Alterations in egg white-related genes expression in response to hormonal stimulation

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Abstract

The reproductive tract in avian females is sensitive to hormonal regulation. Exogenous estrogen induces immature oviduct development to improve egg production after molting. In this process, regressed female reproductive tract is regenerated in response to the secretion of estrogen. However, there is limited knowledge on the physiological mechanisms underlying the regulation of the avian female reproductive system. In our previous study, results from microarray analysis revealed that the expression of genes encoding egg white proteins is affected during molting. Herein, we artificially induced the molting period in chickens through a zinc-containing diet. Subsequently, changes in the expression of genes encoding egg white proteins were confirmed in the oviduct tissue. The levels of *MUC5B*, *ORM1*, *RTBDN*, and *TENP* mRNA were significantly high in the oviduct, and the genes were repressed in the regression phase, whereas these were expressed in the recrudescence phase, particularly in the luminal epithelium and glandular epithelium of the oviduct, during molting. Moreover, we observed that gene expression was induced in the magnum, the site for the secretion of egg white components. Next, differences in expression levels of the four genes in normal and cancerous ovaries were compared. Collectively, results suggest that the four selected genes are expressed in the female chicken reproductive tract in response to hormonal regulation, and egg white protein-encoding genes may serve as modulators of the reproductive system in hens.

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Introduction

Molting is a crucial event in the life cycle of domestic laying hens, similar to other avian species. Molting is a natural physiological process in hens during wintertime. At this time, the hens lose their reproductive function and lay eggs at a low rate (Berry 2003). In addition, hens undergoing molting have a loss of appetite, resulting in reduced feed intake and a weight loss of approximately 20% (Sherry *et al.* 1980). Molting can be artificially induced by modulation of nutrient components in feed and growth conditions, as well as by environmental modulation (Berry 2003). The purpose of artificial molting is to improve egg quality and egg production by rejuvenating laying hens (Bell 2003). In hens after artificial molting, the eggshell strength increases and the red chromaticity and yellow chromaticity are enhanced (Aygün 2013). In particular, molting through high levels of zinc supplementation diet increases egg weight and eggshell thickness during the post-molting period (Berry & Brake 1987). During the molting period, laying hens undergo regression of the reproductive system that follows the recrudescence of tissues along with changes in the concentrations of several endogenous hormones

as well as in gene expression (Hoshino *et al.* 1988, Berry 2003, Jeong *et al.* 2013). In such physiological changes, estrogen acts directly during differentiation and remodeling and affects the formation of egg white proteins via modulation of their components, including ovomucoid, ovalbumin, ovotransferrin, and other physiological proteins and peptides, such as lactoferrin and lysozyme (Palmiter 1972, Seaver & Skafar 1984). Molting has been studied with a specific focus on its effects on egg statistics, external inducing factors, and subsequent economical strategies. Owing to this, there is limited information on the molecular mechanisms followed by the recovery system during molting periods.

In our previous study, several candidate genes that act as latent regulators of the remodeling system of the oviduct and facilitate post-transcriptional regulation in the magnum following the molting of laying hens were identified (Jeong *et al.* 2013). The genes encoding mucin 5B (*MUC5B*), orosomucoid 1 (*ORM1*), retbindin (*RTBDN*), and transiently expressed in neural precursors (*TENP*) were selected in this study as these are commonly related to egg white proteins in the magnum. *MUC5B* encodes alpha-ovomucin that is a component of egg whites; it forms the egg white gel matrix and protects

the embryo from pathogens (Gipson *et al.* 1997, Bansil & Turner 2006, Lang *et al.* 2006, Offengenden *et al.* 2011). *ORM1* is a precursor of ovoglycoproteins such as orosomucoid that is suggested to be similar to alpha 1-acid glycoproteins (α_1 -AGPs) based on its amino acid sequence (Sadakane *et al.* 2002). *RTBDN* is a riboflavin-binding protein that is involved in the transportation of riboflavin for the development of embryos in chickens (Becvar & Palmer 1982). *TENP* is conserved across avian species and was first identified as a putative factor related to early neurological events in post-mitotic cells of chickens (Yan & Wang 1998), while it has a protective antimicrobial function in the egg and oviduct, particularly in egg white (Whenham *et al.* 2014).

Unlike mammals, repetitive ovulation, which is specific to laying hens, increases the risk of developing ovarian cancer (Fathalla 1971). Therefore, laying hen is considered to be a useful animal model for ovarian cancer research due to its natural development of ovarian cancer at a high rate and the histopathology of ovarian cancer similar to that of humans (Johnson & Giles 2013). In addition, in our previous study, many genes that decreased and then increased in the regression and recrudescence phases in the molting process, respectively, were upregulated in ovarian cancer tissues (Jeong *et al.* 2013, Bae *et al.* 2014, Lim & Song 2015). These reports suggest that genes whose expression is regulated during the molting process may be correlated with the risk of ovarian cancer.

Herein, we (1) studied the expression of genes encoding egg white proteins in the reproductive organs of hens; (2) determined the differences in target gene expression during molting; (3) investigated the effects of diethylstilbestrol (DES), a form of synthetic estrogen, on the segments in an immature oviduct; and (4) compared relative gene expression of the four genes between normal and cancerous ovaries in hens. Our study could help identify the egg white-related genes that act as modulators during the molting period and their altered response to estrogen stimulation and ovarian carcinogenesis tissues.

Materials and methods

Animals for experiment and care

The use of chicks, in this study, was approved by the Animal Care and Use Committee of Korea University. We subjected white Leghorn chickens to a 15 h light:9 h darkness cycle. Feed and water were provided *ad libitum*. They were subjected to standard poultry husbandry practices.

Tissue biopsy

Collection of various tissues from male and female chickens

Mature white leghorn (WL) chickens were killed. Tissue samples were collected from the brain, heart, liver, kidneys, muscles, small intestine, gizzard, testes, ovaries, and oviducts and were immersed in paraformaldehyde or frozen in liquid nitrogen

vapor. Subsets of the samples fixed in paraformaldehyde were cut into 10-mm sections and fixed using paraformaldehyde. The other samples were cut to a thickness of at least 5 mm 5 mm and at most 7 mm. Next, all samples were incubated in ethanol for 24 h while enclosed in Paraplast-Plus (Leica MicrosystemsWetzlar). These tissues were further sliced into 5 μ m sections.

Induction of artificial molting period

Molting was induced by adding 20,000 ppm zinc to the feed, as described previously (Berry 2003). Thirty-five laying hens underwent molting by feeding on the zinc diet, and egg laying ceased at 12 days. The chickens were assigned into two groups: a molting-progression group (Group 1) and a post-molting-progression group (Group 2). Group 1 was divided into three subgroups based on the days on which the high zinc diet was fed (normal 0, 6, and 12 days after initial zinc-enriched diet feeding). Group 2 (recrudescence) was divided into four subgroups based on the number of days after cessation of egg production and commencement of normal commercial diet feeding (20, 25, 30, and 35 days after zinc-enriched diet feeding).

DES administration

The sexes of the chickens were determined by PCR analysis using the primer sets for the W chromosome in the boiled blood template from the egg from 2.5- to 3-day-old. (Lee *et al.* 2009). DES was administered as reported previously (Lim *et al.* 2011). Ten milligrams of DES was injected into the abdomen of 1-week-old chickens. After the chicks were treated with DES, all pellets were removed from the chicks. Next, a 10 mg dose of DES was administered to the chicks for 10 days. Five chicks were assigned to the treatment group and five to the control group. For the chicks treated with DES, whole part of oviduct was collected from among the differentiated neonatal oviducts. Conversely, for control chicks, the middle portion of undifferentiated neonatal oviducts was used.

Collection of cancerous ovary tissue

In this study, 136 hens that had stopped laying eggs (at least 24 months in age) were killed to collect the ovaries (cancerous or normal). Cancerous ovary tissues were obtained from ten hens and normal ovary tissues from five hens of comparable age. Hens with ovarian cancer were classified based on the characteristics of ovarian cancer in chicks (Barua *et al.* 2009).

RNA extraction and RT-PCR analysis

Whole RNA was extracted from the fixed tissues using TRIzol reagent in accordance with the manufacturer's instructions. Then, cDNA was synthesized using AccuPower® RT PreMix (Bioneer, Daejeon, Korea). Specific RNA sequences of genes from the chicken oviduct were amplified using primer sets that have been provided in Table 1. PCR amplification was performed using approximately 120 ng of cDNA, and the conditions used and validation required were described previously (Yang *et al.* 2016). Light intensity was detected and measured for appropriately sized bands using a Gel Doc XR+ system. SYBR® Green (Sigma) was used to measure gene expression.

Table 1 Primer list used in quantitative RT-PCR and cloning PCR analysis.

Gene	GenBank accession no.	Sense primer (5' → 3')	Antisense primer (5' → 3')	Product size (bp)
<i>GAPDH</i>	NM_204305	CAC AGC CAC ACA GAA GAC GG	CCA TCA AGT CCA CAA CAC GG	443
		CAT TCC TCC ACC TTT GAT GC	ACC ATC AAG TCC ACA ACA CG	116
<i>MUC5B</i>	NM_204661	GAA CTG GGC TGA CAC TCT CC	ATC TGT ACA TGG GGC TTT GC	577
		GGA GCT GTG GAA GAT TCA	TCC ACA CTG TTT GAG CAA GG	107
<i>ORM1</i>	NM_204541	TCT AGA CCT GCA GGC TCA CC	GGA TGA ACA GGT CCT TGT CG	435
		TGA GGC TGA ATG AAA CAT GC	GGA TGA ACA GGT CCT TGT CG	138
<i>RTBDN</i>	NM_205463	GTC TCA CCC AGA AGG ACA GC	AAC ACC GGT AAA AGC ACT CG	328
		CAA TCA AGC ACC TCC TCT CC	TCT CTT CCC CTT CCT CTT GC	123
<i>TENP</i>	NM_205026	CTG GGA CTG AGC TAC CTT GC	CTG GAG GAC TTG CTT TCT GC	368
		CTG GGA CTG AGC TAC CTT GC	ACA GGT CTG AGG CTC TTT GG	90

To compensate for the variations in loading, *GAPDH* was used between each sample loading. Each gene was analyzed in triplicates; sequences of primer sets used to synthesize the same are provided in Table 1. Based on the standard curve method, we performed PCR amplification using the same conditions as those used in the previous instance (Yang *et al.* 2016). The C_T value, indicated by the cycle number of a reaction, was used to quantify the relative gene expression value based on the $2^{-\Delta\Delta C_T}$ method (Livak & Schmittgen 2001).

Detection of in situ hybridization

In situ hybridization was performed to determine the location of mRNA expression. cDNA was synthesized with the primer used in RT-PCR analysis for the generation of PCR products for forming hybridization probes. The transcripts produced by PCR were extracted and cloned into the TOPO® vector (Invitrogen) after the sequences amplified using the T7- and SP6-specific primers contained in the plasmid were confirmed. Next, RNA probes labeled with digoxigenin (DIG) were transcribed using a DIG RNA labeling kit (Roche Applied Science). Tissue fixation, dehydration, and subsequent incubation for DIG labeling were performed as reported previously (Yang *et al.* 2016). Lastly, the sections were incubated overnight with sheep anti-DIG antibody conjugated to alkaline phosphatase (Roche Applied Science). The signal could be visualized upon exposure to a solution containing 0.4 mM 5-bromo-4-chloro-3-indolyl phosphate, 0.4 mM nitro-blue tetrazolium, and 2 mM levamisole (Sigma).

Statistical analyses

The quantitative PCR data were analyzed using ANOVA based on a linear model (PROC-GLM) to determine whether there was significant differential expression of genes in the chick oviduct in response to DES treatment or molting. Additionally, differences between the gene expression patterns of normal and cancerous ovaries were analyzed using the *F*-test. Differences between means were analyzed using the Student's *t*-test.

Results

Different expression patterns of *MUC5B*, *ORM1*, *RTBDN*, and *TENP* genes in various organs of chickens

First, we conducted semi-quantitative RT-PCR to determine the expression pattern of the four genes

selected by microarray analysis based on previous research on the regulation of egg white formation (Jeong *et al.* 2013) by various organs of chickens. We collected the tissues from male (Fig. 1A) and female (Fig. 1B) chickens and studied the mRNA expression patterns. *MUC5B* expression was detected in the brain, kidney, and oviduct tissue samples from female chickens, while the same was detected only in kidney tissue samples from male chickens. The expression was significantly high in chicken oviduct samples. *ORM1* was expressed in the brain, liver, muscle, gizzard, ovary, and oviduct tissue samples in female chickens and only in liver tissue samples in male chickens. *RTBDN* was expressed in most organs but was not detectable in the heart and

Figure 1 Expression of *MUC5B*, *ORM1*, *RTBDN*, and *TENP* mRNA in various tissues in male (A) and female (B) chickens. The organs were removed to synthesize cDNA in both male and female chickens and analyzed using semiquantitative RT-PCR with *GAPDH* expression as control.

gizzard tissues of male chickens and the heart tissue of female chickens. The expression of *RTBDN* mRNA was considerably high in the oviduct tissues. Although *TENP* was barely expressed in male chickens, its expression was high in the brain, muscle, gizzard, ovary, and oviduct tissues of female chickens. Collectively, the four target genes are highly expressed in chicken oviduct tissues compared to that in other organs.

Altered gene expression during regression and recrudescence period

Next, we investigated whether the regression and recrudescence of oviducts that occur during molting in response to high zinc feeding affect the expression of target genes. Quantitative RT-PCR analysis was used to quantify the transcripts. It was confirmed that *MUC5B*, *ORM1*, *RTBDN*, and *TENP* expression declined in the regression phase (day 0–12) (Fig. 2). However, the original expression levels were gradually recovered as the recrudescence phase progressed and the cell-specific localization in chicken oviducts during regression and recrudescence was observed through *in situ* hybridization analyses. As illustrated in Fig. 3, *MUC5B*, *ORM1*, *RTBDN*, and *TENP* expression declined as the regression phase progressed, which was consistent with quantitative RT-PCR analysis results. Initial mRNA expression levels were restored in the luminal epithelium (LE) and glandular epithelium (GE) of oviducts.

Effects of DES on mRNA expression in the four segments of chicken oviducts

The chicken oviduct consists of the following four parts: infundibulum (site of fertilization), magnum (site for the secretion of egg white components), isthmus (site for the formation of the soft shell membrane), and shell gland (site for the calcification of the eggshell). In a previous study, DES was observed to promote growth and differentiation in chicken oviduct and the four genes were identified as putative regulators that modulate oviduct development in chickens (Song *et al.* 2011, Jeong *et al.* 2013). Using quantitative RT-PCR analysis, we confirmed whether differences in the expression levels of the target genes in the four locations of chicken oviducts resulted from DES treatments. We observed that *MUC5B*, *RTBDN*, *ORM1*, and *TENP* expression levels were high in all subparts of the DES-treated oviducts (Fig. 4). *MUC5B* mRNA expression increased by 24.9-fold in the infundibulum, 482.9-fold in the magnum, 19.6-fold in the isthmus, and 5.8-fold in the shell gland of DES-treated oviducts compared to that in the corresponding parts in control oviducts (Fig. 4A). Similarly, *ORM1* mRNA expression increased by 171.7-fold in the magnum in response to DES treatment (Fig. 4B), whereas *RTBDN* mRNA expression increased by 8.3-fold in the infundibulum,

Figure 2 Effect of regression and recrudescence in chicken oviducts on *MUC5B* (A) *ORM1* (B) *RTBDN* (C) and *TENP* (D) mRNA expression. Quantitative RT-PCR was conducted using cDNA templates from the magnum of hens, dependent on the day after feeding a high-zinc diet. The regression phase represents the period of high-zinc diet (day 6 and 12). Recrudescence phase represents the period of normal diet again (day 20, 25, 30, and 35). These experiments were performed in triplicate and the levels of expression were normalized to that of *GAPDH*. The asterisks represent statistically significant differences (mean $\pm$ S.E.M.; *** P < 0.001).

458.5-fold in the magnum, 6.0-fold in the isthmus, and 2.7-fold in the shell gland in response to DES treatment (Fig. 4C). *TENP* mRNA levels also increased by 2.6-fold in the infundibulum, 15.4-fold in the magnum, 2.3-fold in the isthmus, and 4.1-fold in the shell gland in response to DES treatment (Fig. 4D). In the magnum, where egg white components are secreted, all the genes were significantly upregulated upon DES treatment compared to that in other segments. Additionally, the cell-specific localization was visualized by *in situ* hybridization analysis (Fig. 5). The increase in the intensity corresponding to each mRNA was centered at the LE and GE of oviducts, especially in the magnum.

Differential distribution of mRNAs between normal and cancerous ovaries

Previous studies have suggested that the expression of oviduct-related genes increases in cancerous ovarian tissues (Trevino *et al.* 2010). In the present study, we performed quantitative RT-PCR and *in situ* hybridization analysis to determine whether there are alterations in the expression of oviduct-related target genes between cancerous and normal ovaries. As shown in Fig. 6, the expression of *MUC5B*, *ORM1*, and *RTBDN* mRNA was induced significantly in cancerous ovarian tissues, while *TENP* mRNA expression was inhibited in the cancerous ovaries compared to that in normal ovaries. Moreover, these genes were considerably upregulated in the GE of

Figure 3 Localization of the mRNA of the four target genes in the magnum during regression and recrudescence. *In situ* hybridization analysis indicates the localization of *MUC5B*, *ORM1*, *RTBDN*, and *TENP* mRNA in the magnum during regression (day 6 and 12) and recrudescence (day 20, 25, 30, and 35) upon molting induced by the zinc-enriched diet. LE, luminal epithelium; GE, glandular epithelium.

cancerous tissues, while their overexpression was barely detected in normal tissues (Fig. 6E).

Discussion

Herein, our results revealed the specific expression patterns of *MUC5B*, *ORM1*, *RTBDN*, and *TENP* genes in the oviduct and their fluctuations during the regression and recrudescence phase of chicken oviducts following molting periods. The four genes were upregulated in response to DES treatment, especially in the magnum. However, compared to those in normal tissues, only *TENP* was downregulated in ovarian cancerous tissues. Under the same conditions, the other genes were upregulated in the cancerous tissues. These results suggest that *MUC5B*,

ORM1, *RTBDN*, and *TENP* are closely associated with restoration of the oviduct in response to estrogen exposure and molting of laying hens.

The four genes used, in this study, were related directly to the formation of egg white proteins in the magnum of oviducts. *MUC5B* encodes alpha-ovomucin, which is one of the two ovomucin components in egg white (Omana *et al.* 2010, Offengenden *et al.* 2011). Its high viscosity facilitates gel formation and provides structural protection against pathogens, including bacteria and viruses. Ovomucin is similar in terms of genetic, rheological, and structural characteristics with gel-forming mucins that are secreted to protect organ surfaces, including the reproductive tract (Gipson *et al.* 1997, Offengenden *et al.* 2011); the *MUC* gene family,

Figure 4 Relative expression of target genes in the four segments of the oviduct (infundibulum, magnum, isthmus, shell gland) in response to DES treatment. Quantitative RT-PCR analysis was conducted to quantify *MUC5B* (A) *ORM1* (B) *RTBDN* (C) and *TENP* (D) mRNA expression using cDNA templates synthesized from samples of DES-treated and non-treated oviduct from chickens. These experiments were performed in triplicate, and the expression levels were normalized to that of *GAPDH*. The asterisks represent statistically significant differences (mean $\pm$ S.E.M.; *** P < 0.001, ** P < 0.01, and * P < 0.05).

which includes *MUC5B* in chickens, is located in the corresponding locus encoding human mucins in chromosomes 5 and 11 (Gipson *et al.* 1997). *RTBDN* encodes a riboflavin-binding protein present in egg whites in the magnum of the oviduct (Hamazume *et al.* 1984, Tian *et al.* 2010). Avian *RTBDN* is divided into two types based on the producing organs: yolk-RBP in liver and egg white-RBP in oviduct, which are biosynthesized from the same gene (Norioka *et al.* 1985). This glycoprophosphoprotein consists of ovomucoid type sugar chains that reversibly bind to vitamin B2 (riboflavin) and transport it from the serum and egg white to the developing embryo (Becvar & Palmer 1982, Hamazume *et al.* 1984, Tian *et al.* 2010). *ORM1* in chicken is known to encode an ovoglycoprotein present in egg whites (OGCHI). *ORM1* is an ovoglycoprotein precursor gene with high expression levels that are maintained during egg white formation (Yin *et al.* 2020). It has been used to separate drug enantiomers because its chiral recognition ability arises from a protein domain and not from a sugar (Haginaka *et al.* 2000, Sadakane *et al.* 2002). Results of the study suggested that the amino acid sequence of *OGCHI*, which is associated with lipocalin-transporting hydrophobic molecules such as steroid hormones, and the mature form of the gene share 31–32% of their identities with the corresponding counterparts of rabbit and human α_1 -AGPs, which suggests that *OGCHI* encodes the members of α_1 -AGPs family in mammals.

TENP has been suggested to share a divergent orthology with human *LPUNC2*, a member of the *PLUNC* family that is structurally homologous to BPI proteins, which have anti-bacterial functions depending on N- or C-terminal type (Beamer *et al.* 1998, Chiang *et al.* 2011). Moreover, *TENP* is widely present in eggs and was initially identified in the retina and brain in association with early neurological events in post-mitotic cells before the extensive differentiation of precursor cells (Yan & Wang 1998, Whenham *et al.* 2014). In adult hens, *TENP* is expressed locally in the magnum, and its levels are higher in laying hens than in non-laying hens (Whenham *et al.* 2014). These results suggest that *TENP* has a relatively close association with the reproductive system in adult hens and also plays a role in the development of oocyte/embryo cells in eggs, and these sequential events occur in the magnum of the oviduct. In our study, the genes were upregulated in the oviduct than in other organs, while their expression was downregulated in the regression phase. This reduction was reversed gradually as the recrudescence phase progressed in the molting period. Consistent with this result, a similar trend was noted in the GE and LE of oviducts. These results imply that *MUC5B*, *ORM1*, *RTBDN*, and *TENP* encode components of egg whites and are associated with secretory functions. Moreover, their functional roles in oviductal remodeling during the regression and recrudescence period of molting were assumed.

DES is synthesized as a non-steroidal estrogen that targets the estrogen receptor in avian reproductive organs and enhances the secretion of egg white proteins such as SERPINB1, SERPIN14B, ovalbumin, and ovomucoid (Song *et al.* 2011) and regulates nervous and endocrine system development, hormone interactions, and tissue remodeling in chick oviduct, similar to endogenous estrogen (Seaver & Skafar 1984, Song *et al.* 2011). Estrogen affects the oviduct-related genes and enhances the overall secretion of the major components of egg white proteins, including ovalbumin, ovomucoid, and conalbumin (Kohler *et al.* 1968), and regulates the development and functions of the reproductive system (Okada *et al.* 2005). We investigated the effects of DES on the oviduct based on observations from various segments of oviducts and by comparing normal and cancerous ovaries. In our study, the four genes were significantly upregulated in the magnum in response to DES treatment compared to that in other segments, with specific localization detected in GE and LE. The results provide evidence that egg white protein genes are mediators that regulate the development and remodeling of oviducts affected by estrogen primarily present in the magnum.

The risk of developing ovarian cancer in hens is closely related to the number of ovulations (Risch 1998). The pathogenesis of ovarian cancer in hens is not clearly understood, but repeated ovulation processes cause disruption, including genetic damage to the ovarian epithelium, and induce neoplastic transformation,

Figure 5 Cell-specific localization of the mRNA of each gene was detected by *in situ* hybridization analysis in immature oviduct after DES treatment. The cross-sections of oviducts from chicks after DES or vehicle treatment were hybridized with antisense or sense cDNA probes of *MUC5B*, *ORM1*, *RTBDN*, and *TENP*. LE, luminal epithelium; GE, glandular epithelium.

Figure 6 Comparative mRNA expression of the four genes in normal and cancerous ovaries. Quantitative RT-PCR analyses were performed to quantify *MUC5B* (A) *ORM1* (B) *RTBDN* (C) and *TENP* (D) mRNA using cDNA templates derived from normal and cancerous ovaries of chickens. These experiments were performed in triplicate, and the expression levels were normalized to that of *GAPDH*. The asterisks represent statistically significant differences (mean $\pm$ S.E.M.; *** P < 0.001 and ** P < 0.01). (E) Cell-specific localization of *MUC5B*, *ORM1*, and *RTBDN* mRNA was detected by *in situ* hybridization analysis. F, follicle; GE, glandular epithelium.

similar to the pathogenesis of ovarian cancer in women (Lowry *et al.* 1991). Moreover, similar to women, the frequency of p53 alteration in hen tumors has been reported to correlate with the number of ovulation processes (Hakim *et al.* 2009). These previous studies suggest that disruption and repair of the ovarian epithelium during frequent ovulation in hens may contribute to the proliferation of ovarian cells based on genetic defects. Indeed, inhibition of ovulation through calorie restriction in hens significantly lowered the risk of ovarian cancer (Carver *et al.* 2011). Microarray analysis found that 40% of the oviduct-related genes were upregulated in the ovarian cancer tissue of hens (Trevino *et al.* 2010). Moreover, the hen oviduct is highly responsive to estrogen and are thought to contribute to the development of ovarian cancer (O'Malley *et al.* 1967, Kohler *et al.* 1968). In addition, The gene whose expression is increased in response to estrogen in the oviduct is also highly expressed in ovarian cancer tissues of hens (Jeong *et al.* 2012, Lim & Song 2013). Furthermore, ovarian tumors from hens are also observed in the oviduct, which is similar to the fact that many aggressive ovarian cancers are observed in the oviduct in women (Kim *et al.* 2012). For these reasons, genes whose expression decreases during the molting period and then recovers are predicted to be involved in the risk of ovarian cancer, as well as the reproductive function of hens, which is affected by hormones and ovulation. In our study, the relation between the oviduct and ovaries was established as the egg white protein genes, except *TENP*, were upregulated in cancerous tissue compared to that in normal tissue. We did not determine the reason for this inconsistency, and presumed that it could be attributed to the unknown aspects of *TENP*, which has a bidirectional function in adults and in the immature chicken embryo, as revealed in a study by Song *et al.* (2011) where genes were observed to be upregulated/downregulated in response to DES treatment under the same conditions.

In conclusion, the results of our study revealed that the expression of genes encoding egg white proteins plays a crucial role in the regression and recrudescence of the oviduct during molting owing to their sensitivity to estrogen levels. In particular, in the parts of the magnum involved in egg white formation, expression of these genes is highly sensitive to estrogen. Moreover, their expression patterns vary distinctly between cancerous ovary tissues and normal samples. Collectively, the egg white protein genes could be utilized as a tool for a thorough understanding of the reproductive system in chickens.

Declaration of interest

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

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Data availability statement

The authors confirm that the data supporting the findings of this study are available within the article.

Author contribution statement

W L and G S designed and directed the study. M L and C Y performed experiments, wrote and prepared the manuscript. M L and C Y designed the figures. All authors provided critical feedback and helped to shape the manuscript.

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