GENETICS AND MOLECULAR BIOLOGY

Ovary removal modifies liver message RNA profiles in single Comb White Leghorn chickens

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ABSTRACT Ovaries produce sex hormones, and ovariectomized animals are often used as models for ovarian dysfunction. The liver is a vital organ involved in metabolism and immunity. In the present study, we conducted experiments to investigate the effects of ovariectomy on transcription and metabolic processes in the liver in chicken. Eight Single Comb White Leghorn (SCWL) female chickens were ovariectomized at 17 wk of age, and 8 intact SCWL females served as controls. At 100 wk of age, all chickens were euthanized. High-throughput transcriptome sequencing was performed on liver RNA obtained from ovariectomized and intact females. A total of 267 differentially expressed genes (DEG) were identified in our study. After analysis using DAVID functional annotation tool, one significant Kyoto Encyclopedia of Genes and Genomes pathway, the phosphatidylinositol signaling pathway, was clustered. Gene Ontology enrichment analysis yielded 46 significant Gene Ontology terms. Among terms describing biological processes, the glycerolipid metabolic and lipid localization processes were dominant. The anabolic genes, *PEPCK* and *GK5*, and the catabolic genes, *VTG1*; *VTG2*; *PLD5*; *DGKQ*; *DGKE*; and *FABP3,* were detected in ovariectomized chickens. Differentially expressed genes such as *ENSGALG00000000162*, *IL-1B*, *SVOPL,* and *CA12* implied that livers in ovariectomized chickens were subjected to strong inflammatory reactions, whereas defenses against endogenous materials were compromised. A comprehensive view of gene expression in the liver of ovariectomized chickens would advance our understanding of lipid metabolism, glycometabolism, and their relationships to pathologies induced by absence of the ovary. The identified DEG indicated that ovariectomy disturbed lipid metabolism in the liver and was accompanied by an increase in hepatic gluconeogenesis and reductions in phosphatidic acid synthesis and lipid carrier capacity.

Key words: ovariectomy, liver mRNA profile, single Comb White Leghorn chicken, RNA-seq

INTRODUCTION

Ovaries in chickens develop unilaterally on the left side of the body and produce sex hormones including estrogen, progesterone, and small amounts of androgen. Estrogen and androgen, the 2 most active gonadal steroids, function in sexual reproduction by facilitating the maturation of sexual organs, promoting the development of secondary sexual characteristics, maintaining sexual function, and regulating many other physiological processes (Matute and Kalkhoff, 1973; Aoki et al., 2016). Estrogen and androgen are also involved in processes that are not immediately related to reproduction. Estrogen helps control blood pressure in premenopausal women by modulating the renin–angiotensin–aldosterone system, which reduces the risk of cardiovascular, inflammation, and atherosclerosis and promotes vasodilation (Xue et al., 2013; Prabhushankar et al., 2014). It also has profound effects on learning and memory (Mukai et al., 2010). Estrogen impacts lipid metabolism in women by regulating liver lipases directly and by acting indirectly to release hormones that increase hormone-sensitive lipase activity in adipose tissue (Szafran and Smielak-Korombel, 1997). The function of androgen in females is also well studied. In addition to its roles in estrogen synthesis, follicular

development, and fertility, androgen appears to be associated with β-cell function, type II diabetes, and systemic oxidative stress (Liu et al., 2010; Sen et al., 2010, 2014).

Ovariectomized animals are often used as models for ovarian dysfunction. Ovariectomy dramatically disturbs sex hormone homeostasis and causes serum estrogen levels to drop precipitously (Cui et al., 2016). The impact of ovariectomy on androgen function is controversial, but there is no dispute that androgen levels are very low in ovariectomized chickens (Abraham et al., 1969; Baum et al., 1978; Nishino et al., 1998; Turdi et al., 2015). Because steroid hormones have diverse activities, ovarian dysfunction is associated with a variety of pathologies, such as glucose and lipid metabolism disorders and immune system dysregulation (Knöferl et al., 2001; Wood et al., 2008; Burra et al., 2010; Kharb et al., 2015). Abdominal fat, insulin, and blood glucose levels are often significantly higher in ovariectomized rats, as are levels of liver lipids, total cholesterol, and triglycerides, and the effects can be reversed by estrogen replacement therapy (Liu et al., 2004; Wang et al., 2004; Choi and Song, 2009; Leite et al., 2009). Ovariectomized rats also exhibit depressed immunity and accelerated aging of the nervous and immune systems (Baeza et al., 2010). In chicken, ovariectomy increases intramuscular fat and reduces muscle shear and muscle fiber diameter, resulting in meat that is more tender (Shao et al., 2009; Cui et al., 2016).

The liver is critical for proper metabolic and immune functions (Nemeth et al., 2009). In chickens, almost all *de novo* lipogenesis proceeds in liver tissue. Because ovariectomized chickens tend to accumulate more fat than intact females, it is likely that ovaries exert an effect on liver function. In this study, transcription profiles were generated from the livers of ovariectomized and intact female Single Comb White Leghorns (**SCWL**) and then analyzed to identify differentially expressed genes (**DEG**). The DEG revealed physiological processes in the liver that are sensitive to ovary removal.

MATERIALS AND METHODS

Chickens and Sample Preparation

Female SCWL were provided by the Poultry Genetic Resources and Breeding Station of China Agricultural University. All animal procedures were approved by the Animal Welfare Committee of China Agricultural University.

Eight females were ovariectomized at 17 wk of age, and 8 intact females served as controls. Surgical ovariectomy was used in this study. After 12 hours fasting, chickens were immobilized with the left sides of the bodies up. Following that, ovaries were removed from 1 to 1.5 cm incisions between the last 2 ribs. The ovariectomized chickens were given 0.25 mg of antibiotic for anti-inflammatory and raised in cages separately. All

Table 1. Components of diet.

Ingredient	Proportion (%)	
	7–19 wk	>19 wk
Water	≤13.5	≤13.5
Crude protein	≥16.0	≥16.5
Crude fiber	≤6.0	≤6.0
Crude ash	≤8.0	≤13.0
Calcium	0.8–1.20	3.20–4.40
Total phosphorus	≥0.60	≥0.5
Salt	0.2–0.79	0.2–0.7
Methionine + cystine	≥0.55	≥0.64

chickens were reared to 100 wk of age and fed *ad libitum* (Table 1). Liver tissues were collected and stored at –80°C.

RNA Extraction, Library Construction, and Sequencing

Total RNA was extracted from liver tissue using TRIzol reagent (15596026; Invitrogen, Carlsbad, CA) and the RNAprep pure Tissue Kit (DP431; Tiangen, Beijing, China). One ml TRIzol was added to 20 mg liver tissue for disruption using a horizontal oscillating tissue cracker. The tissue suspension was allowed to stand for 3 min on 4°C and then centrifuged to remove debris. 200 μL trichloromethane was added to the supernatant and mixed completely. All remaining steps followed the manufacturer's protocol. RNA quality was assessed using agarose gel electrophoresis and an Agilent 2,100 Bioanalyzer system (Agilent, Santa Clara, CA). RNA purity was assessed using a NanoDrop spectrophotometer (Thermo Fisher Scientific, Waltham, MA), and RNA concentration was determined using the Qubit 2.0 Flurometer (Thermo Fisher Scientific).

Each RNA sample divided into 2 samples, 1 for RT-qPCR and the other for making pools for RNA sequencing. Equal amounts of liver mRNA from the 8 ovariectomized chickens were combined into 2 pools, each representing 4 individual animals. RNA from the 8 intact chickens was treated similarly, yielding 2 control pools. Each pool was used to construct a library, following the protocol provided by Illumina. Briefly, total mRNA was isolated by using Dyna Oligo(dt) beads and then fragmented by using fragmentation buffer. The first cDNA strand was synthesized using random hexamers and reverse transcriptase, and the second cDNA strand was synthesized using dNTPs and DNA polymerase I. Double-stranded cDNA was purified using AMPure XP beads, followed by an end repair reaction, poly(A) addition, Illumina adapter ligation, and fragment size selection. Library construction was completed by PCR enrichment of specific fragments. Library concentration was determined using a Qubit 2.0 fluorometer, and insert size range was measured using an Agilent 2,100 Bioanalyzer. Libraries with effective concentrations greater than 2 nM were analyzed using an Illumina HiSeq 2,000 sequencing system.

Transcriptome Data Analysis

Raw data quality was assessed based on error rate and A/T/G/C bases distribution. Reads containing adapter sequences consisting of more than 10% uncertain bases, or containing 50% or higher bases of low quality, were excluded from subsequent analysis. Clean reads were aligned against the chicken reference genome (Gallus_-gallus-5.0, GCA_000002315.3; obtained from NCBI) using TopHat2 (Kim et al., 2013) with default parameters. Transcripts were assembled using Cufflink (Trapnell et al., 2010).

The union model of HTSeq was used to analyze transcript abundance. Reads per kilo bases per million reads (**RPKM**) was calculated to represent gene expression level. The read counts were used as input to DESeq to identify DEG. Gene Ontology (GO) annotation and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses were conducted using DAVID v6.7 with default parameters.

Validation of DEG Identification by RT-qPCR

Ten mRNAs identified as differentially expressed between ovariectomized and intact hens in the high-throughput sequencing data set were selected for verification using RT-qPCR. 1 μg RNA from each sample of 8 ovariectomized and 8 intact hens was used for cDNA synthesis using reverse transcription. *HPRT* was set as control for *PPAF, ESR, DGKE, AGPAT,* and *DEGQ*; *GAPDH* was a control to *PLCXD3, LPIN,* and *PDE10 A*. Primers (Supplementary Table 4) were designed using Primer Premier 5.0 (Premier Biosoft International, San Francisco, CA) and synthesized by Sangon. qPCR was performed on a CFX96 TM Real-Time PCR Detection System (Bio-Rad, Hercules, CA) with SYBR Green Mix (FP202, TIANGEN, Beijing, China). PCR cycling conditions were as follows: 95°C for 3 min, 39 cycles of 95°C for 0.5 min, annealing for 0.5 min, then elongating at 72°C for 0.5 min.

Amplification efficiency for all genes was maintained at $100 \pm 10\%$, and differences between target and reference genes was lower than 10%. Relative expression was calculated using ratio (reference/target) = $2^{Ct(reference)-Ct(target)}$. All data were processed with ANOVA analysis variance. *P < 0.05* was defined as significant difference, and *P < 0.01* was very significant difference. Expression change folds of above selected 8 genes were calculated according to average Rpkms in RNA-Seq data and average relative expressions in RT-qPCR results separately and compared to assess the accuracy of RNA-Seq data.

RESULTS

Liver RNA Library Construction and Sequencing

To detect transcriptional changes that occur in response to ovary removal, 4 RNA sample pools were prepared.

Each pool contained liver RNA from ovariectomized (**Tr_O**) or intact female SCWL (**Co_I**) chickens (2 pools of each type; 4 animals per pool). The RNAs were used to construct cDNA libraries, which were then analyzed using the Illumina HiSeq 2,000 high-throughput sequencing system. We obtained 246,870,718 paired-end reads, with error rates below 0.04% and Q20 (Percentage of bases with a Phred value of at least 20) values higher than 94% in all 4 libraries. After excluding low-quality reads, 239,972,412 clean paired-end reads remained. Read statistics are summarized in Table 2.

Assembly and Mapping to Reference Genome

Clean reads were aligned to the chicken reference genome (Gallus_gallus-5.0, GCA_000002315.3) from NCBI using TopHat2 and default parameters. 84.42 to 86.01% of the sequences in the 4 libraries mapped uniquely to the genome (Supplementary Table 1). Uniquely mapped reads were assembled into gene transcripts using Cufflinks and then compared to annotated sequences by Cuffcompare. As a result, there were 15,508 known genes being annotated and 6,243 novel genes being presumed (Supplementary Table 2).

Totally, 19,113 genes exceeded an expression threshold of RPKM ≥ 0.1 (Reads Per Kilo bases per Million reads). Of these, 12,870 were annotated genes. 10,354 genes in the annotated group exhibited RPKM levels exceeding 1, and 1,027 (9.92%) were highly expressed (RPKM > 60). KEGG pathway analysis suggests that many of these are ribosome components or are involved in carbohydrate metabolism, fatty acid metabolism, or drug metabolism. Of the novel genes, 3,659 had RPKM expression levels >1, and 82 had levels >60. Gene annotation data are shown in Supplementary Table 2. The Pearson correlation coefficients between the 2 control pools and between the 2 ovariectomized chicken pools were calculated with R package (Version 3.0), and the results showed the high reproducibility between the same type pools (Supplementary Figure 1).

Differentially Expressed Genes in Ovariectomized vs. Intact Chickens

Differentially expressed genes were classified using DESeq, based on a comparison of read counts in ovariectomized and intact females, with an adjusted *P*-value (padj) of 0.05 or less as a criterion for significance. The analysis yielded 267 DEG, of which 175 were known. Using gene expression levels in the intact group as a reference, 49 genes are expressed at higher levels and 218 at lower levels, in ovariectomized chickens (Figure 1A). Among the genes with higher levels of expression, *Novel06151* expression was observed only in ovariectomized animals. *PEPCK* (*ENSGALG00000007636*) exhibited the largest (19.33-fold) expression ratio, followed by *Novel06147* and *Novel06150*. Of the genes

Table 2. RNA-Seq yield and quality statistics.

Groups	Sample name	Raw reads	Clean reads	Q30 (%)[1]	GC content (%)
Tr_O	O_L1_1	34,383,572	33,467,089	93.70	47.82
	O_L1_2	34,383,572	33,467,089	89.75	48.03
	O_L2_1	31,385,403	30,473,980	93.64	47.82
	O_L2_2	31,385,403	30,473,980	90.49	48.03
Co_I	I_L1_1	26,036,236	25,396,442	93.90	46.99
	I_L1_2	26,036,236	25,396,442	91.14	47.19
	I_L2_1	31,630,148	30,648,695	93.80	48.09
	I_L2_2	31,630,148	30,648,695	90.78	48.27

[1]Q30: percentage of bases with a Phred value of at least 30.

expressed at lower levels in ovariectomized animals, *SVOPL* (*ENSGALG00000012812*), *SLITRK4* (*ENSGALG00000007242*), *CA12* (*ENSGALG00000003456*), and 13 other novel genes were expressed only in intact animals (Figure 1B). With these genes excluded, *VTG1* (*ENSGALG00000008900*) and *RNF186* (*ENSGALG00000026524*) exhibited the largest expression ratios 2,130.53 fold and 1,167.34 fold, respectively.

GO Enrichment and KEGG Pathways Analyses for DEG

To identify the liver physiological processes most affected by ovariectomy, 267 DEG were subjected to GO enrichment and KEGG pathways analyses using the DAVID v.6.7 online tool and default parameters. A total of 46 GO terms were enriched (*P*-value < 0.1; Supplementary Table 3), and 16 were significant (*P*-value < 0.05, fold enrichment > 2) as shown in Figure 2. Differentially expressed genes most frequently clustered in lipid metabolism (primarily glycerolipid metabolic process GO:0046486) and lipid localization (GO:0010876). Enrichment was also observed for glycerophospholipid metabolic process (GO:0006650), lipid transport (GO:0006869), and regulation of lipid transport (GO:0032368). In the cellular component category,

plasma membrane (GO:0044459) was enriched and was associated with processes such as signal transduction, material channels, and cellular homeostasis maintenance. Nutrient reservoir activity (GO:0045735), associated with 3 genes, was the most enriched term within molecular function, followed by lipid transporter activity (GO:0005319). KEGG pathway analysis identified only 1 significantly enriched pathway, the phosphatidylinositol signaling system (gga04070), associated with 4 genes.

Transcriptome Sequencing Validation by RT-qPCR

To verify the accuracy of sequencing and the initial differential expression analysis, qPCR was performed to examine expression of *AGPAT2, DGKE, DGKQ, ESR, PPAPDC1A, PLCXD3, PLIN1,* and *PDE10 A* in livers from ovariectomized and intact animals. Consistent with the results from RNA-Seq, the relative levels of *AGPAT2, DGKE, DGKQ, ESR, PPAPDC1A,* and *PLCXD3* were lower, whereas *PLIN1* and *PDE10 A* levels were higher in ovariectomized animals (Figure 3A). The expression fold changes of above genes were compared with those generated using the RNA-Seq data except for *AGPAT2* and *PLCXD3* (Figure 3B), demonstrating good agreement between the 2 methods.
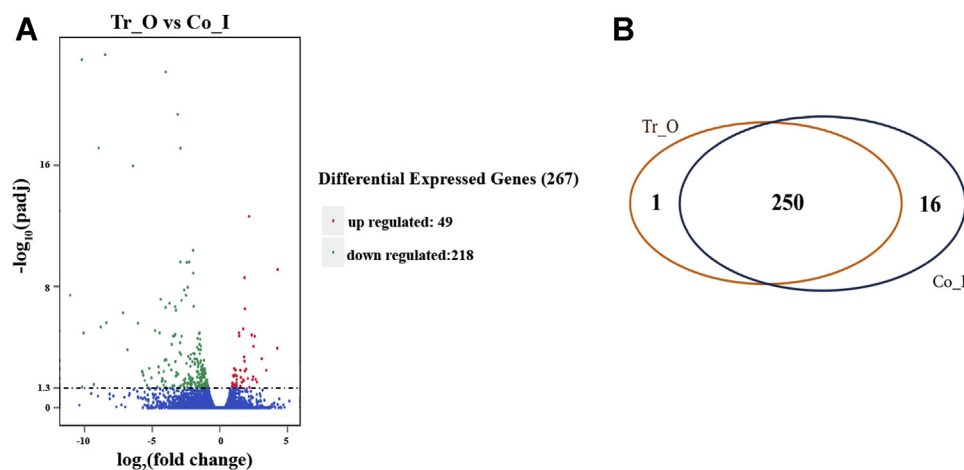
Figure 1. Differentially expressed genes in ovariectomized (Tr_O) vs. intact (Co_I) hens. (A) Volcano plot showing differentially expressed genes. The x-axis shows $\log_2$ of the expression ratio, and the y-axis shows adjusted *P*-value. Red dots represent genes that are expressed at significantly higher levels in ovariectomized animals, and green dots represent genes with significantly lower relative expression. Genes classified as having no significant differences in gene regulation are represented using blue dots. (B) Venn diagram representing 267 DEG, identified in: ovariectomized and intact animals (250), ovariectomized animals only (1), and intact animals only (16). Abbreviation: DEG, differentially expressed gene.
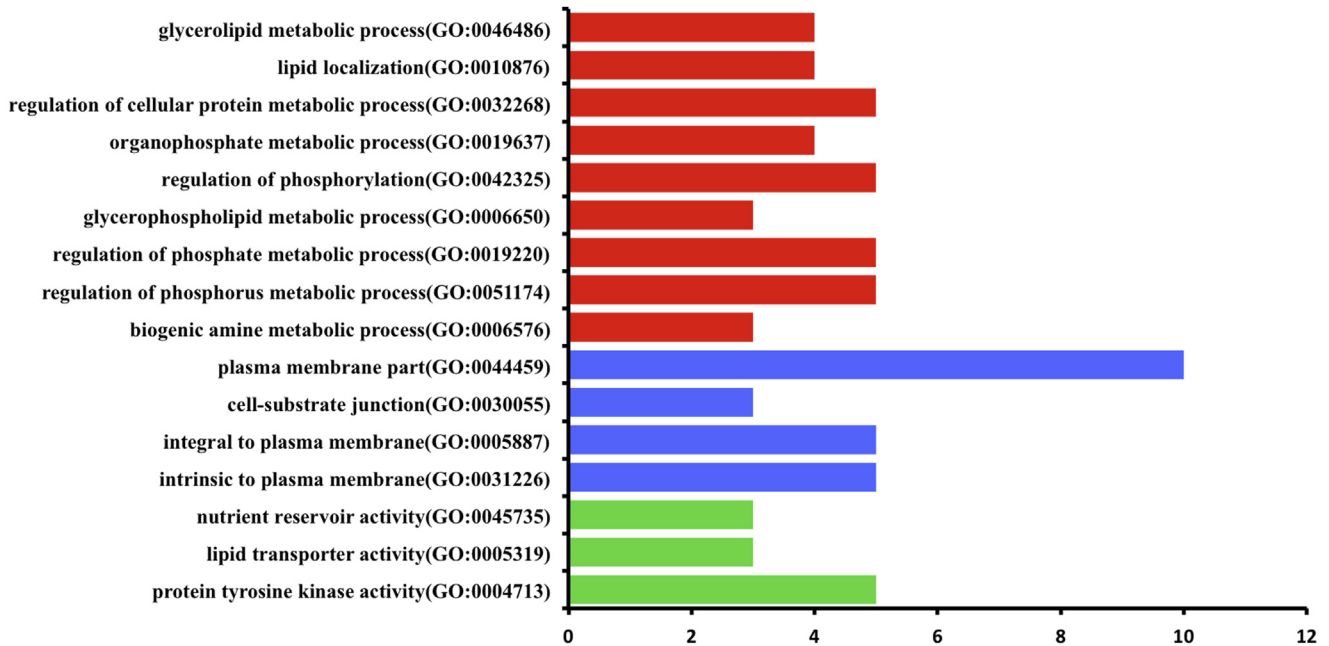
Figure 2. Most significantly enriched GO terms associated with differentially expressed genes between ovariectomized and intact females. *P*-value of 0.05 and Fold Enrichment above 2 were used as threshold for significant enrichment. The x-axis shows the number of genes in each category. Red, biological processes; blue, cellular components; green, molecular functions.

DISCUSSION

Ovariectomy is often used to simulate *in vivo* estrogen deficiencies, but little research has been conducted in the chicken to determine the effects of ovary removal. Because the liver is a vital organ involved in metabolism and immunity, we conducted experiments to determine how it responds to ovariectomy. Liver mRNA from intact vs. ovariectomized animals was subjected to high-throughput sequencing, revealing 267 DEG involved in a variety of physiological processes.

KEGG pathway analysis identified a single pathway (gga04070: phosphatidylinositol (**PI**) signaling system) that was significantly associated with the DEG of padj 0.05. In the PI signaling system, extracellular signals are transmitted into cells by G protein-coupled receptors, which initiate production of 2 second messenger molecules, inositol 1,4,5-trisphosphate, and diacylglycerol (**DAG**) (Berridge, 2014). Inositol 1,4,5-trisphosphate, a polyfunctional signal molecule involved in 64 signaling pathways in the KEGG database, monitors intracellular calcium and activates various calmodulin-dependent protein kinases. Its signal terminator, inositol-trisphosphate
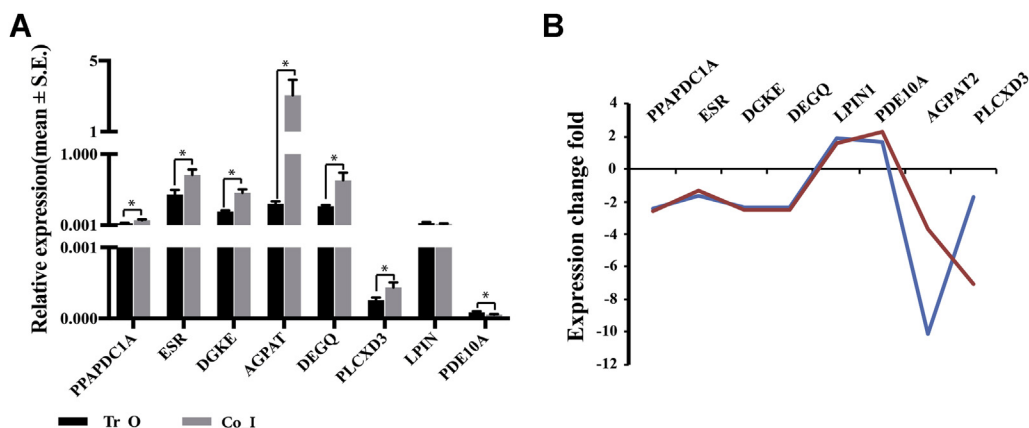
Figure 3. Quantitative validation of differentially expressed genes between ovariectomized and intact females. (A) Relative expression for 8 selected genes. Black blocks mean gene relative expressions in ovariectomized group, grey blocks mean gene relative expressions in intact group. * means significant difference ($P < 0.05$). (B) Comparison of RT-qPCR and RNA-Seq results. Expression change folds of the 8 selected genes were calculated according to RNA-Seq and RT-qPCR results separately. Y-axis shows gene expression change folds of ovariectomized relative to intact group. Positive means gene expressions were increased in ovariectomized group. Negative means gene expressions were reduced in ovariectomized group. Red line shows gene expression change folds from RNA-Seq, blue line shows gene expression change folds from RT-qPCR.

3-kinase b (*ITPKB*), exhibited increased expression in ovariectomized chickens, which likely impacts inositol 1,4,5-trisphosphate–transduced pathways such as the estrogen signaling pathway, the MAPK signaling pathway, and insulin secretion. Incidentally, MAPK signaling pathway (gga04010) and insulin resistance (gga04931) were enriched most obviously when we filtered the DEG with padj threshold 0.2. The second messenger molecule DAG regulates cell secretion, proliferation, and differentiation by activating protein kinase C (Macnicol et al., 1990). Protein kinase C is involved in EGFR tyrosine kinase inhibitor resistance, the estrogen signaling pathway, the MAPK signaling pathway, fat digestion and absorption, and insulin signaling in diabetic humans (Idris et al., 2001). There are 2 established mechanisms to terminate signal transduction by DAG: phosphorylation via diacylglycerol kinase (**DGK**) and hydrolysis by diacylglycerol lipase (Newton, 1995). In the ovariectomized animals, we found that *DGKQ* and *DGKE* were reduced in expression 5.4 fold and 2.7 fold respectively, and protein kinase C (*PRKCE*) was reduced in expression more than 2-fold (identified as a DEG by increasing the padj threshold to 0.07). We did not observe changes in the expression of DAG lipase. The regulation of *DGKQ* and *DGKE* by ovariectomy has not been reported; however, the expression of DGKA, another member of the DGK family, is thought to be induced by E$_2$ (Filigheddu et al., 2011).

The GO enrichment analysis returned 46 significant terms. Twenty are shown in Figure 2, and 11 of these are identified as associated with "biological processes" (blue line). Glycerolipid metabolic process and lipid localization were the most significant of these processes. These 2 terms, together with 3 others that were significantly enriched (glycerophospholipid metabolic process, lipid transport, and regulation of lipid transport), strongly suggest that lipid metabolism is considerably affected by ovary removal. Sex hormones are known to regulate lipid metabolism, but the regulatory mechanisms are not well understood, and the underlying physiological modulators remain to be elucidated (Wang et al., 2011; Varlamov et al., 2015). Changes in the fatty acid profile and in the thickness of intramuscular fat have been observed in ovariectomized chickens, but the mechanism of these findings have not been elucidated (Cui et al., 2016). Obesity induced by ovariectomy is attributed not only to lipogenesis but also to decreasing oxygen consumption and decreased expression of lipogenesis-related transcription factors such as peroxisome proliferator activated receptor (*Ppar*), acetyl CoA carboxylase (*Acc*), and fatty acid synthase (*Fas*) (Kamei et al., 2005). However, fat deposition patterns are likely to be different between mammals and chickens, because nearly all fat in the chicken is synthesized by the liver.

Several genes appear to be strong candidates as likely contributors to the liver pathologies observed in ovariectomized chickens. Cholesteryl ester transfer protein, associated by GO enrichment analysis with the terms glycerolipid metabolic process and lipid localization biological process, was 4.5-fold more abundant in ovariectomized animals. Cholesteryl ester transfer protein functions in the transfer of cholesteryl esters from high-density lipoprotein to very low-density and low-density lipoproteins (Mazzucco et al., 2010). The overexpression of cholesteryl ester transfer protein is associated with high levels of low-density lipoprotein cholesterol, reduced levels of high-density lipoprotein cholesterol and contributes to hepatic steatosis and fatty liver development (Blake et al., 1994; Boekholdt et al., 2004; Nseir et al., 2012). Another gene enriched in our screen *FABP3*, associated with the GO term glycerolipid metabolic process, exhibited 19.7-fold lower levels in ovariectomized females. *FABP3* encodes a fatty acid transporter of long-chain fatty acids and is related to the PPAR/RXR signaling pathway. *FABP3* participates in lipid β-oxidation and has been linked to cardiovascular risk (Jordal et al., 2006; De Lange et al., 2008; Zhang et al., 2013; Thumser et al., 2014; Catalucci et al., 2015). However, *FABP3* is associated with carcass back fat thickness and intramuscular fat content in the pig, suggesting that *FABP3* is also involved in fat metabolism (Cho et al., 2011; Diaz et al., 2015; Shimada et al., 2015). The estrogen responsive genes *VTG1* and *VTG2* were also enriched in the lipid transport category. The vitellogenins probably function as lipid transport proteins, given their similarity to *APOB-100* (Mann et al., 1999). The level of APOB-100 after ovariectomy was 5-fold lower than in intact chickens (using a threshold of padj = 0.11). *VTG1* and *VTG2* mRNA levels decreased 2,130 and 33-fold in ovariectomized chickens, respectively. These results suggest that lipid transport is compromised by ovary deficiency, consistent with the decreased estrogen levels in these animals (Yamaguchi et al., 2009).

PEPCK exhibited the most obvious increase in ovariectomized animals, with a 19-fold change. *PEPCK* is a rate-limiting enzyme in gluconeogenesis and helps maintain blood glucose and supplies glycerol-3-phosphate for glycerolipid synthesis, especially during long-term starvation (Prentki and Madiraju, 2008). Whether *PEPCK* expression changes in response to ovariectomy is unclear. *PEPCK* mRNA levels in the liver are elevated in ovariectomized rats and arsenic-treated ovariectomized mice, but Tomaz *et.al.* reported that *Pepck* expression does not change in the livers of estrogen sulfotransferase (*Est*) transgenic mice or ovariectomized rats (Khor et al., 2010; Tomaz et al., 2016). Our results are consistent with the former and also with the conclusion that estradiol can suppress hepatic gluconeogenesis in female rats following 21 D of treatment with exogenous estrogen and progesterone (Matute and Kalkhoff, 1973). Intriguingly, in our previous study, we found that *PEPCK* is also overexpressed in castrated male chickens, and levels are restored to normal levels after treatment with testosterone (Duan et al., 2013). We therefore suggest that either male or female hypogonadism facilitates *PEPCK* transcription, although the regulatory mechanism probably differs between the sexes.

Our study implicated other potentially important DEG in lipid metabolism. Glycerol kinase 5 (**GK5**), which encodes an enzyme with phosphotransferase activity, catalyzes the degradation reaction of glycerol into *sn*-glycerol-3-phosphate, which functions as a precursor in either *de novo* lipogenesis or gluconeogenesis in hepatocytes. The more than 2-fold increase in *GK5* expression in ovariectomized chickens reflects that ovariectomy affects gluconeogenesis and triacylglycerol biosynthesis in female chickens. Genes with reduced expression in ovariectomized chickens, *PLD5*, *DGKQ*, and *DGKE*, encode enzymes involved in the synthesis of phosphatidic acid, which participates in transmitting signals, generating adipose, and facilitating the enlargement of lipid droplets (Winter et al., 2010; Fei et al., 2011). The oxysterol-binding protein-like 10 (OSBPL10) inhibits the delivery of phosphatidylserine to the plasma membrane (Maeda et al., 2013). The decreased expression of *OSBPL10* in ovariectomized chickens would be expected to result in increased lipid biosynthesis in the liver.

Hormones, lipid, and carbohydrate are effective immunomodulators in innate and adaptive immune systems (Choi et al., 2015). Because we observed changes in expression in genes related to lipid metabolism and glycometabolism in response to ovariectomy, it is likely that the ovary ordinarily has a regulatory function in immunity. Consistent with this, the expression of genes such as *ENSGALG00000000162* and interleukin 1β (*IL-1B*) in ovariectomized chickens increased notably. *ENSGALG00000000162* is predicted to encode MHC class I/II-like antigen recognition protein, which is associated with adaptive immune responses (Choi et al., 2015). IL-1B is a key proinflammatory cytokine and indicator of autoimmune and inflammatory diseases and a proposed sensor that directly detects pathogen-associated proteolysis (LaRock et al., 2016). The same study suggested that the inflammatory response is triggered in the liver by ovariectomy and is consistent with an earlier report that correlates a decrease in ovarian function with induction IL-1B (Johnson et al., 2006). Our results show that *SVOPL* and *CA12* are expressed only in intact chickens. SVOPL is predicted to encode a synaptic vesicle glycoprotein that functions as an organic ion transporter, mediating the uptake, and excretion of endogenous toxins (Jacobsson et al., 2007; Olender et al., 2016). CA12 is thought to be involved in the respiration tract antibacterial defense system by regulating ion balance (Kumar and Teckman, 2015). Thus, we speculate that ovariectomy may impact these defense capabilities.

It should be noted that liver development is affected by ovariectomy although genes involved in liver development process, such as *NF1*, *JUN*, *WNT4,* and *PCSK9*, were not identified as DEG unless the threshold padj value was at least 0.1. However, *LRP6* expression decreased significantly in ovariectomized chickens. The LPR5 and LPR6 co-receptor is the essential initiator of the canonical WNT signaling pathway, which is required to induce the development of hair follicles and feather buds (Noramly et al., 1999; Andl et al., 2002). This is likely to be related to our observation that the feathers of ovariectomized females are highly masculinized.

CONCLUSIONS

A comparison of the liver transcriptomes of ovariectomized and intact chickens indicates that ovariectomy primarily disturbs the transmembrane signal transduction system, suggesting possible disturbances in lipid metabolism, glycometabolism, and immune response systems. After ovariectomy, mRNA levels for *PEPCK* and *GK5* increased, whereas levels for *PLD5*, *DGKQ*, *DGKE*, *FABP3*, *VTG1*, and *VTG2* decreased. The aberrant fat metabolism induced by ovariectomy is associated with enhanced hepatic gluconeogenesis and reduced phosphatidic acid synthesis and lipid carrier capacity. The increased expression of *ENSGALG00000000162* and *IL-1B* and the undetectable expression of *SVOPL* and *CA12* in ovariectomized chickens suggest that ovariectomized chickens are likely to have abnormal inflammatory responses. Interestingly, levels of the key glycogen synthesis enzyme *PEPCK* increase in both castrated chickens that are treated with testosterone and in ovariectomized chickens. Further investigation is required to understand how *PEPCK* is regulated and functions in these 2 scenarios. Finally, the data reported here may provide insight into the mechanisms by which ovarian failure leads to liver disease and help explain how natural hormonal disorders influence lipid metabolism, glycometabolism, and other processes.

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SUPPLEMENTARY DATA

Supplementary data associated with this article can be found in the online version at https://doi.org/10.1016/j.psj.2019.12.036.

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