH, respectively. However, Shyam (2012) reported thickness of wall as 105.50 µm and 133 µm in Aseel and RIR respectively at 20 weeks of age. Also, Ingole (1985) noticed it as 273.60 µm and 279.30 µm in Narmada XI and normal WLH birds at 18 weeks of age. At 48 weeks of age the thickness of infundibulum wall was 420.75±3.01 µm and 213.92±1.79 µm in Kadaknath and WLH respectively. However, Shyam (2012) reported it as 243.00 µm and 282.00 µm in Aseel and RIR, respectively at 13 months of age.
Magnum

At 48 weeks of age thickness of magnum wall was 685.67±1.71 µm and 448.54±3.02 µm in Kadaknath and WLH respectively. However, at 13 months of age Shyam (2012) reported magnum wall length as 400.25 µm and 444.25 µm in Aseel and RIR respectively. The mean height of epithelium at 48 weeks of age was 17.56±0.45 µm in Kadaknath and WLH respectively. However, Shyam (2012) reported it as 22.53 µm and 19.06 µm in Aseel and RIR respectively. At 24 weeks of age height of epithelium in Kadaknath was 15.05±0.46 µm which was greater than present findings with an average of 24.60 µm.

The epithelium was ciliated simple columnar with goblet cells. The present observation is in line with Islam et al. (2002) and (Bharti and Gautam, 2013) as they recorded simple columnar ciliated epithelium. However, Mehta et al. (2005) reported pseudostratified columnar epithelium with goblet cells. Primary and secondary folds were observed in present study in Kadaknath and WLH except in 24 weeks of Kadaknath. The present finding is in agreement with Garg (2006) and (Bharti and Gautam, 2013). But (Nickel et al., 1977; King, 1975 and Gilbert, 1970) found only primary folds.

In propria submucosa of Kadaknath and WLH well developed long branched coiled tubular glands were present except 24 weeks of Kadaknath. The present study is in accordance with the (Bharti and Gautam, 2013) who also observed long branched coiled tubular glands in propria submucosa in indigenous chicken of Assam.

Isthmus

In 24-48 weeks of WLH and 32-48 weeks of Kadaknath the mural folds were longitudinally oriented flat leaf like or angular in shape. The present study is in accordance with the Garg (2006).

The thickness of isthmus wall was 527.00±4.44 µm and 488.75±3.01 µm at 48 weeks of Kadaknath and WLH respectively. However, Shyam (2012) recorded it as 436.15 µm and 310.25 µm in Aseel and RIR at
13 months of age. At 32 weeks in Kadaknath, the thickness of wall was 382.50±1.81 µm which is in agreement with the result of Garg (2006) as he reported it as 387.80 µm at 26 weeks of age in Kadaknath.

The thickness of isthmus wall was increased with the progression of age in Kadaknath. This indicates that the glandular portion increases with age. However, in WLH it increased up to 40 weeks of age showed less glandular activity. The height of epithelium was maximum in 48 weeks of Kadaknath and 40 weeks of WLH show maximum activity of isthmus. The epithelium was simple columnar ciliated with goblet cells. The present study is in line with Naragude et al. (2000). However, Garg (2006) and Shyam (2012) reported ciliated pseudostratified columnar epithelium.

Shell gland

At 24 weeks of Kadaknath wide, short and discoid primary folds with occasional secondary folds were present. However, Islam et al. (2002) reported it as dome shaped at 12 weeks and elongated at 19 and 30 weeks old desi chicken. From 32 weeks onward in Kadaknath the folds became extensively longer, narrower and pedunculated with variable number of secondary folds corroborates with the findings of Mehta et al. (2005) and Mishra et al. (2014).

The average epithelial height at 48 weeks of age was 25.08±0.34 µm and 19.35±0.46 µm in Kadaknath and WLH respectively. However, Shyam (2012) observed it as 21.06 µm and 23.73 µm in Aseel and RIR respectively at 13 months of age. The epithelium was ciliated pseudostratified columnar in the present study. This study is in line with Hodges (1974). Shyam (2012) reported pseudostratified columnar epithelium at some places.

From 32 weeks of Kadaknath and all groups of WLH had well developed branched coiled tubular glands in propria submucosa. This study is in alignment with the results of Bradley and Grahame (1960).

Vagina
The thickness of vaginal wall was 942.08±1.79 µm and 504.33±2.26 µm in Kadaknath and WLH respectively at 48 weeks of age. However, Shyam (2012) reported it as 564 µm and 629.50 µm in Aseel and RIR at 13 months of age.

The height of epithelium was maximum in 48 weeks of Kadaknath i.e. 32.97±0.43 µm. However, Shyam (2012) reported it as 26.13 µm in Aseel. The epithelium in the present study was ciliated pseudostratified columnar with few goblet cells. This result is in agreement with the Ferdous et al. (2011). However, Nickel et al. (1977) reported tall ciliated columnar epithelium.

**Histochemistry**

Network of fine reticular fibers were seen in the central part of core of folds of oviduct and in the basement membrane of the glands in both the breeds. In Kadaknath these can be differentiated from fibromelanin as reticular fibers were thin whereas, fibromelanin were in the form of thick shreads.

Mild to moderate PAS reaction was seen in the epithelium of glands of magnum and isthmus in all the groups. The present observation might be due to presence of neutral mucopolysaccharides and glycogen in this part. The present observation is in line with the findings of Mehta and Singh (2003). Bharti and Gautam (2013) observed intense PAS positive reaction in glands of magnum.

The luminal content of the magnum showed mild to moderate reaction for the Ninhydrin Schiff’s might be due to the presence of NH$_2$- protein in the luminal content.

Sudan black- B reaction in the follicular content of large follicle and in follicular epithelial cells confirmed the presence of lipid.

Epithelium of shell gland showed positive reaction for calcium might be due to presence of calcium salts in the apical part of the epithelium of shell gland.
6. SUMMARY, CONCLUSIONS AND SUGGESTIONS FOR FURTHER WORK

6.1 Summary

The present experiment was conducted on gross and histological observation of female reproductive system of 48 layers of Kadaknath and WLH with 24 in each breed. These birds were divided into 04 groups viz; group I (24 weeks), group II (32 weeks), group III (40 weeks) and group IV (48 weeks). The Kadaknath fowl were procured from All India Coordinated Research Project on Poultry Farm, Krishinagar, Adhartal, Jabalpur. However, WLH fowl were collected from Phoenix Poultry Farm, Jabalpur. The birds were dissected under day light and served to pick up reproductive system of female layers and studied for gross and histological parameters. The histoarchitectural observations of ovary and oviduct in Kadaknath and WLH layers were performed from the sections stained with Haematoxylin and Eosin, Gomori’s silver impregnation, Weigert’s elastic stain with Van Gieson’s stain, Periodic Acid Schiff’s stain, Mowry’s colloidal iron, Sudan black B, and Ninhydrin Schiff’s stain.

6.1.1 Gross

Ovary

Gross observation revealed that the shape of the ovary was elongated triangular at 24 weeks of Kadaknath and in the form of bunch of grapes in later groups. However, in the same age group in WLH it was in the form of bunch of grapes.

The colour of the ovary in Kadaknath was grayish in all the groups. However, in WLH it was pinkish white to yellow in colour. Per cent left ovarian weight to body weight was maximum in group II of Kadaknath fowl. Although it was maximum in group I of WLH.

Growth spurt of ovary showed that the length was increased only in group I-II in both the groups. However, the values decreased in later
groups. The decrease of length was maximum in group I-II of WLH. The width of the ovary showed increasing trend which was maximum (70.61%) from group I-II of Kadaknath. However, in WLH it showed decreasing trend. The maximum decrease of width was from group II-III. The maximum increase (704.27%) in weight of left ovary was from group I-II of Kadaknath and maximum decrease was noticed from group III-IV in WLH.

Oviduct

The left oviduct extended caudally from the left ovary and opened in the urodeum of cloaca lateral to the left ureter. The coils of the oviduct were not seen in group I of Kadaknath whereas 4-5 coils were observed in rest groups of Kadaknath and all the groups of WLH. The colour of the oviduct was grayish in all the groups of Kadaknath. However, in group IV the colour intensity was reduced. In WLH, it was white in colour in all the groups.

Growth spurt of length and weight of oviduct in Kadaknath and WLH revealed the maximum increase of length (391.19%) and weight (975.24%) from group I-II in Kadaknath breed of fowl.

6.1.2 Histology

Ovary

Histological observation of the ovary showed that the division of the ovary into cortex and medulla was evident in group I of Kadaknath. However, in later groups of Kadaknath and all the groups of WLH the division between cortex and medulla was not distinct.

The surface epithelium was made up of single layer of cuboidal cells with spherical to oval nuclei. However, squamous epithelium was also noticed at some places. Developing primordial and primary follicles were seen in the outer cortical zone just beneath the germinal epithelium in group I of both the breeds. Proliferation was noticed in germinal epithelium at several places and cells were seen migrating from here in to the cortical area.
Occurrence of follicles between 51-100 µm (primary follicles) were maximum (37.93 %) in group III of Kadaknath. However, in WLH it was maximum in group I (7.14 %). These follicles were lined by single layer of cuboidal epithelium. This layer is surrounded by flattened/ stromal cells and these cells formed almost continuous layers.

In all the groups of WLH post ovulatory follicles were present. The ovary contained only few large irregular follicles. These post ovulatory follicles were filled with vacuolated cells and stromal cells. The yellow pigment (hemosiderin) was also seen in some of the post ovulatory follicles.

Oviduct

The left oviduct consisted of five regions namely infundibulum, magnum, isthmus, shell gland and vagina. These divisions have structural differences.

In group I the infundibulum had dorso-ventrally elongated lumen with average diameter of 896.43±1.54 µm and 1604±1.67 µm respectively in Kadaknath and WLH. The wall thickness was less in all the groups of WLH and Kadaknath in comparison to other parts of oviduct. The mural folds were less developed in group I of Kadaknath fowl however, in WLH these folds were well developed, long with primary, secondary and tertiary folds.

The wall of the magnum presented tall, thick longitudinal primary and secondary folds in all the groups of WLH and Kadaknath except in group I of Kadaknath where these folds were short, blunt/ broad with few secondary folds. Developing glands were seen in the propria submucosa of group I of Kadaknath. However, in WLH well developed long, branched, coiled tubular glands were observed in the propria submucosa which completely filled the propria submucosa with intense staining affinity. In group II highly developed long, branched, coiled tubular glands are observed which completely packed the propria submucosa of the magnum. The density of glands decreased from group III onwards in Kadaknath and group II onwards in WLH.
In group I of Kadaknath developing glands in isthmus were seen in the propria submucosa of core of the folds. However, in WLH well developed branched, tubular glands were observed which completely filled the propria submucosa. Glands were less pronounced in all the groups with progression of age.

The epithelium was ciliated pseudostratified columnar in all the groups of shell gland. Few developing glands were seen in a outer zone of propria submucosa in group I of Kadaknath. In the later groups well developed branched, coiled, tubular glands were observed which completely filled the propria submucosa.

6.1.3 Histochemistry

In the oviduct all the three types of fibers were noticed among them collagen fibers were short present in the propria submucosa and extending into the core of mucosal folds. Mild to moderate PAS reaction was seen in the epithelium of the glands of magnum and isthmus.
6.2 Conclusions

The following conclusions are drawn from the result of this investigation on ovary and oviduct in laying Kadaknath and White Leghorn fowl.

- In Kadaknath at 24 weeks of age the ovary was triangular with small follicles and segmentation of oviduct into five parts was ill defined suggesting that up to 24 weeks of age laying was not started in Kadaknath.

- In Kadaknath at 24 weeks of age mural folds in all the parts of oviduct and proliferating glands indicate that developing phase of reproductive system in Kadaknath at 24 weeks of age.

- At 32 weeks of age most of the parameters of the ovary showed maximum value reflects the peak stage of ovarian development. However, the different segments of oviduct showed higher values at 48 weeks of age.

- Fibromelanosis in left ovary and oviduct of Kadaknath reduced as per the advancement of age.

- Per cent ovarian weight to body weight was maximum at 32 weeks of age in Kadaknath. However, in WLH it was maximum at 24 weeks of age is suggestive of highest ovarian activity (among study groups) of WLH at 24 weeks of age.

- Predominance of the post ovulatory follicles in the left ovary of WLH after 24 weeks of age and reduction in the most of the parameters of oviduct indicate the decline phase of egg production in WLH.
6.3 Suggestions for Further Work

- Histoenzymatic study can be made on developing left ovary and oviduct in pre and posthatch period.

- Histological variations can be seen in the left ovary and oviduct by using hormonal preparations in posthatch Kadaknath fowl.

- Immunohistochemical study may be made in the left ovary and oviduct of Kadaknath during pre and post hatch period to see the development of specific receptors.

- Ultrastructural studies of left ovary and oviduct of Kadaknath can be performed in different stages of development.

- Genetic correlation can be studied for egg production and fibromelanosis.


livelihoods of tribals through niche market opportunities. Potential Good Practice Note, Delhi, India, 13 p.


VITA

The author Shashi Bharti D/O Mr. Bhaiya Lal Patel was born on 28th October 1990 in Rewa, Madhya Pradesh. She passed higher secondary examination in 2008 with first class. She was awarded her B.V.Sc and A.H. degree in 2015 from College of Veterinary Science and A.H., NDVSU, Jabalpur, Madhya Pradesh with OGPA 7.678. The author was awarded with gold medal for securing highest marks in subject of Animal Reproduction, Gynaecology and Obstetrics (AGRO) in her B.V.Sc. degree programme. She took admission in post graduation programme at Department of Veterinary Anatomy, College of Veterinary Science and A.H., NDVSU, Jabalpur. The author had published 01 popular article and 03 research article. The author qualified the ICAR National Eligibility Test in the year 2017.

PERMANENT ADDRESS:

Vivekanand Nagar, East Land, Ordnance Factory, Katni (M.P.)
Pin code-483503
Email: shashivet3548@gmail.com