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Histological Study of Ilam Native Hens Reproductive System

S. Soltani*, E. Baba Ahmadi, B. Akbari
Department of Histology, Faculty of Veterinary Medicine, Ilam University, Ilam, Iran.

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Abstract

Considering the importance of the chicken female reproductive system in egg production and reproduction and production of chickens as well as the development of breeding to preserve six pieces of chicken the native species of Ilam, its histological study is necessary to know more about this system and provide a basis for histological studies. In this study, native chickens of Ilam were prepared, ovaries and oviduct samples fixed and studied after H&E staining. Results showed tonica albugina, a thin layer of dense connective tissue, containing the ovarian capsule. The ovarian surface epithelium appears as Simple columnar epithelium, cubic or short columnar cells. The follicles are surrounded by a layer of granulosa cells around each follicle. The magnum is covered by anastomotic protrusions and mucosal folds. The tubular glands completely close in the mucosal layer in each crease. The superficial epithelium of the isthmus is of the ciliated, secretory-like cylindrical type. The short tubular glands and the epithelium are simple cubic glands. The lining of the uterus is a quasi-layered cylindrical type of eyelashes. Mucosa-submucosa has ruptured tubular glands. The muscular layer, especially the annular layer of the vagina, is well developed and the surface of the vagina is covered with mucous-like cylindrical epithelium-like cylindrical epithelium. Tubular glands are located inside the vaginal mucosal connective tissue near the junction with the uterus. In general, there are very close similarities between the reproductive tissues of the native chicken of Ilam and other chickens.

1. Introduction

The oviduct is a complex biological organ that undergoes a series of hormonal, neurological, biochemical, and cellular changes during egg formation. This is of particular interest to the commercial egg industry. Any alteration or deviation in the function of the oviduct of a laying hen can directly affect the quality of the egg and its shell. The reduction in egg and shell quality costs the egg industry millions of dollars each year. The oviduct has been extensively studied in some poultry, especially domestic chickens. The general morphology and overall function of the oviducts, especially in Gallus domesticus, have been studied for many years. Information on the histomorphology of the avian oviduct is still incomplete (Mohammadpour *et al.*, 2012).

Considering the importance of the chicken reproductive system in egg production and chick reproduction and production, as well as the development of breeding to preserve the native Ilam species, it seems necessary to study its histology to further understand this system and provide a basis for histological studies. Histological research has played a significant role in the diagnosis, etiology, and prevention of diseases in most

*Corresponding author: S. Soltani (Assistant Professor)
E-mail address: Sa.soltani@ilam.ac.ir
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cases, directly and indirectly.

2. Materials and Methods

For this study, local laying hens without any signs of disease and clinically completely healthy were selected. Environmental conditions during the experiment were as follows: a light schedule of 12 hours of light and 12 hours of darkness, controlled ambient temperature, and unrestricted access to water and food.

First, the test poultry was euthanized in accordance with ethical standards, and tissue samples were separated from the female reproductive organs and transferred to the histology laboratory for tissue slide preparation and histological study. For this purpose, after separating the tissue samples, they were fixed in 10% buffered formalin solution. To prepare microscopic sections from the samples, tissue sections were prepared using the usual and standard method. Then, the tissue structure of different parts of the prepared tissue sections was evaluated.

Tissue passage steps and preparation of tissue sections:
Since tissue samples contain a large amount of water and because the material used for paraffin embedding in this method is insoluble in water, excess water must be removed during the passage steps using materials that have the power to completely remove the water present in the tissue so that the samples can be prepared for the next steps. In this study, dehydration was performed by placing tissue samples in containers containing ethanol with increasing concentrations of 50, 70, 80, 90 and then two containers of absolute ethanol alcohol. In this study, all stages of dehydration, clarification and tissue embedding in paraffin were performed by an automatic Histokinet device.

3. Dehydration

To prevent the mixing of alcohol with xylol in the clarification stage, the samples were placed in an alcohol-xylol mixed solution so that the samples would also become clear while removing the alcohol and preventing the decrease in the xylol concentration in the next stage.

4. Clarify

In the method of preparing tissue sections using paraffin, an intermediate step is required between the two stages of dehydration and embedding because the alcohol used for dehydration does not mix with molten paraffin. Therefore, before embedding, the dehydrated tissue must be placed in a solution that can replace alcohol and mix with paraffin, and also has the property of removing turbidity and increasing the transparency of the tissue. In this study, the clarification process was performed using a xylol solution and by placing the studied tissue samples in two containers containing xylol for one hour each.

5. Embedding of Tissue with Paraffin

The step after clearing is the embedding step. The material used for this purpose is paraffin with a melting point of 56-58°C. In this step, the samples were placed in paraffin that had been melted at 60°C for at least 10 hours, during which the paraffin replaced the xylene and stabilized the internal structure of the cells, allowing the preparation of microtome sections of the tissues.

6. Embedding of Tissue in Paraffin

After the tissues were embedded in paraffin, they were immediately embedded. For this purpose, the paraffin-embedded tissues were placed in special molds whose inner surfaces were greased with glycerin and contained molten paraffin, from the surface intended for cutting. While cooling, the specimen number was placed on them. After cooling, the mold containing the tissue was frozen and ready for modification.

7. Microtomy and Transferring the Sections onto the Slide

The molded specimens that were placed in the refrigerator were cut using a rotary microtome at this stage. The sections prepared from the tissue were 6 micrometers thick. Then the sections were placed on the surface of a 52-53°C hot water bath to remove the wrinkles on their surface. Then, by dipping the slides soaked in albumin glue under the straightened sections, the sections were transferred onto the slide and in order to adhere and fix the sections, the slides and the sections on them were placed in laboratory conditions for several hours (Kalantari-Hesari *et al.*, 2022).

8. Histological Studies

There are several methods for preparing tissue sections from the studied samples, but the method used in this study was to perform conventional methods of preparing sections, including dehydrating the samples using ascending concentrations of ethanol, clarifying them with xylene, and embedding and embedding with paraffin. Sections of 6 μm thickness were prepared from each sample using a microtome. Then, the samples were stained with H& E, and after attaching the coverslip and mounting, they were studied under a light microscope.

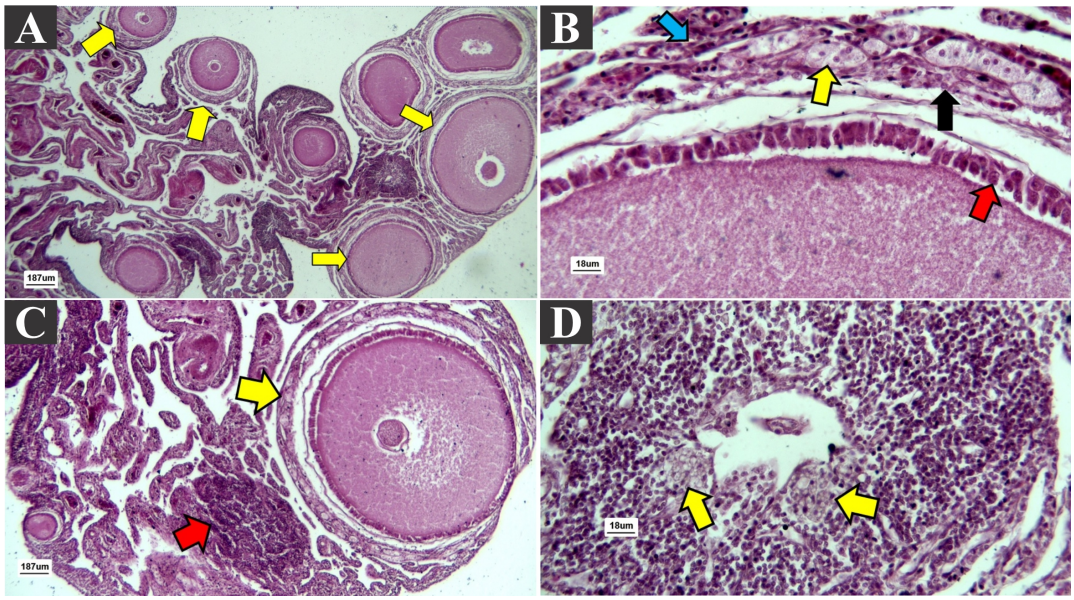

Fig. 1. A) Ovary. Cortical section containing mature follicles (yellow arrow) (H& E stain) Magnification ×4. B) Ovary, showing part of the wall of a developing follicle. Inner single cells (black arrow) - outer single cells (blue arrow) - interstitial cells (yellow arrow) - granulosa layer (red arrow), (hematoxylin-eosin stain. Magnification) ×40. C) Ovary, growing follicle (yellow arrow) ,atrophied follicle (red arrow) (hematoxylin-eosin stain). Magnification ×4. D) Ovary, vacuolated cells (yellow arrow). The vacuolated cells contain pyknotic, lipid-filled nuclei, which is a collapsed follicle after ovulation (hematoxylin-eosin stain), 40× magnification.

9. Staining

Since the cellular elements of the tissue sections prepared in the study cannot be seen and distinguished with a light microscope, it is necessary to stain them before performing a microscopic study so that the different parts of the tissue and the cellular elements that make it up can be separated and studied. In this study, general hematoxylin-eosin staining was used to study the tissue structure of different parts of the female reproductive system.

Hematoxylin-eosin staining is the most common and widely used staining in histology. In this staining, the cell nucleus takes on the purple color of hematoxylin and the cytoplasm takes on the pink color of eosin. The method of preparing the dyes and the different stages of staining were carried out in a standard and usual way (Galloway *el al.*, 2000).

A histological study was conducted to evaluate the condition of the cells and structure of the reproductive tract of Ilam native laying hens.

10. Results

After preparation, slide preparation, and hematoxylin and eosin staining of the reproductive organs of native chickens, ovary and oviduct samples (including the infundibulum, magnum, isthmus, uterus, and vagina) were studied and examined, as follows: Cortical section containing mature follicles. Ovary, showing part of the wall of a developing follicle. Inner single cells, outer single cells, interstitial cells, granulosa layer and atrophied follicles. Ovary contains some vacuolated cells. The vacuolated cells contain pyknotic, lipid-filled nuclei, which is a collapsed follicle after ovulation (Fig. 1).

Section of the infundibulum tissue showed that base between the primary and secondary fold has glandular groove and simple ciliated columnar epithelium (Fig. 2(A)). Section of the magnum shown pseudostratified columnar epithelium with cuboidal secretory cell epithelium and activated glandular epithelium (Fig. 2(B)). Section of the isthmus showed primary fold with pseudostratified columnar epithelium (Fig. 2(C)). Section of the final part of oviduct showed primary and secondary fold and muscular layer (Fig. 2(D)). Section of the vagina showed long primary folds and thick muscle layer (Fig. 2(E)).

11. Discussion

Based on the studies Nugrahini *et al.*, 2018, conducted in evaluating the reproductive performance of Magellanic ducks by tissue preparation, he showed that the type of housing has an effect on the reproductive performance of ducks. In this study, external factors such as nutrition and nature were not studied. Evaluation of internal factors by histochemical staining proved that the closed housing system showed more reproductive tissue characteristics (Nugrahini *et al.*, 2018).

Inother report, shown the vagina in poultry is a place for sexual intercourse and sperm accumulation.

In poultry, this part has ridges that serve as a resting place for sperm before fertilization. Mucopolysaccharides secreted from this part provide nutrition for sperm. The vagina is composed of pseudostratified columnar epithelial cells that secrete mucopolysaccharides and serves as a storage site for sperm (Wani *et al.*, 2017).

Noor and Nisa (2014) showed the isthmus are a secretory tissue that forms the thin shell on the egg. In animals kept in confinement, it has been shown that the lumen is opened and the size of the epithelial cells of the lumen is increased. The isthmus is a developed magnum that secretes active filaments and keratin to form the thin egg shell (Noor and Nisa, 2014).

Mohammadpour *et al.*, 2012 by studying the reproductive organs, they stated that these organs in female ducks consist of two important parts: the ovary as the site of egg production and the oviduct as the egg duct, which includes five parts: the infundibulum, magnum, isthmus, uterus, and vagina. The reproductive organs of ducks that are traditionally kept are lighter than those that are kept in a closed system. Most ducks in this type of traditional keeping are non-productive. The difference in production in these two keeping methods is due to the difference in the active tissue of the reproductive organs, which are longer due to secretory activity. Secretory organs in poultry have secretory cells that make secretory compounds for egg production. In the production stage, the tissues in the isthmus and magnum have thick secretory cells and a mucous layer (Mohammadpour *et al.*, 2012).

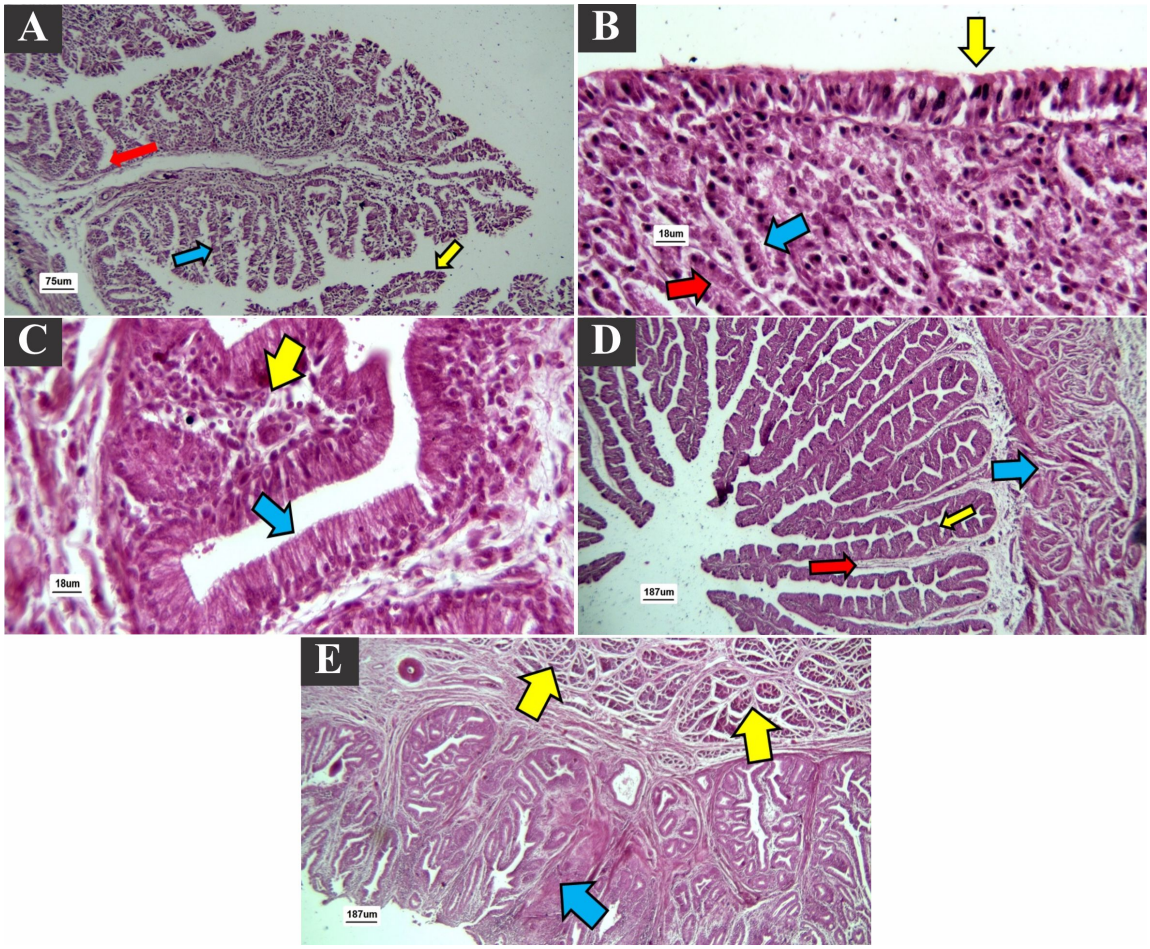

Fig. 2. A) Transverse section of the infundibulum opening, base between the folds, glandular groove (red arrow). Simple ciliated columnar epithelium (yellow arrow), secondary fold (blue arrow) (hematoxylin-eosin stain). Magnification ×10. B) Transverse section of the magnum, oviduct, pseudostratified columnar epithelium (yellow arrow). Cuboidal secretory cell epithelium (blue arrow), activated glandular epithelium (red arrow) (hematoxylin-eosin stain). Magnification ×40. C) Transverse section of the isthmus, oviduct, pseudostratified columnar epithelium (blue arrow). Primary fold (yellow arrow) (hematoxylin-eosin staining). Magnification ×40. D) Transverse section of the oviduct. muscular layer (blue arrow) - primary fold (red arrow) - secondary fold (yellow arrow) (hematoxylin-eosin staining). Magnification ×4. E) Transverse section of the vagina, long primary folds (blue arrow), thick muscle layer (yellow arrow) (hematoxylin-eosin staining). Magnification ×4.

Shahroz in 2013 stated that the magnum has a thick wall with long and thick primary and secondary mucosal-submucosal folds. Albumin is added to the egg in the magnum region. The integumentary tissue of the magnum is of a simple cylindrical type with an almost equal number of ciliated and goblet-shaped cylindrical cells. The loose connective tissue of the parenchyma-submucosa densely contains long and branched, convoluted tubular glands. The glandular cells are pyramidal and filled with coarse basophilic granules (Shahroz, 2013).

Rezaian in 2013 stated that the isthmus is relatively short and has a smaller diameter than the magnum. The surface epithelium is of a pseudostratified cylindrical type with cilia and is secretory. The tubular glands are short and the epithelium is simple cuboidal glands. These glands are responsible for the secretion of the shell around the albumen. Muscles contain two layers, an inner circular and an outer longitudinal, and serosa covers the outer surface of the isthmus (Rezaian, 2013).

Dellman et a., 1998 by studying the uterus, stated that the uterus or squamous gland is a sac-like or thick-walled extensible part. The primary and secondary longitudinal folds are replaced by primary and secondary annular folds. The epithelium is of a ciliated, pseudostratified cylindrical type. The parenchyma-submucosa contains confluent branched tubular glands. The cells of these glands are pyramidal in shape and have a diffuse, granular cytoplasm with vacuoles (Dellman *et al.,* 1998).

William et al., 2000 By studying the reproductive organs, they stated that the muscular layer, especially the annular layer of the vagina, is well developed and the surface of the vagina is covered with a ciliated, pseudostratified cylindrical epithelium with mucous cells. The tubular glands are present within the connective tissue of the vaginal mucosa near the junction with the uterus (William *et al.*, 2000).

Sultana *et al.*, 2003 showed in ducks kept in a closed system, the development of ovarian tissue is clearly visible in the eggs ready for oviposition. The magnum is the next part of the reproductive organ that secretes egg albumin. In ducks kept in a conventional way, the magnum is lined with ciliated secretory cells. This tissue consists of a prealbuminous layer that contains polysaccharides and glycoproteins. In this study, there was no histological difference between the vaginas of ducks kept in a conventional and closed system (Sultana *et al.*, 2003).

Klomp *et al.*, 1991 showed Great Skuas that have previously bred have smaller nest sizes and lower egg laying success than their peers. This study showed that improvements in nest size and egg size were associated with increased breeding experience, with evidence of improvements from the first breeding attempt to the second attempt, but not from the second to the third attempt. These findings suggest that the additional benefits of previous breeding experience diminish after the first breeding attempt. Longitudinal analyses showed that the increase in nest size and egg size with experience was due to improvements in the birds' performance (Klomp *et al.*, 1991).

Klomp *et al.*, 1991 showed Individual Great Skuas tended to have small territories close to their nest sites, but they moved farther away from the club and increased range size on successful breeding attempts. Since experience and age are closely related, the increase in breeding experience could be due to age and the acquisition of foraging skills. Breeding experience improved nest size more than foraging in Great Skuas, and similar studies in other species have shown that experience can affect breeding independently of age, while other studies have shown that age is more involved than experience in improving breeding performance (Klomp *et al.*, 1991).

Pyle *et al.*, 1991 With a study on Western Gulls Larus occidentalis showed that age improved breeding success in males, as males forage more, while females lay and guard the nest and therefore have more experience. Great Skuas with previous breeding experience, with a nest size of one egg, are less likely to breed again. Young birds with low breeding success are less likely to return to breed. It is possible that low-quality phenotypes have poor reproductive success and are less likely to breed again, perhaps due to higher mortality rates or a higher probability of delayed breeding. In cross-sectional studies, breeding performance can increase with increasing experience, as a result of differential mortality, because only birds with high breeding performance will be present in the breeding population after the first year of breeding. One explanation for delayed breeding is that breeding effort reduces the value of remaining breeding stock through increased mortality, and the loss of reproductive potential cannot be compensated for by lower reproductive success in young breeding chicks. Olso, they stated in the prediction that reproductive success increases with experience, the optimal reproductive strategy is to breed at low intensity early in life to benefit from the reproductive advantage and avoid the costs of breeding. In situations where breeding improves with age, not experience, a long period of delayed breeding would be desirable to gain search experience to ensure that the cost of breeding is justified by the high breeding success. In Great Skuas, breeding experience appears to be more important than age in determining nest size, but birds that breed at very young ages have higher mortality rates. This represents an initial fitness cost and therefore the age of first breeding in Great Skuas is a matter of trade-off between delayed breeding to maintain future breeding potential and low intensity breeding to improve nest size through experience. The final equilibrium of this substitution relationship de-

pends on the relative contributions of these parameters to reproductive success over the lifespan (Pyle *et al.*, 1991).

Perche *et al.*, 1990 stated the laying hen (Gallus domesticus) is a powerful animal model for epithelial ovarian cancer. The use of animal models is very important in identifying early markers of the disease and conducting chemotherapy trials (Perche *et al.*, 1990).

Giles *et al.*, 2010 stated Albumin, the major protein secreted by the primary ovary in chickens, is expressed in EOC tumors in chickens regardless of the presence of tumors in the ovary (Giles *et al.*, 2010).

Trevino *et al.*, 2010 In one study, 273 genes were identified that were differentially expressed between normal and cancerous ovaries of laying hens. Ten of the 25 overexpressed genes were associated with ovarian function. None of these genes were significantly expressed in normal ovarian surface epithelium (Trevino *et al.*, 2010).

O'Shannessy *et al.*, 2013 stated Expression of genes such as folate receptor and CA125 by EOC tumors may not be a case of overexpression. Rather, these genes are normally expressed relative to the cell of origin (ovarian epithelium) and are only abnormally expressed relative to OSE gene expression (O'Shannessy *et al.*, 2013). It is suggested that the tissue structure of the reproductive system of native chickens be studied by electron microscopy.

Other organs of native chickens of Ilam, such as the lymphatic system, be evaluated histologically.

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