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# Genetic and Hormonal Regulation of Egg Formation in the Oviduct of Laying Hens

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## Abstract

The chicken oviduct is a unique organ in which ovulated yolk transforms into a complete egg. Ovarian hormones induce the cellular and biochemical changes in the oviducts during the egg formation and oviposition. Estradiol regulates the folliculogenesis, accumulation of yolk in the follicles, ovulation, and development of oviducts. Estradiol also induces glandular development and expression of the genes responsible for egg white proteins. Progesterone induces the ovulation of yolk from the ovary, and development of oviductal glands. In addition, several genes are spatiotemporally expressed in the magnum for albumen synthesis and deposition around the yolk, in the isthmus for shell membranes synthesis, and in the uterus for eggshell biomineralization. This chapter highlights the involvement of hormones, genes/proteins, and their interaction for egg formation in the oviduct of laying hens.

**Keywords:** oviduct, hormone, gene expression, albumen, eggshell

## 1. Introduction

The poultry oviduct provides the biological environment for the egg formation and fertilization of ovulated oocyte. The hens are born with a pair of ovary and oviduct, however, the development of the right ovary and oviduct cease and gradually regress. The left ovary and the oviduct remain functional and contribute in the egg formation. The oviduct is a long tubular structure consisting of five functionally and histomorphologically distinct segments namely: the infundibulum (site of fertilization), the magnum (production of components of egg white), the isthmus (formation of the egg shell-membranes), the shell gland or uterus (formation of calcified eggshell), and the vagina (oviposition or egg laying). Following ovulation, the ovum passes through the entire length of the oviduct, where the constituents of the egg are secreted and deposited from respective parts of the oviduct. The yolk enters the oviduct, and in about 24–28 h, a complete egg is formed. While the egg traverses through the oviduct, each segment of the oviduct either produces a component of the egg or has a vital non-secretory role. Besides environmental, nutritional, and pathological conditions, oviductal functions also govern the egg production and quality. The formation of the egg inside the oviduct is highly complex and is under genetic and hormonal control. There are several genes and biological pathways involved in the egg formation [1, 2]. The purpose of this

chapter is to provide updated information on the role of hormones, genes/protein, and their interaction that trigger histomorphological and biochemical changes in the segments of oviduct for egg formation.

## **2. Histomorphology and functions of the oviduct**

The infundibulum in hens encloses the whole ovary and has two distinctions: the membranous and the muscular infundibulum. The membranous infundibulum covers the ovarian cluster, while the muscular infundibulum is lined by ciliated cells and acts as a passage for the yolk inside the oviduct. The egg remains for a very brief period (15–30 min) in the infundibulum and then descends in the magnum where albumen is deposited around it. The infundibulum, therefore, is also the site for any potential fertilization of the ovum. The magnum is the largest segment of the oviduct and produces the egg-white proteins which surround the yolk. The glandular epithelial cells of the magnum synthesize the different egg-white proteins, store them, and secrete only for the 2–3 h duration when the egg is present in it, whereas, the ciliated epithelial cells aid in egg transport. The egg-white is rich in protein and is the main source of nutrient for the embryo during development. It also contains some antimicrobial proteins that protect the embryo from pathogenic microbes. The albumen constitutes more than 60% of the total egg, so determines the egg weight and hatchling weight. Later, the egg moves down in the isthmus, the bridging segment between the magnum and the shell gland, where it remains for 1–2 h. In the isthmus, the outer and inner eggshell membranes (ESM) are formed around the egg albumen. The eggshell membranes are fibrous networks holding the jelly egg-white in the center and also provide the site for initiation of eggshell mineralization. After being enveloped by the ESM, the egg moves in the shell gland and sits there for nearly 18–22 h during which the calcite crystals are deposited on the ESM to form the eggshell. The eggshell is 95% calcium by composition and thus, is the main source of calcium for the growing embryo. The organization of the eggshell prevents the passage of external microbes inside the egg while allowing the movement of air inside the egg for the inchoate embryo to breathe. Eventually, after complete mineralization of the eggshell, the egg is held momentarily in the vagina. Pigmentation of eggs, in some birds, is completed in the vagina and finally, the egg is laid out.

## **3. Genetic regulation of egg formation**

The egg formation is regulated through the spatiotemporal expression of genes/proteins and biological pathways in the segments of oviduct. The protein-coding genes expressed in the oviduct regulate the movement of egg, deposition of the egg constituents, and ensure the formation of quality eggs. The genetic regulation of egg formation in the oviduct is discussed below based on the genesis of each egg component.

### **3.1 Genetic regulation of albumen formation**

The albumen, also known as egg-white, is the protein-rich jelly portion of a fresh egg. It is a composite of nearly 148 different proteins that are vital for the survival and growth of the chicken embryo. The fundamental proteins include ovalbumin (OVAL), conalbumin (TF), ovomucoid (OVM), ovomucin (MUC), and lysozyme (LYZ), among others. OVAL is a structural protein making up about 54% of the total egg-white protein. Ovalbumin X, a homolog of the OVAL protein,

has antimicrobial property [3]. TF also has some antimicrobial action [4, 5]. OVM is a trypsin inhibitor and an antimicrobial agent [6]. MUC is a mucoprotein having anti-bacterial and anti-viral activity [7, 8]. LYZ has some very well-known antibiotic effects. Most of these fundamental albumen proteins are synthesized in the tubular gland cells of the magnum. The amino-acids required for the genesis of these proteins are transported from the circulation across the epithelial membrane into the gland cells by special transporter genes; the solute carriers (SLCs). The expression of many SLC mRNAs is increased in the magnum epithelium during the egg formation (Sah et al., unpublished). The synthesis of OVAL, TF, OVM, and LYZ proteins occur in a single cell-type (gland cells) continuously at a proportional rate to their abundance in the egg-white [9]. The expression of OVAL, TF, OVM, and LYZ mRNA is upregulated in the magnum of laying hens during 4–23 h post-ovulation [10].

Once the egg enters the magnum, it creates a mechanical distention of the magnum wall which elicits the stimulus to trigger the release of the stored proteins. One such molecule that provokes the secretion of the proteins from the epithelial cells is relaxin (RLN3). Expression of *RNL3* mRNA is increased in the magnum with the presence of an egg in laying hens (Sah et al., unpublished). The renin-angiotensin system (RAS), besides its renal function, participate in the protein secretion-signaling pathway. The OVAL, TF, OVM, and LYZ proteins are released in secretory granules from the glands and deposited over the yolk. Some other proteins that get incorporated in the egg-white for its defense are avian beta-defensins, cystatin, and avidin [11–13].

### 3.2 Genetic regulation of eggshell membrane formation

The eggshell membranes are fibrous networks arranged in outer and inner layers interconnected with fibers making up a highly cross-linked fibrous meshwork. This meshwork provides the nucleation sites for the initiation of eggshell mineralization. Disruption in the formation and organization of these cross-linked fibers can negatively impact the eggshell strength [14]. The expression of several genes and proteins, when the egg is in the isthmus, is critical to the formation of the ESM. Collagens are the fundamental fibrous components of the ESM. The expression of *collagen X (COL10A1)* mRNA is higher in the isthmus of laying hens [14]. The collagen X proteins are homotrimer of  $\alpha$ -1 chains secreted from the tubular gland cells of the isthmus [15] that provide the structural integrity to the ESM. Beside collagens, the ESM formation depends on other proteins such as fibrillin-1, cysteine-rich eggshell membrane protein (CREMP), lysyl oxidases, quiescin Q6 sulfhydryl oxidase 1 (QSOX1), and thioredoxin [1]. The fibrillin-1 is a microfibrillar glycoprotein whose mRNA is over-expressed only in the isthmus [14]. Fibrillin-1 gives the elastic nature to the ESM. The major constituency of cysteine in the ESM comes from CREMPs which are expressed most in the isthmus. The CREMP also has some antibacterial effect in the egg. Lysyl oxidases, on the other hand, are enzymes found in the ESM that mediate the formation of cross-links between collagen and ESM fibrillar proteins [16]. The QSOX1 protein also mediates the genesis of ESM meshwork and regulates the integrity of the ESMs [17]. The enzyme thioredoxin catalyzes the formation of disulfide cross-links between fibrillar proteins.

### 3.3 Genetic regulation of eggshell biomineralization

The chicken eggshell, the outermost calcified layer, is very critical for the safety of the eggs. The roles of several genes and proteins in synthesis and mineralization of the eggshell has widely been explored. The eggshell mineralization is activated with

the formation of calcite nodules on the outer ESM and is continued with deposition and elongation of calcium carbonate crystals. The mineralization process occurs in an acidic medium in the extracellular matrix uterine fluid. Matrix proteins such as ovocleidins, ovocalyxins, and osteopontin have well-established roles in the organization of the calcite crystals during eggshell calcification. Other localized proteins of the uterine epithelium such as, calbindin, calcitonin, otopetrin, and ATPases, as well, have crucial functions in ion-regulation across the uterine epithelium for the mineralization of egg.

For the eggshell formation, huge amount of calcium is required which is supplied partly through dietary sources and mostly through the mobilized calcium ions from medullary bones. The ion-transporting proteins, otopetrin-2, and ATPase 2C2, actively aids in the transfer of the  $\text{Ca}^{2+}$ -ions from the blood circulation into the uterine epithelial cells [1]. Calcium is also imported in the uterine epithelium passively via calcium-ion channels. Calcium-transporting ATPase (ATP2C2) and calcitonin-related polypeptide- $\beta$  (CALCB) trigger the intracellular release of  $\text{Ca}^{2+}$ -ions from calcium reserve pools such as Golgi apparatus and endoplasmic reticulum [2]. The increased concentration of intracellular  $\text{Ca}^{2+}$ -ions in the uterine epithelium is maintained by calbindin 1. Calbindin-1 facilitates the transport of intracellular  $\text{Ca}^{2+}$ -ions to the extracellular matrix (ECM) in the uterine lumen [18]. Plasma membrane Ca-ATPases (PMCA) and sodium calcium exchangers (NCX) are the essential proteins necessary for the efflux of  $\text{Ca}^{2+}$ -ions into the uterine fluid [18]. Both PMCA and NCX transport one molecule of Ca-ion with a simultaneous import of one  $\text{Na}^+$ -ion in the uterine epithelium. ATPases such as ATP2B1 and ATP2B2 also transport  $\text{Ca}^{2+}$ -ions at the expense of  $\text{H}^+$ -ions import [2, 19]. The resultant increased in cellular  $\text{Na}^+$ -ions is offset with the efflux of those excess ions by ATP1A1, ATP1B1, and NKAIN4 but with the contemporaneous influx of  $\text{K}^+$ -ions in the uterine epithelium. Again, the elevated concentrations of K-ions are nullified by efflux via  $\text{K}^+$ -ion channel proteins such as KCNH1 or KCNJ2 [2, 19]. As such, the transport of  $\text{Ca}^{2+}$ -ions across the uterine epithelium requires the balance of  $\text{Na}^+$ -,  $\text{K}^+$ -, and  $\text{H}^+$ - ions which are regulated by ATPases, ion-channel and some other proteins. Bicarbonate ( $\text{HCO}_3^-$ )-ions are equally important in the mineralization of eggshell. The enzyme, carbonic anhydrase, catalyzes the formation of cellular  $\text{HCO}_3^-$ -ions from carbon dioxide and water. The  $\text{HCO}_3^-$ -ions are then carried out into the uterine fluid by special transporter proteins, the solute carriers. These  $\text{HCO}_3^-$ -ions eventually combine with the free Ca-ions in the fluid bathing the egg to make calcite crystals.

Ovocleidins (OC) are eggshell matrix proteins which regulate the crystallization phenomenon in the uterus. The OC-17 catalyzes the mineralization of amorphous calcium carbonate to calcite crystals [20]. OC-116 regulates the organization of calcite crystals in the eggshell. Ovocalyxins (OCX) has three major member proteins which participate in eggshell mineralization. The OCX-32 controls the morphology of the calcite crystals and has a rather anti-mineralization function during the termination phase of calcification [21]. The direct role of OCX-36 in eggshell calcification has not been established, however, it protects the egg from microbial invasion [22]. Another member of the ovocalyxins, OCX-21, ensures the quality eggshell formation by providing a conducive environment [23]. Osteopontin, known as a secreted phosphoprotein, is also a negative regulator of calcification and determines the form and shape of the eggshell [24].

### **3.4 Ubiquitous proteins of the oviduct in the regulation of egg formation**

Matrix metalloproteases (MMPs) are ubiquitous proteases that are known to degrade different extracellular matrix proteins (ECM) [25]. Cells in the body are surrounded by ECM, and cellular growth, proliferation, and differentiation are regulated by ECM degradation and remodeling through MMPs [25]. MMPs

are detected in the whole oviduct, and mostly in the magnum and uterus [1]. The cells of the magnum and uterus are highly secretory in nature, which require the proliferation of epithelium. The MMPs degrade the ECM surrounding the oviductal epithelium and help in cellular migration, proliferation, and differentiation [25]. Different MMPs (MMP-2, -7, and -9) are actively expressed in the oviduct during molting, while downregulated during the shift from immature to adult hens [26, 27]. Expression of MMP-1 and -10 is highest in laying hens in comparison to non-laying and molting hens (Sah et al., Unpublished). MMP-1 degrades interstitial collagens (type I, II, and III). MMP-2 degrades type IV collagens and induces angiogenesis. MMP-7 is also known as matrilysin which degrades casein, fibronectin, elastin and proteoglycans. MMP-9 is a gelatinase that also provokes the formation of new vasculatures [28]. MMP-10 is a stromelysin enzyme that can breakdown proteoglycans and fibronectins. The various matrix degrading roles of aforementioned MMPs ultimately ensures proper reproductive functions of the oviduct.

The solute carriers (SLCs) are another group of ubiquitous proteins found throughout the chicken oviduct. The SLCs are specialized molecular transporting proteins that are largely expressed on the plasma membrane. The expression of more than dozen of SLCs is evident in the oviduct [1, 2]. Several SLCs transport inorganic ions and amino acids in the magnum during albumen formation [29]. Some SLCs are mitochondrial carriers and are over-expressed in the uterus [19]. The SLCs are also upregulated at the uterovaginal junction to ensure the survival of the chicken sperm during storage [30, 31].

## **4. Hormonal regulation of egg formation**

Egg formation in the laying hen is an intricate process involving the interplay of different molecules and hormones. Hormones are of cardinal significant in every process of egg formation; from the development of the reproductive tract, ovulation, albumen synthesis, eggshell formation, and finally to the oviposition of eggs. Major hormones that play a crucial role in the egg formation in laying hens are discussed below.

### **4.1 Role of gonadotropin-releasing hormone (GnRH) in egg formation**

The GnRH in hens is released from the hypothalamic/portal system in response to the photo-stimulation and rising concentration of the progesterone. Two chemical forms of the GnRH are present in the avian species: chicken GnRH-I (cGnRH-I) and chicken GnRH-II (cGnRH-II) [32]. These two forms of the GnRH play different roles in the avian species. GnRH-I is vital for stimulating synthesis and release of anterior pituitary hormones, GnRH-II, on the other hand, is involved in mating and courtship behavior [33]. GnRH in hens are regulated by catecholamine, vasotocin, vasoactive intestinal peptide, neuropeptide Y and opioid peptides [34]. Recently, we detected the GnRH receptor in the oviduct of laying hens; however, its functional role in the egg formation is completely unknown.

### **4.2 Role of gonadotropins in egg formation**

The gonadotropins; follicle-stimulating hormone (FSH) and luteinizing hormone (LH) are produced at the anterior pituitary in response to the GnRH from the hypothalamus. FSH in the hen is responsible for the recruitment and granulosa cell development of the small follicles. FSH acts mainly on the granulosa layer of the small yellow follicles, and the sixth (F6) to third (F3) largest follicles. It also

stimulates progesterone production in granulosa cells from F6 to F3 follicles [35]. Sustained plasma concentration of the FSH remains throughout the ovulatory cycle except for a small increase at around 12 h before ovulation [36]. The LH in hens, unlike other mammalian species, does not luteinize the follicles, rather they are involved in ovulation and steroidogenesis [37]. Plasma concentration of the LH peaks at around 4–6 h before ovulation (coincide with the peak rise of progesterone), whereas lowest plasma concentration of LH is observed at 11 h before ovulation [38]. The primary target for the LH is larger preovulatory follicles.

### **4.3 Role of estrogen in egg formation**

Estrogens are mainly produced by the theca cells of the small follicles. The highest plasma concentration of the estradiol occurs 4–6 h before ovulation although some small rise in estrogen is also observed at 18–23 h before ovulation. Estrogen plays a crucial role in the egg yolk formation by stimulating the avian liver to produce the yolk precursor, vitellogenin and very-low-density lipoprotein, the primary source of yolk protein and lipid, respectively [39]. Estradiol also sensitizes hypothalamus to the positive feedback effect of the progesterone. Besides the essential role of estradiol for the growth, and development of the oviduct, it also regulates calcium metabolism for the eggshell formation and development of secondary sex characters. [37]. Albumen is mainly synthesized in the tubular gland cells in the magnum and comprises mainly of ovalbumin, conalbumin, ovomucoid, and lysozyme. Estrogen is found to be associated with the synthesis of these molecules and thus, plays a crucial role in egg-white formation [40].

### **4.4 Role of progesterone in egg formation**

Progesterone along with its cognate receptor regulates the female fertility [41, 42]. Progesterone is mainly produced by the granulosa cells of the larger follicles (F1–F3). The peak plasma concentration of the progesterone occurs 4–6 h before ovulation [38]. During the time of preovulatory LH surge, only the largest preovulatory follicles secrete progesterone. This increase in progesterone creates a positive feedback response to the hypothalamus, which in turn increases the secretion of GnRH into the hypothalamic-pituitary portal system producing the surge in LH from the anterior pituitary. This LH causes rupture and release of yolk (ovum) from the mature follicles (F1). Progesterone is also associated with the avidin production, contraction of the myometrium and eggshell formation [41].

### **4.5 Role of androgens in egg formation**

Androgen is produced in theca and granulosa cells of both small and large follicles. Peak preovulatory concentration of testosterone occurs 6–10 h prior to ovulation, whereas the highest concentration of the 5 $\alpha$ -dihydrotestosterone occurs 6 h before ovulation [41]. Role of androgen in ovulation is still obscured. Androgen is found to regulate ovomucoid and ovalbumin gene expressions in the oviduct of the chicken [43]. Androgens also help in the development of the secondary sexual characters in hens such as growth and coloring of combs and wattle.

## **5. Conclusion**

In conclusion, hormones are required for the timely ovulation of yolk from the ovary, and preparation of oviduct for egg formation. Gene expressions in the

different segment of oviducts help in the cellular remodeling, secretion, synthesis and transport of essential molecules for the egg formation. Understanding of this information will be helpful in developing persistence layers with quality eggs.

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## **Conflict of interest**

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of these information.

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