# PREHATCHING AND POSTHATCHING STUDIES ON THE MORPHOGENESIS OF THE OVIDUCT IN THE LAYING HEN

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## ABSTRACT

*Investigation of the reproductive tract of the laying hen has been considered in the past few decades. Such strategy aims to increase the egg and the meat production in the poultry industry. However to understand the physiology of reproduction, it is important to explore the developmental changes prior to the egg production. Therefore, we examined the morphometrical and the developmental changes taken place during the development of the oviduct. The results demonstrated that the development of the oviduct in the laying hen divide into two consecutive stages. Prehatching stage (five-21 days-old chick embryos): this period was characterized by the formation of the Mullerian duct as cellular mass thickening and invagination from the coelomic epithelium. Such cellular mass became canalized and lined with single layer of simple columnar epithelium. Furthermore, there was complete absence of the mucosal folds. Posthatching stage (one day-24 weeks old chicks): this period was characterized by dramatic increase in the height of the mucosal fold. Epithelial transformation from simple columnar to pesudostratified differentiated into ciliated and non-ciliated cells. The muscular layer development from thin strip of smooth muscle into well developed layer of inner circular and outer longitudinal smooth muscle layers.*

## INTRODUCTION

Studies on the anatomy of the reproductive tract of the laying hen are important approach to understand the physiology and function of the reproduction. The avian oviduct could be divide into five distinguishable regions: Infundibulum, magnum, isthmus, uterus (shell gland) and vagina **(Sturkie, 1976 and Khokhlov and kuznetcov, 2007)**. The oviduct performs several functions. Infundibulum engulfs the ovum after ovulation, where the fertilization takes place and produces the vitelline membrane **(Bakst and Howarth, 1977)**. Magnum produces the egg albumin and certain amount of calcium. Isthmus forms the outer and the inner shell membrane, uterus provides the calcium for shell calcification and vagina has no role in egg formation but facilitates the egg expulsion **(Romanoff and Romanoff, 1949)**.

It is well established that the female reproductive tracts in chicks develop from two pairs of genital ducts, the Müllerian and the Wolffian ducts. Müllerian ducts (paramesonephric ducts) develop adjacent to the Wolffian ducts (mesonephric ducts) **Gruenwald, 1941)**. In the chicken, both female and male embryos initially have a pair of undifferentiat-

ed gonads and Müllerian ducts that develop in the coelomic cavity from day 5 of incubation and grow to the region of the cloaca **(RomanoV, 1960)**. Both the left and right Müllerian duct engage in a linear development until day 12 of incubation **(Teng, 1987)**. Subsequently, the right Müllerian duct completely disappears at the time of hatching. Conversely, the left Müllerian duct continues to grow and develops into the oviduct (**RomanoV, 1960**).

The basic structure of the oviduct in domestic fowls is formed of mucosa that was consisted of pseudostratified epithelium and a glandular lamina propria. The mucosa formed mucosal folds with various degrees of height and thickness. The tunica muscularis is composed of a few smooth muscle bundles in the infundibulum to two distinct layers, inner circular and outer longitudinal in rest of the oviduct **(Mohammad pour and Keshtmandi 2008)**.

The avian oviduct is a complex biological organ that undergoes a series of hormonal, neural, biochemical and cellular changes during the formation of the egg. The morphology of the mature oviduct has been described by **Richardson, 1935; Aitken and Johnston 1963; Johnston et al. 1963; Breen and Debruyun 1969; Wyburn et al. 1970; Draper et al. 1972; and Mohammad pour and Keshtmandi, 2008)**. Furthermore, the development of the post hatching reproductive tract has been investigated by **Fouad (1970)** in fayoumi chicks, **El-habbak (1990)** in pekin duckling, **El-bargeesy (1990)** in immature turkey, **Kelany et al. (1993)** in chick and **Sayed (2000)** in immature quail. However and due to lack of information regarding the morphometrical changes during the development of the oviduct in the laying hen, this study aims to shed the light on the development of the epithelial and the glandular structure of the oviduct through using the histological and the morphometrical analysis.

## *MATERIAL AND METHODS*

This study was carried out on 60 of chick embryos and foetuses (five days-21$^{st}$ days-old) and chicks (one day-24 week old). The embryos and the chicks were obtained from the local farms **(Abd Elsalam Hegazy breed)** in El-Dakahlya Province through personal communications with the farms. The chick embryos were carefully removed from the egg and fixed in Bouin's solution for 12 hours. Then it was transferred to 10% neutral buffered formalin. The caudal part of the embryo was divided into 3 parts, cranial part toward the kidney, middle part and caudal part toward the cloaca. The post hatching chicks were sacrificed through neck dislocation. The left oviduct was distinguished into five regions (infundibulum, magnum, isthmus, uterus and vagina (Fig. 1). The oviduct was carefully dissected using the dissecting microscope, fixed in Bouin's followed by 10% neutral buffered formalin. Specimens were dehydrated through ascending grades of ethanol, cleared in benzene, embedded in paraffin wax and sectioned at 5 µm using rotatory microtome. General histological examination was performed through using Ehrlich's Haematoxylin and Eosin, Periodic acid Schiff technique (PAS), Alcian blue and Masson's trichrome stain adopted by **(Bancroft and Stevens, 1990)**. All slides were examined and photographed by using the ordinary microscope

provided with digital camera. Analyses of the morphometry of the oviduct were performed using ANOVA.

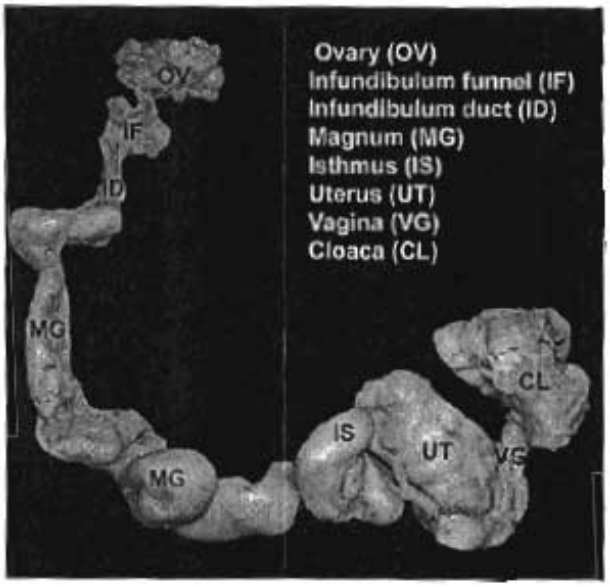



**Figure (1)** Photomacrograph of the various compartments of the reproductive tract of the laying hen.

## *RESULTS & DISCUSSION*

Morphogenesis of the reproductive tract in the laying hen was chronologically subdivided into two sequential stages;

**1- Pre-hatching stage (five days-21st. day) of egg incubation:**

The primitive mullerian duct was recognized in the fifth day of egg incubation as a thickening in the coelomic epithelium (placode). Such thickness resulted from cellular proliferation dorsolateral to the mesonephric duct followed by invagination of these cellular mass (Fig. 2). In six day old chick embryo, the coelomic invagination was deepens in the placode forming a solid cellular mass that loss the connection with the coelomic epithelium (Fig. 3). In seven day-old chick embryo, this solid mass became canalized forming a tubal structure. Furthermore, the duct was lined with columnar epithelium resting on a basement membrane (Fig. 4). In eight day-old chick embryo, the canalization became more obvious and the duct was lined by columnar epithelium (Fig. 5). In 10 days-old embryo a mesenchymal constriction was observed between the mullerian duct and the mesonephros (Fig. 6). By age advancing, in 13 days-old embryo, the lumen of the mullerian duct became wide. A thin layer of coelomic epithelium covered the duct externally as well as it remained connected with mesonephric duct by a thin mesenchymal strip (Fig. 7). At the level of cloaca, the mullerian duct (paramesonephric duct) became ventral to the Wolffian duct (Fig. 8). In 18-day-old chick embryo, the lumen of the mullerian duct became wide and the epithelium was short columnar with large spherical nuclei (Fig. 9).

**2- Post-hatching stage (one day-24 weeks-old chicks):**

In one-day old chick, the mucosa of the oviduct (cranial part) was lined by a single layer of high cuboidal epithelial cells, large spherical nuclei average height 8.44±0.6µm (Fig. 10). However, in the middle and caudal parts, the epithelium was columnar with elongated oval nuclei. Such epithelial cells were resting on ill-defined basement membrane. Highly cellular connective tissue layer surrounded the epithelium (Fig. 11). In three-days-old chick, the cranial part of the oviduct demonstrated mucosal protrusion into the lumen which permits shallow grooves in between. Such mucosal folds with the grooves gave the mucosa its wavy appearance. Moreover, the

lamina epithelialis composed of high cuboidal to low columnar epithelial cells with an average height 10.53±0.5µm (Fig. 12). In the middle and caudal parts of the oviduct, the mucosal protrusions became clear and frequent forming short and broad mucosal folds (Fig. 13). By the end of the first week post hatching, the mucosal folds became higher and the epithelial grooves were more numerous and deeper compared to the previous ages (Fig. 14). By age advancement, there were detectable differences in the structure and significant morphometrical changes of the oviduct. In two weeks-old chicks, the mucosal layer was folded, subdivided and higher with average height (41.9 ± 5.1 µm). The lamina epithelialis changed into tall columnar epithelium (16.18 ± 0.7 µm). In three weeks-old chicks, the mucosal folds were subdivided into primary and secondary folds. The secondary folds were clearly observed and the underlying layers became well differentiated specially in the caudal part of the oviduct (Fig. 15). From the fourth week-old chick, the oviduct was divided anatomically into five regions (infundibulum, magnum, isthmus, uterus and vagina) in order to describe the developmental changes in each region. In four weeks-old chicks, the mucosa of the infundibulum was arranged into a few short pyramidal and wide mucosal folds. The average folds height and width were 26.5±2.6µm and 58.8±3.6µm respectively (Fig. 16). In eight -12 weeks-old chicks, the mucosal folds of the infundibulum displayed remarkable increases in the height and the width of the fold 33.2±3.9µm and 64.1±2.9 µm respectively. In 12 weeks-old chicks, the average height of the epithelial cells was increased (18.7±1.3 µm) leading to deep branched epithelial grooves (Fig. 17). Significant increases was found in the height and the width of the mucosal fold up to 37.7±2.7µm 135.7±1.7µm. In 16 weeks-old chicks, primitive cilia was observed on the luminal border of the dark cells with average height 3.4±0.2µm. Remarkable increases were detected in the height and the width of the mucosal folds to be 51.5±3.2µm and 173.5±2.2 µm respectively (Fig. 18).

In 17-20 weeks-old chicks, the premature pattern of the reproductive tract development was attained. In the tubular part of the infundibulum, the lamina propria was endowed with tubular glands. Each gland was lined with single layer of cuboidal or pyramidal epithelium with rounded and basally located nuclei (Fig. 19). The glandular epithelium demonstrated weak to moderate PAS positive reaction and AB negative reaction. The non-ciliated cells exhibited a strong PAS positive and AB negative reactions in their apical parts (Fig. 20). In 21-24 weeks-old chicks, the mature pattern of the infundibulum became clearly developed. The mucosal folds were large and extensively branched. Each fold was branched into primary, secondary and tertiary smaller fold. The terminal branches were communicated which results in extensive and complex networks. There were significant increases in the height and width of the mucosal folds (295.1±11.9 µm and 264.15±2.9 µm) respectively compared to the previous ages. Highly significant increase in the height of the epithelial layer (24.1± 2.9 µm) and the cilia (7.1 ± 0.6 µm) respectively were seen. The ciliated epithelial cells were the predominant cell type in the funnel part of the infundibulum (Fig. 21).

**Plate (1):**

**Fig. (2):** Photomicrograph of 5-days-old chick embryo showing, the primordia of the paramesonephric duct (pd) dorso-lateral to the mesonephric duct (md). Note the mesonephric tubules (mt). H&E. Stain.X40.

**Fig. (3):** Photomicrograph of 6-days-old chick embryo showing, the paramesonephric duct (pd), celomic epithelium (arrow head), mesonephric duct (md). Note mesonephric tubules (mt). H&E. Stain.X100.

**Fig. (4):** Photomicrograph of 8-days-old chick embryo showing, canalized paramesonephric duct (pd), the coelomic epithelium (arrow head) and mesonephric duct (md). H&E. Stain. X400.

**Fig. (5):** Magnification of fig. (4) Showing, paramesonephric duct (pd) lined by simple columnar epithelium displaying mitotic figure (arrow head) resting on cellular lamina propria (p), mesonephric duct (md) and coelomic epithelium (arrow). H&E. Stain. X1000.

**Fig. (6):** Photomicrograph of 11-days-old chick embryo showing, a mesenchymal constriction (arrow) between the paramesonephric duct (pd) and the mesonephric duct (md), the mesonephric tubules (mt) and the coelomic cavity (asterisk) .H&E. Stain.X100.

**Fig. (7):** Photomicrograph of the caudal part of 13-days-old chick embryo showing, wide lumen of the paramesonephric duct (pd) and cellular condensation around the epithelium covered by celomic epithelium (arrow head), a constriction (arrow) separate the paramesonephric duct from the mesonephric duct (md) and coelomic cavity (asterisk). H&E. Stain. X400.

**Fig. (8):** Photomicrograph of 13-days-old chick embryo at the level of the cloaca (c) showing, paramesonephric duct (pd), note mesenchymal condensation around the duct (arrow) and mesonephric duct (md). H&E. Stain. X100.

**Fig. (9):** Photomicrograph of 18-days-old chick embryo showing, great increase in the lumen of the paramesonephric duct (pd), short columnar epithelium lies on a highly cellular lamina propria (p) and ureter (u). H&E. Stain. X400.

**Plate (2):**

**Fig. (10):** Photomicrograph of the cranial part of the oviduct of 1-day-old chick showing, a single layer of high cuboidal epithelium with large spherical nuclei (arrow head) surrounded by highly cellular lamina propria (p).H&E. Stain. X400.

**Fig. (11):** Photomicrograph of the middle part of the oviduct of 1-day-old chick showing, columnar epithelium with elongated oval nuclei (arrow), rested on ill-defined basement membrane and highly cellular lamina propria (p). H&E. Stain. X400.

**Fig. (12):** Photomicrograph of the cranial part of the oviduct of 3-days-old chick showing, wavy appearance of the mucosa composed of cuboidal to low columnar epithelium (arrow head) and cellular condensed lamina propria (p). H&E. Stain. X400.

**Fig. (13):** Photomicrograph of the middle

part of the oviduct of 3-days-old chick showing, numerous mucosal folds (arrow), cuboidal to low columnar epithelium (arrow head) and cellular condensed lamina propria (p). H&E. Stain. X400.

**Fig. (14):** Photomicrograph of the middle part of the oviduct of 7-days-old chick showing, mucosal folds (arrow) with deep grooves in between (arrow head) and cellular condensed lamina propria (p). H&E. Stain. X100.

**Fig. (15):** Photomicrograph of the caudal part of the oviduct of 21-days-old chick showing, high mucosal folds (arrow), small secondary folds (arrow head) lined with columnar epithelium (EP) and lamina propria (P). H&E. Stain. X400.

**Plate (3):**

**Fig. (16):** Photomicrograph of the infundibulum of 4 weeks old chick showing, short and broad mucosal folds (mf), glandular grooves between the folds (arrow), pseudostratified columnar epithelium (EP) and lamina propria (p). H&E. Stain. X400.

**Fig. (17):** Photomicrograph of the infundibulum of 12 weeks old chick showing, pesudostratified columnar epithelium (EP), the mucosal folds (MF) and small blood vessels (arrow head) in the lamina propria (p). H&E. Stain. X400.

**Fig. (18):** Photomicrograph of the infundibulum of 16 weeks old chick showing, the mucosal folds (mf), epithelial grooves (arrow) and lamina propria (p). H&E. Stain. X40. Intersect: magnification showing, the lining epithelium (EP) with primitive cilia (arrow). H&E. Stain. X 1000.

**Fig. (19):** Photomicrograph of the tubular part of the infundibulum of 20 weeks old hen showing, mucosal folds (MF), pseudostratifed columnar epithelium (EP), the lamina propria (p) endowed with tubular glands (G), their acini were lined with cuboidal or pyramidal cells (arrow head) and well differentiated muscular layer (M). H&E. Stain. X400.

**Fig. (20):** Photomicrograph of the tubular part of the infundibulum of 20 weeks old hen showing, strong PAS positive reaction at the apical parts of the non-ciliated cells (arrow head), weak to moderate reaction in the tubular glands (arrow) in lamina propria (p). PAS. Stain. X400.

**Fig. (21):** Photomicrograph of the funnel part of the infundibulum of 24 weeks old hen showing, extensively branched mucosal folds, primary fold (pf), secondary folds (sf) and tertiary folds (tf), the lining epithelium (EP), the ciliated cells carry a long well developed cilia (arrow head) and muscular layer (M). H&E. Stain. X400.

Our results showed that the primitive mullerian duct developed early during the embryogenesis. In 5 days-old chick embryos, the mullerian begins as a placode-like thickening in the coelomic epithelium. Such thickness was deepened to form a solid cellular mass dorsolateral to the mesonephric duct. This result was in agreement with **RomanoV, (1960)** in chick embryos. It was found that this cellular mass originating from the Wolffian duct **(Mullers, 1830)** in contrast, Orvis and

Behringer **(2007)** observed that this cellular mass originating from the coelomic epithelium without contribution from the wolffian duct.

This study showed that the primordia of the mullerian duct became canalized and the lumen was formed of simple columnar epithelium at 7-11 days-old chick embryos. In the same line with these results, it has been described that, the mullerian cord is canalized sequentially cranio-caudally then sinks beneath the surface of the genital ridge **(Jacob et al. 1999)**.

In accordance with **Guioli et al. (2006)** in 13- 21 days-old chick embryos, the mullerian duct displayed full pattern of organization; it was formed of wide lumen lined by short columnar epithelium with large spherical nuclei and it was surrounded by a condensed mesenchymal tissue.

The present investigation showed that at 1 day - 7 days old chick, the mucosa of the cranial part of the oviduct was lined by a single layer of cuboidal epithelium. However, in the middle and caudal parts, the epithelium was columnar. These results are in agreement with **Kohler et al. (1968)** in 5 days old chick stated that the mucosa was lined by a single layer of primitive undifferentiated stem cells. However, **Kelany et al. (1993)** in 1 day old chicks reported that the epithelium was simple columnar or pesudostratified columnar.

This work revealed that at 14 days-old chicks, the lining epithelium of the oviduct was changed into tall columnar epithelium differentiated into light and dark cells. Similar finding was mentioned by **(El-bargeesy, 1990)** in turkey chicks and **(Sayed, 2000)** in 1-3 weeks old quail.

In accordance with, **El-bargeesy (1990)** in immature turkey, **El-habbak (1990)** in Pekin ducklings and **Kelany et al. (1993)** in fowl and **Sayed (2000)** in 1-3 weeks old quails. The results showed clearly that the primitive mucosal folds was observed at 3 days-old chicks and continuously increased in the height and the width by ageing. Moreover, the mucosal folds were subdivided into primary and secondary folds at 21 days old chicks.

The results showed that the mucosa of the infundibulum in 4-16 weeks old chick was formed of short and wide mucosal folds. These folds were increased in height and width by ageing. This observation is in agreement with **Fouad (1970)** in 3-4 month fayoumi chicks and **El-argeesy (1990)** in sexually mature turkey who reported that the mucosa of the infundibulum had low primary mucosal folds accompanied with very small secondary folds in between.

The present investigations showed that the mucosa of the infundibulum in 20-24 weeks old hen displays complex pattern of organization, where it showed an extensive network of long, convoluted and highly branched mucosal folds, primary folds are provided with secondary and tertiary folds the mucosal folding increased toward the caudal part of the infundibulum. This result is in line with that mentioned by **El-bargeesy (1990)** in sexually mature turkey, **Mohammadpour and Keshtmandi (2008)** in mature pigeon, **Saber et al. (2009)** in sexually mature ostrich.

This work revealed that, the epithelial pattern of the infundibulum in 4-24 weeks old hen was formed of pseudostratified columnar epithelium. This result is similar to that observed by **Saber et al. (2009)** in mature ostrich and **Youssef (1995)** in mature quail. However, **Mohammadpour and Keshtmandi (2008)** in mature pigeon and turkey who stated that the epithelium was formed of ciliated simple columnar type in mature pigeon but simple cuboidal to ciliated simple columnar in mature turkey. On the other hand, it was described by **Muwazi et al. (1982)** in mature turkey, stated that it was formed of simple columnar with some area of pseudo stratification.

The present finding showed a remarkable absence of the glands in the lamina propria of the funnel part of the infundibulum. These results are in agreement with **Fouad (1970)** in fayoumi chicks and **Rai et al. (1980)** in fowls. On the other hand, in 20-24 weeks old hen, the infundibulum showed tubular glands in the tubular part of infundibulum. These glands were lined with cuboidal or pyramidal cells. This simulates the finding of **El-bargeesy (1990)** in mature turkey and Mohammadpour and **Keshtmandi (2008)** in mature pigeon.

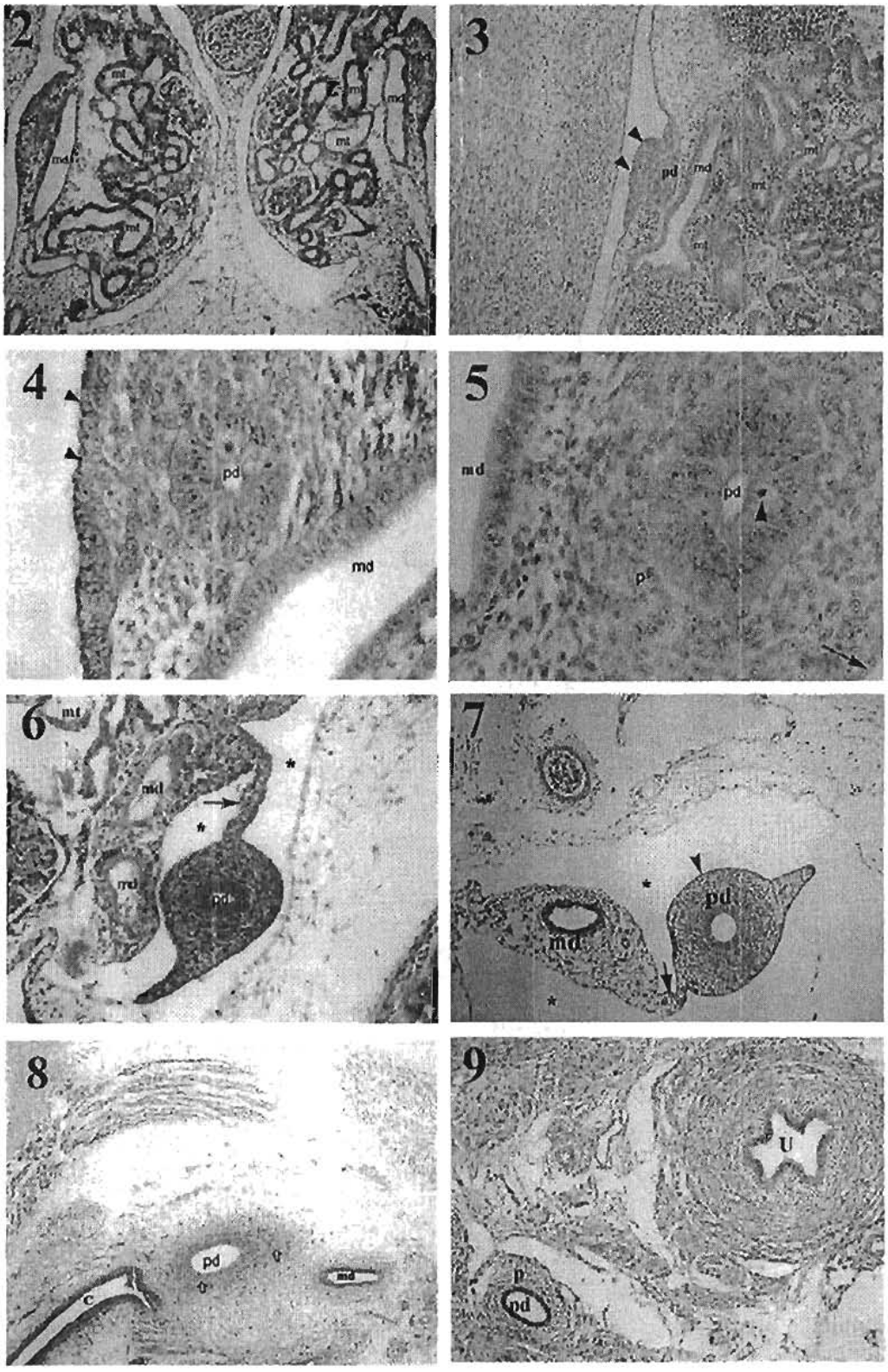
Our results showed that the mature muscular layer of the infundibulum in 20-24 weeks old hen was formed of outer longitudinal and inner circular smooth muscle fibers similar results were reported by **(El-bargeesy, 1990)** in mature turkey.

The present study demonstrated clearly that the epithelium of the infundibulum in 4-16 weeks old chicks gave negative reaction to PAS and AB staining. In contrast in 20-24 weeks old hen, the glandular epithelium demonstrated weak to moderate PAS positive reaction and AB negative. Furthermore, the non ciliated cells gave strong PAS positive reaction and AB negative. These results suggest that the oviduct remains inactive without any secretory product however at 20 weeks old (pre-laying stage) the sexual maturity starts therefore the secretory granules in the non-ciliated cells gave PAS positive reaction. These results in agreement with the statement of **Solomon (1971)** and **Aitken (1971)** in domestic fowls stated that the non-ciliated cells of the infundibulum give positive reaction to PAS. However, **El-bargeesy (1990)** in mature turkey and **Sharaf (2005)** in ostriches mentioned that the surface epithelium was positive to PAS and AB staining.

**Table 1&2** Illustrate the average epithelial height, length and width of mucosal folds.

| Measurement (µm) | 1-day | 3-days | 5-days | 14-days | 21-days |
|---|---|---|---|---|---|
| *Height of the epithelium* | 8.44±0.6 | 10.53±0.5 | 15.5±1.1 | 16.18±0.7 | 18.7±0.9 |
| *Length of the mucosal fold* | -- | 36.1±3 | 41.1±4.2 | 41.9±5.1 | 61.3±6.2 |
| *Width of the mucosal fold* | -- | 43.1±4.2 | 52.8±11.7 | 55.5±8.1 | 62.8±7.8 |

| Measurement (µm) | 1-Month | 2-Month | 3-Month | 4-Month | 5-Month | 6-Month |
|---|---|---|---|---|---|---|
| *Length of the mucosal fold* | 26.5±2.6 | 33.2±3.9 | 37.7±2.7 | 51.5±3.2 | 74.5±3.8 | 295.1±11.9 |
| *Width of the mucosal fold* | 58.8±3.6 | 64.1±2.9 | 135.7±1.7 | 173.5±2.2 | 217.5±3.8 | 264.15±2.9 |
| *Height of the epithelium* | 12.3±0.2 | 13.2±1 | 18.7±1.3 | 18.4±2.2 | 22.5±2.3 | 24.1±2.9 |
| *Height of the cilia* | -- | -- | -- | 3.4±0.2 | 5.5±0.3 | 7.1±0.6 |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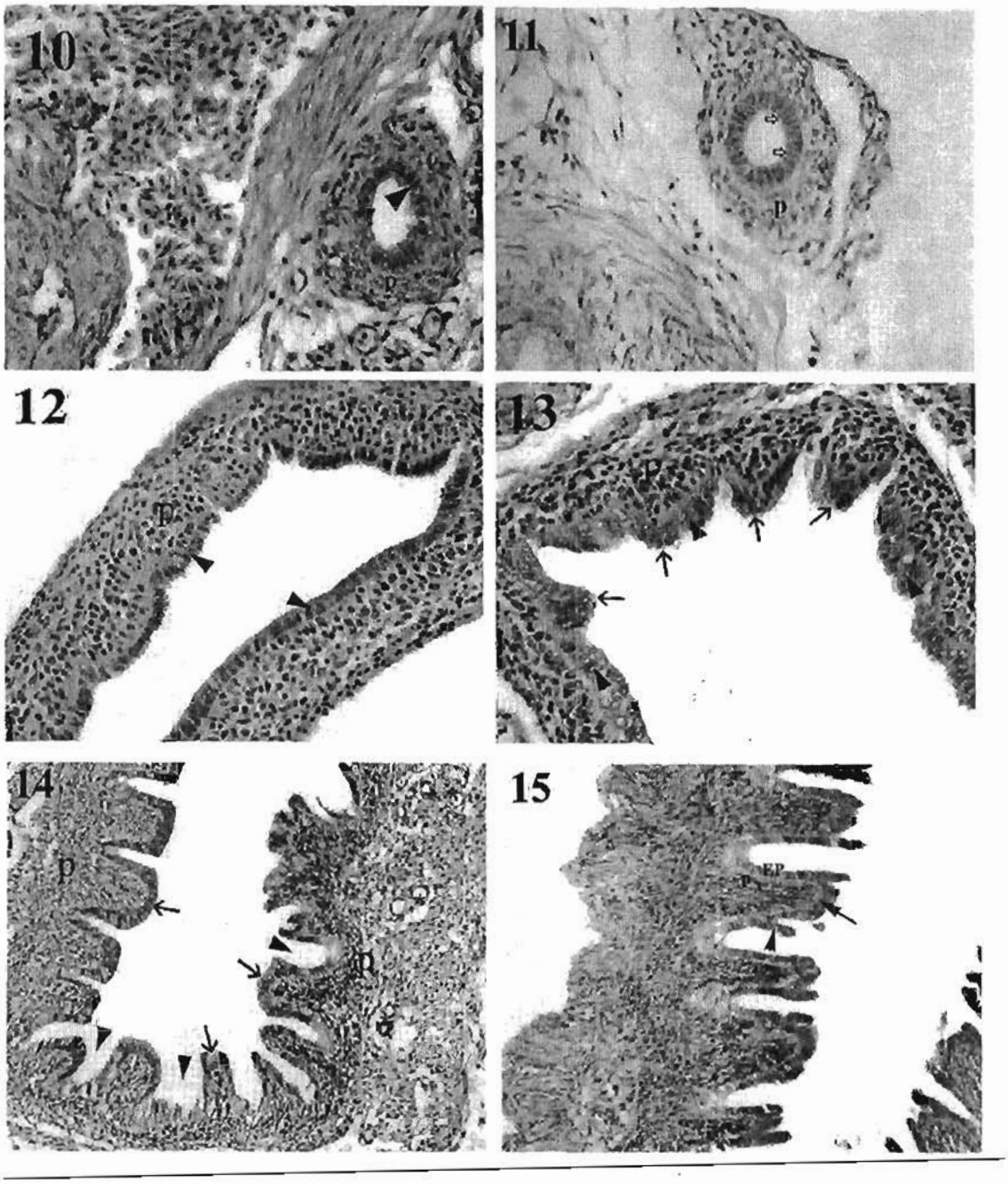
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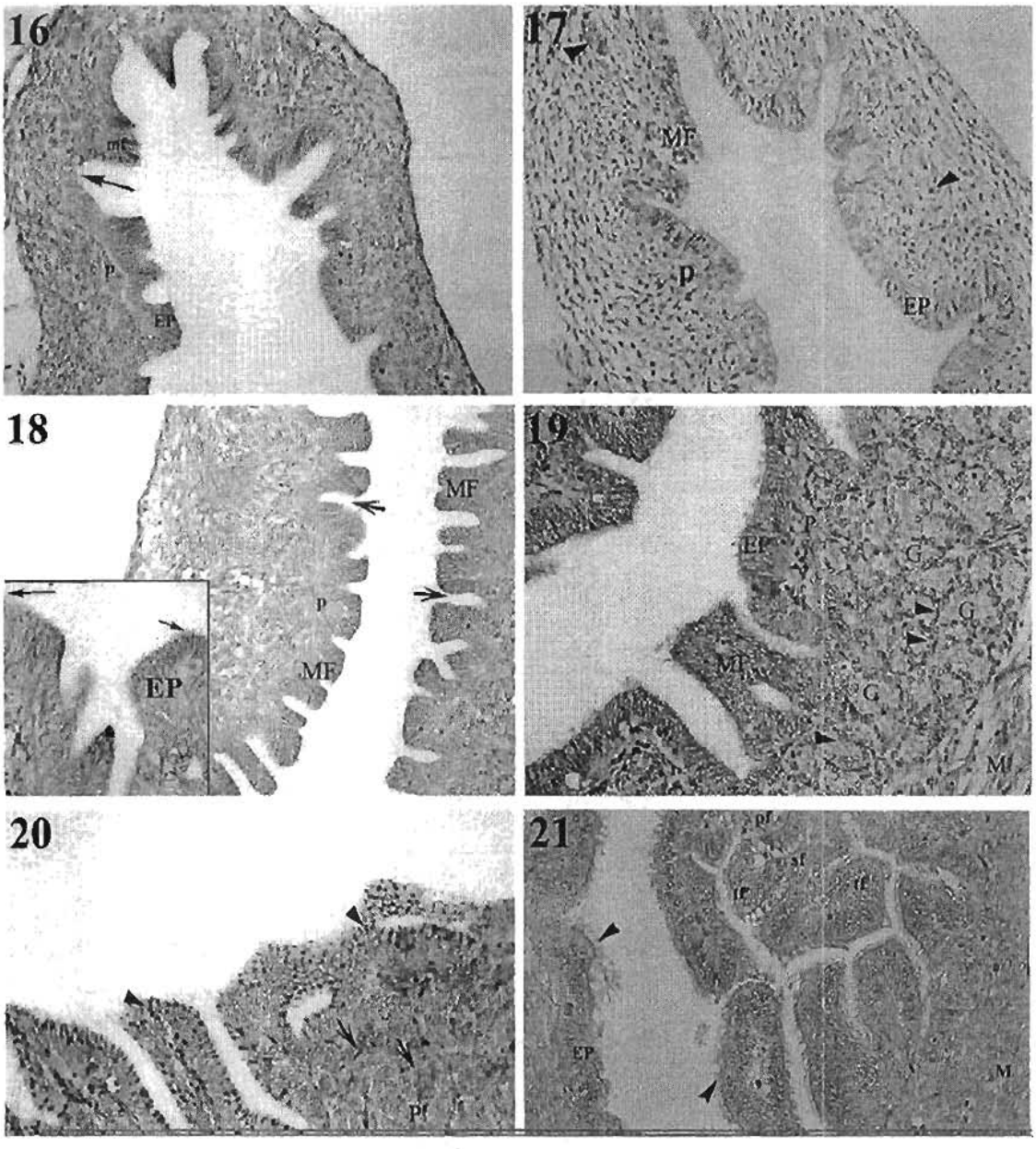
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# الملخص العربى

## دراســـات على تطـور قناة البيض فى الدجـاج البيـاض قبل وبعـد الفقس

صلاح المرسى فرج صفوت عبادة محمد أسماء عوض الداعى

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لقد تمت دراسة الجهاز التناسلى للدجاج البياض في العقود القليله الماضية، تهدف هذه الاستراتيجيه الى زيادة انتاج البيض و اللحوم في صناعة الدواجن. ولكي يتم الفهم الكامل لفسيولوجيا التكاثر و انتاج البيض فانه من المهم استكشاف التغيرات الشكلية والنسيجية خلال المرحلة الجنينية ومرحلة النمو. وقد اظهرت النتائج ان تطور قناة البيض فى الدجاج البياض تنقسم الى مرحلتين متتاليتين ;مرحلة ماقبل الفقس (اجنة الدجاج عمر ٥ -٢١ يوم): تميزت هذه المرحلة بتكون قناة مولر عن طريق سماكة الغشاء الطلائي للتجويف الجنينى وانبعاجه الى الداخل لتكوين بدايات قناة البيض و كانت مبطنة بطبقة واحدة من النسيج العمودى. اما مرحلة مابعد الفقس (كتاكيت الدجاج عمر ١ يوم - ٢٤ اسبوع) و قد تميزت هذه المرحلة بزيادة تدريجية فى ارتفاع الثنايا المخاطية، تحول النسيج الطلائى من عمودى بسيط الى نسيج كاذب عديد الطبقات مكون من خلايا مهدبة و غير مهدبة مع تغير العضلات من طبقة رقيقة الى طبقة متطورة مكونة من نسيج عضلى دائرى داخلى ونسيج عضلى طولى خارجى.