INTRODUCTION

Follicle-stimulating hormone (FSH) is a member of the glycoprotein hormones synthesized and secreted by gonadotropes in the anterior pituitary gland and is comprised of two dissimilar subunits, alpha and beta, encoded by separate genes (Jameson et al., 1988; Gharib et al., 1989). Within a species, FSH shares the identical α subunit to luteinizing hormone (LH) and thyroid-stimulating hormone (TSH), while it has its own β subunit that is different and determines both hormonal and species specificity (Pierce and Parsons, 1981). It acts in an endocrine manner and play an essential role in the reproductive system, including steroidogenesis, folliculogenesis and follicular maturation (Choi et al., 2005), and in regulating gonadal endocrine function (Moyle and Campbell, 1996). The latest studies have revealed that FSH exists in two glycoforms: diglycosylated FSH (DiGlycFSH) and tetraglycosylated FSH (TetGlycFSH). It also specifically functions to stimulate estrogen synthesis and serves as a selection factor for dominant follicles, which are essential to maintain fertility (Bousfield et al., 2007; Tran et al., 2008). In humans, DiGlycFSH involves expression of recombinant hFSHβ, separation, purification from soluble and insoluble fractions, folding, and re-association with human chorionic gonadotropin (hCGα) (Tran et al., 2008).

Cloning and Expression of FSHβ Gene and the Effect of FSHβ on the mRNA Levels of FSHR in the Local Chicken*

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ABSTRACT : Follicle-stimulating hormone (FSH) is a pituitary glycoprotein hormone that is encoded by separate alpha- and beta-subunit genes. It plays a key role in stimulating and regulating ovarian follicular development and egg production in chicken. FSH signal transduction is mediated by the FSH receptor (FSHR) that exclusively interacts with the beta-subunit of FSH, but characterization of prokaryotic expression of the FSHβ gene and its effect on the expression of the FSHR gene in local chickens have received very little attention. In the current study, the cDNA fragment of the FSHβ gene from Dagu chicken was amplified using reverse transcription polymerase chain reaction (RT-PCR), and inserted into the pET-28a (+) vector to construct the pET-28a-FSHβ plasmid. After expression of the plasmid in E. coli BL21 (DE3) under inducing conditions, the recombination protein, FSHβ subunit, was purified and injected into the experimental hens and the effect on the mRNA expression levels of the FSHR gene was investigated. Sequence comparison showed that the coding region of the FSHβ gene in the local chicken shared 99%-100% homology to published nucleotides in chickens; only one synonymous nucleotide substitution was detected in the region. The encoded amino acids were completely identical with the reported sequence, which confirmed that the sequences of the chicken FSHβ gene and the peptides of the FSHβ subunit are highly conserved. This may be due to the critical role of the normal function of the FSHβ gene in hormonal specificity and regulation of reproduction. The results of gene expression revealed that a recombinant protein with a molecular weight of about 19 kDa was efficiently expressed and it was identified by Western blotting analysis. After administration of the purified FSHβ protein, significantly higher expression levels were demonstrated in uterus, ovary and oviduct samples (p<0.05). These observations suggested that the expressed FSHβ protein possesses biological activity, and has a potential role in regulation of reproductive physiology in chickens. (Key Words : Chicken, FSHβ Gene, Expression, FSH-receptor)
was earlier determined from human pituitary glands, and consists of 118 amino acid residues with a predominant proportion of molecules having 108 residues due to microheterogeneity at the NH2 and COOH termini (Saxena and Rathnam, 1976). The FSHb gene encoding FSHβ has been isolated in the human, that encoded a 18 amino acid signal sequence and a 111 amino acid apoprotein (Watkins et al., 1987; Jameson et al., 1988); in the rat, that encoded a 20 amino acid signal peptide followed by a mature protein of 110 amino acid residues (Maurer, 1987; Gharib et al., 1989), in the porcine (Kato, 1988), in the bovine (Esch et al., 1986; Maurer and Beck, 1986), in Chinese hamster (Keene et al., 1989), in the ovine (Guzman et al., 1991), and the equine (Saneyoshi et al., 2001). In the chicken, the FSHβ precursor molecule consisted of 131 amino acids with a signal peptide of 20 amino acids followed by a mature protein of 111 amino acids that was encoded by FSHb cDNA, which was composed of 2,457 bp nucleotides, including 44 bp nucleotides of the 5'-untranslated region (UTR), 396 bp of the open reading frame, and a long 3'-UTR of 2,001 bp nucleotides followed by a poly(A) tail (Shen and Yu, 2002). The effects of FSH on ovarian follicular development and growth have given rise to the hypothesis that FSH functions to enhance laying performance, or egg production, in the chicken, but the beta subunits of FSH vary in different species. It confers its specific biologic action and is responsible for interaction with the FSH receptor (Simoni et al., 1997).

The FSH receptor (FSHR) is a member of the superfAMILY of G-protein-coupled receptors; it was reported to be expressed exclusively on granulosa cells of the ovary in females and on sertoli cells of the testis in males, which mediate FSH signal transduction through the cAMP pathway (Griswold et al., 1995; Simoni et al., 1997). FSH indirectly influences spermatogenesis and oogenesis by exerting influence on the corresponding somatic cells (Griswold et al., 1995). Both granulosa cells and immature sertoli cells are capable of synthesizing estrogen from an exogenous source of androgens (Fritz, 1982). Regulation of the expression of FSHR and its connection with specific signal transduction pathways are key points in regulating folliculogenesis (Findlay and Drummond, 1999). With respect to regulation of the FSHR gene expression and effects on the expression level of FSHR mRNA, extensive studies have been undertaken on the granulosa cells of the ovary and sertoli cells of the testis in the rat (Camp et al., 1991; Heckert and Griswold, 1991), mouse (Yaron et al., 1998), sheep (Tisdall et al., 1995), bovine (Xu et al., 1995), human (Oktary et al., 1997; Findlay and Drummond, 1999), and chicken (Yamamura et al., 2001; Woods and Johnson, 2005). Of these, most experiments were focused on response of the sampled tissues or cells to FSH and its roles in folliculogenesis and spermatogenesis. While in the tissue distribution and expression levels of FSHR mRNA, and response to FSHβ subunit, very little information is available in Chinese indigenous chickens. As for the preparation of recombinant FSHβ subunit and identification of its biological activity, work has been reported rarely in chickens. The objective of this study was to investigate the expression characteristics of FSHR mRNA in the different tissues sampled, to obtain the purified recombinant FSHβ subunit, and to evaluate the effect of muscular injection of a dose of the FSHβ on the mRNA expression levels of FSHR in Chinese indigenous layers. The results will hopefully establish a foundation for study of the physiological activity and characteristics of chicken FSHβ subunit in regulation of egg performance.

MATERIALS AND METHODS

Animals and RNA isolation

In this study, pituitary gland, uterus, ovary, oviduct, pancreas, glandular stomach and kidney samples were collected at 18 weeks of age from Dagu chicken, a Chinese indigenous breed, and the tissues were snap-frozen in liquid nitrogen within 5 min of sacrifice. Total RNA was extracted using TRIzol Reagent according to the manufacturer’s protocols (Gibco BRL, Grand Island, NY), the concentration of total RNA was quantified by measuring its absorbance at 260 nm, and the purity was detected from the ratio of absorbance at 260/280 nm.

RT-PCR analysis

To identify the tissue specificity of chicken FSHb mRNA expression, RT-PCR analysis of FSHb and constitutively expressed β-actin was carried out as described previously (Zheng et al., 2006; Wu et al., 2008). The cDNA was synthesized by reverse transcription using 1 μg total RNA according to the instructions of the reagent kit (Reverse Transcriptase kit, Takara). The PCR reaction was performed in a volume of 20 μl consisting of 1 μl of RT-PCR product, 1×PCR buffer, 1.5 mM MgCl2, 0.2 mM dNTPs, 0.3 μM each primer and 1 U Taq DNA polymerase (MBI Fermentas). The PCR conditions were 95°C for 5 min followed by 30 cycles of denaturing at 95°C for 40 s, annealing at 59°C for 40 s, extension at 72°C for 40 s, and final extension at 72°C for 4 min, and finally kept at 4°C. The amplification was performed on a PTC-100TM programmable thermal controller (MJ Research, Inc.). The β-actin gene was used as an internal control. The following sets of oligonucleotide primers were used: for FSHb, 5'-GCAACAGATAACACCTCTTCC-3' and 5'-AGGCA TTCCTCTTCCGTCAG-3'; for β-actin, 5'-GACA GGAGGTT-3' and 5'-CGGACCAACAG AAGTGGA-3'. The primers were designed according to the

published sequences of chicken FSHb (Accession No. NM_204257) and β-actin (M26111) mRNA in the NCBI GenBank database. The reaction products (4 μl) were visualized and photographed following electrophoresis on 2% agarose gels stained with 0.25 μg/ml ethidium bromide, and the expected product was extracted and verified by direct DNA sequencing. To verify the specificity of the bands shown, a negative control was performed using an aliquot of a cDNA synthesis reaction where no reverse transcriptase enzyme was added.

Cloning of the encoding region of chicken FSHb gene

The total volume of the PCR reaction system was 50 μl, containing 1 μl of the above synthesized cDNA template, 0.25 μM of each primer, and 1 U of Taq DNA polymerase, to amplify 396 bp of the full encoding region cDNA of the chicken FSHb gene. The conditions for PCR were pre-amplification denaturation at 94°C for 5 min followed by 30 thermal cycles of 94°C for 30 s, 56°C for 30 s, 72°C for 30 s, and a final extension at 72°C for 4 min. The primers were also designed on the basis of the aforementioned FSHb mRNA sequence (NM_204257) and optimized for E. coli codon usage. EcoRI and SalI restriction enzyme sites were incorporated in the primers: the forward primer 5’-TCGGAATTCTAGAAAGACACTTAACTGT-3’, and the reverse primer 5’-AGGTCGACTCATGGATTGCTTCCA TTG-3’, corresponding to the 45-440 nt of follicle stimulating hormone, beta polypeptide (FSHb) mRNA. The PCR products were isolated with 1.5% agarose gel electrophoresis and purified with a DNA purification kit (MiniBEST DNA Purification Kit, Takara). The amplified fragment was inserted into pGEM-T easy vector using T4 DNA ligase (Promaga); recombinant DNAs were introduced by heat shock into E. coli DH5α for propagation. Positive clones with correct insertions were identified using PCR and restriction analysis of plasmids carrying the recombinant cDNAs. The recombinant plasmids were then subjected to DNA sequence analysis, and selected for construction of expression vector with the obtained FSHb fragment.

Sequence alignments, translations and comparisons were carried out using MEGA program Version2.1 (Kumar et al., 2001), and the deduced amino acids of the FSHβ subunit corresponding to the 21 sequences of FSHb mRNA from 13 species (Table 1) were used to perform sequence comparison.

Expression of chicken FSHb gene in Escherichia coli BL21 (DE3)

The chicken FSHb cDNA was cloned into EcoRI and SalI restriction sites of the pET-28a (+) vector to construct the pET-28a-FSHb plasmid. The recombinant plasmid was confirmed by restriction analysis and transformed into E. coli BL21 (DE3) cells. The positive clone was selected for incubation overnight at 37°C with shaking in LB medium (10 ml) containing 34 mg/L kanamycin. This culture was used to inoculate the pre-warmed new LB medium containing 34 mg/L kanamycin with a 1:100 dilution at 37°C with shaking. When the culture had grown to an OD600 of 0.6-0.8, the expression of recombinant FSHβ was induced by adding isopropyl-β-D-thiogalactopyranoside (IPTG) at 0.1 M final concentration, and incubating for 1, 2, 3 and 4 h at 37°C. The cultures were collected and analyzed by 12% SDS-PAGE as described by Laemmli (Laemmli, 1970). The fusion protein was identified using a commercially available monoclonal anti-FSHβ protein (Santa Cruz Biotechnology) by Western blotting assay (Barnes et al., 2002; Lu et al., 2007).

Recombination protein extraction and purification

The following purification was performed using cells derived from one liter of IPTG-induced bacterial culture. The fusion protein was purified from E. coli BL21 by the methods described previously (Dai et al., 2007). In brief, the cultured cells were collected by centrifugation and resuspended. The samples was lysed by ultrasonication. The lysate was centrifuged at 12,000 rpm for 30 min and 2% sodium deoxycholate was added to the inclusion body; the

<table>
<thead>
<tr>
<th>Species</th>
<th>GenBank Accession No.</th>
<th>Sequence homology (%)</th>
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<tbody>
<tr>
<td>Chicken</td>
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<td>100</td>
</tr>
<tr>
<td></td>
<td>AB077362</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>AF467082</td>
<td>99</td>
</tr>
<tr>
<td>Broiler chicken</td>
<td>BJ292995</td>
<td>100</td>
</tr>
<tr>
<td>Japanese quail</td>
<td>AB086952</td>
<td>97</td>
</tr>
<tr>
<td>Domestic duck</td>
<td>DQ232890</td>
<td>95</td>
</tr>
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<td>Chinese goose</td>
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<td>Crested ibis</td>
<td>AB089502</td>
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<td>Equine</td>
<td>AB029157</td>
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Table 1. Homologies of the coding regions of FSHβ genes and the deduced amino acid sequences of FSHβ subunits used in this study.
body was collected by centrifugation and re-suspended. The sample was centrifuged at 12,000 rpm for 30 min; all steps were performed at 4°C and then 20% PEG-4000, 50 mM oxidized glutathione, and 100 mM reduced glutathione were added to the supernatant to final concentrations of 0.2%, 1 mM and 2 mM, respectively. The expressed protein was then concentrated by PEG-20000.

Effect of FSHβ on the expression of FSHR mRNA in hens

Ten healthy Dagu hens at 23 weeks of age were divided randomly into two groups. They were housed in individual cages and raised under the same conditions of management and nutrition on the farm of the Jilin Agricultural University. One group was administered a pectoral muscle injection of 10 μg/ml of purified FSHβ protein dissolved in 0.9% sodium chloride. The first administration of FSH was equal to a dose of 5 μg, the second of 15 μg per bird. At the same time, the other group was used as the control and injected with an equal amount of 0.9% sodium chloride. After two successive injections, given 3-4 h apart based on the half-life of FSH (Laster, 1972), tissue samples of pituitary gland, uterus, ovary, oviduct, pancreas, glandular stomach and kidney were collected from the sacrificed hens. Before treatment, tissues from another five non-treated Dagu chickens at 23 weeks of age were collected to reveal expression of the FSHR gene. To measure mRNA expression level of the chicken FSHR gene in the tissues, semi-quantitative RT-PCR analysis of FSHR and β-actin was performed as described above. The primers were designed on the basis of published chicken FSHR (GenBank accession No. NM_205079) mRNA sequences: 5’- TAAGAGCCAGGTCTACATACA -3’ and 5’- GTGGGTGTTCCAGTGATAG -3’, corresponding to the 786-1,200 nt of follicle stimulating hormone receptor (FSHR) mRNA sequences: 5’- TAAGAGCGAGGTCTACATACA -3’ and 5’- GTGGGTGTTCCAGTGATAG -3’. Sequence analysis revealed that the FSHβ gene of poultry had a length of 396 bp nucleotides encoding a peptide with 131 amino acid residues; rat and mouse had a 393 bp the coding region of FSHβ encoding 130 amino acids; but in each of human, hamster, porcine, bovine, ovine and equine had the same length of 390 bp nucleotides encoding a peptide with 131 amino acid residues. By alignment, the inserted nucleotide fragment shared 99% and 100% identities to reported chicken FSHR sequences (AF467082 and NM_204257); only one synonymous nucleotide substitution, A345→G, was detected in the region. The deduced amino acid sequence from the newly isolated development, higher FSHβ mRNA expression levels were observed in the pituitary gland tissues of Dagu chickens sampled at 18 weeks of age. However, FSHβ mRNA expression in other tissues sampled, namely uterus, ovary, oviduct, pancreas, glandular stomach and kidney, was not observed when an equal amount of total RNA was used under the same RT-PCR reaction conditions. In combination with the results previously reported by Shen and Yu (2002) that RNAs from other tissues (brain, heart, thyroid, liver, testis, and adipose) did not produce this cDNA fragment under the same RT-PCR conditions, this observation indicated that the FSHβ mRNA was specifically expressed in the pituitary gland tissue of the chicken.

Sequence analysis and expression of chicken FSHβ gene

A cDNA fragment, which comprised an open reading frame of 396 bp encoding 131 amino acid residues (with the exception of a stop codon), was amplified from first strand cDNA prepared from total RNA of chicken pituitary gland. The cDNA sequence carried in the recombinant plasmid with correct insertion was identified by PCR and restriction analysis (Figure 2). Sequence of the coding region with its 3’-untranslated region of the chicken FSHβ gene was submitted to the NCBI GenBank and assigned the accession number of GQ856365. Sequence analysis revealed that the FSHβ gene of poultry had a length of 396 bp nucleotides encoding a peptide with 131 amino acid residues; rat and mouse had a 393 bp the coding region of FSHβ encoding 130 amino acids; but in each of human, hamster, porcine, bovine, ovine and equine had the same length of 390 bp nucleotides encoding a peptide of 129 amino acids. By alignment, the inserted nucleotide fragment shared 99% and 100% identities to reported chicken FSHβ sequences (AF467082 and NM_204257); only one synonymous nucleotide substitution, A345→G, was detected in the region. The deduced amino acid sequence from the newly isolated

![Figure 1](image-url)
Figure 1. The mRNA expression of chicken FSHβ in the tissues sampled. Lane1: presenting the controls: group +, using total RNA from chicken pituitary gland sample without primers for β-actin; Lane 2: group -, performed using an aliquot of a cDNA synthesis reaction where no reverse transcriptase enzyme was added; Lane 3: pituitary gland, lane 4: uterus, lane 5: ovary, lane 6: oviduct, lane 7: pancreas, lane 8: glandular stomach, and lane 9: kidney samples. A: 647 bp band for FSHβ, B: 461 bp band for β-actin.

RESULTS

The tissue specificity of FSHβ mRNA expression analyzed by RT-PCR

As shown in Figure 1, during chicken ovary

Statistical analysis

All quantified results are expressed as means±SE. The results were assessed using the Kruskall-Wallis non-parametric test by the software package SPSS11.0 (Conover, 1999; Lin et al., 2002). Statistical significance was indicated with a p value <0.05.

![Image](image-url)

Fragment shared 100% similarity to those published from the chicken. This result confirmed that the sequences of the chicken FSHb gene and the peptides of the FSHβ subunit are highly conserved. Moreover, the sequence homologies of the cloned chicken segment with quail, duck, goose and other animals are listed in Table 1. The comparison of the deduced amino acids of the FSHβ subunit corresponding to the 17 sequences of FSHb mRNA from 13 species is shown in Figure 3. It shows the two asparagine (Asn) at position 5 and 22 of the mature chicken FSHβ subunits for the N-linked glycosylation (Ulloa-Aguirre and Timossi, 1998); and 12 cysteine (Cys) sites for disulphide bonds (Ryan et al., 1987), and the regions forming a ‘seatbelt’ (Chopineau et al., 2001).

The positive E. coli BL21 (DE3) cells harboring pET-28a-FSHb were cultured. The expression of recombinant FSHβ protein is shown in Figure 4. On SDS-PAGE analysis under the induced conditions, the recombinant protein was highly expressed in E. coli BL21 after inducing expression by IPTG at 0.1 M final concentration for 3 h and 4 h. A recombinant protein band with molecular weight of about 19 kDa was detected, which corresponded to the estimated molecular weight of the chicken FSHβ subunit of about 17.4 kDa. The expressed FSHβ from E. coli BL21 was purified according to the method aforementioned (shown in Figure 4, lane 7).

As shown in Figure 5, the expressed recombinant molecule was identified using a commercially available monoclonal anti-FSHβ protein by Western blotting analysis. The expressed protein was recognized by the monoclonal antiserum. The purified and un-purified recombinant

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Table 1. Sequence homologies of the cloned chicken segment with quail, duck, goose and other animals.

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**Figure 2.** Identification of the recombinant plasmid pGEM-cFSHb using PCR and digestion of endonucleases of EcoRI and SalI. M, DL 2000 marker; Lane1, PCR product of recombinant pGEM-cFSHb; Lane 2, digestion of pGEM-cFSHb with enzyme EcoRI; Lane 3, digestion of pGEM-cFSHb with SalI; Lane 4, digestion of pGEM-cFSHb with EcoRI and SalI.

**Figure 3.** Comparison of the deduced amino acid sequences of FSHβ subunits corresponding to the 17 sequences of FSHb mRNA from 13 species. The asterisk (*) and exclamation mark (!) at top indicate the position of the first amino acid residues of the mature FSHβ subunits and the last residue of the signal peptides, respectively. The arrow (↓) donates the position of N-linked glycosylation, the arrowhead (▼) marks the 12 cysteine (Cys) sites for disulphide bond, the dot (•) shows identity to the peptide sequence of the FSHβ subunit, and the boxes indicate the regions forming a ‘seatbelt’ (Chopineau et al., 2001).
proteins are shown in lane 1 and 2, respectively, of Figure 5.

Effect of FSHβ subunit on the expression of FSHR mRNA in hen

To understand the effects of the FSHβ subunit on the expression of FSHR mRNA in hens, semi-quantitative RT-PCR was developed to characterize expression of the FSHR gene in pituitary gland, uterus, ovary, oviduct, pancreas, glandular stomach and kidney tissues. The results showed expression of FSHR mRNA in uterus, ovary, oviduct, pancreas and glandular stomach in the three groups; whereas the expression levels of FSHR mRNA in the non-gonadal tissues were much lower than in the ovary. The levels of mRNA expression in the control chickens were not significantly different from those detected before treatment by injection of FSHβ protein or 0.9% sodium chloride (p>0.05), but significantly higher expression levels were demonstrated in uterus, ovary and oviduct samples of the trial group than in the control (p<0.05). No expression was detected in pituitary gland and kidney tissues of the three groups (Figure 6).

DISCUSSION

Chicken FSH plays an essential role in the control of pubertal maturation and reproductive processes. Specifically, it initiates ovarian follicular development and stimulates the maturation of germ cells in hens (Rao et al., 1978; David et al., 2007). Related studies showed that there was a firm positive correlation during laying between chicken FSH levels in plasma and egg laying performance (Yang et al., 2007). It is well accepted that multiple regulatory mechanisms govern the release of FSH. The levels of FSH secretion are mainly regulated by the hypothalamic-pituitary-gonadal axis (HPGA) (Padmanabhan et al., 2002). Extensive studies have been made of the physiological function of FSH and associated regulative factors and stimuli (activin) or inhibins (Lovell et al., 2001), but several questions have still received very little attention.

Considering that FSH is comprised of two dissimilar subunits, α and β, encoded by separate genes, of which the FSHβ subunit is responsible for interaction with the FSHR gene.
(Tomas et al., 1990; Simoni et al., 1997), then what effect does a single FSHβ subunit exclusively have on the mRNA expression of the FSHR gene? Furthermore, how does the FSHβ gene contribute to the diversification of ovarian follicular development and egg production in the chicken?

In this study, we have firstly isolated the coding region of the FSHb gene encoding the FSH beta-subunit from Dagu chickens at 18 weeks of age. The high identities of the coding region sequence of the FSHb gene and the deduced amino acid sequence from the Dagu breed to those of chicken (broiler), quail, domestic duck, goose and crested ibis, indicated that the coding region of the chicken FSHb gene and the amino acid sequence of the FSHβ subunit were highly conserved. This may be due to the crucial role of the normal function of the FSHβ gene in the hormonal specificity and regulation of reproduction within a species. However, why do, the various effects of the FSHβ gene on laying performance of chickens exist? Possible reasons may be explained by the following: i) Polymorphism of the chicken FSHβ gene mainly comes from its un-translated regions except for its two introns in the genomic sequence. In Dagu chicken, the 3'-untranslated region of FSHb mRNA showed 97.98% similarity to each other within the breed sampled (unpublished material). In Taihu and Wenchang chicken, six nucleotide variations were identified in the promoter region of the FSHb gene; some of them were significantly correlated with egg production of layers (Hong, 2004; Han, 2006). ii) The activity of the FSHβ subunit determines its specific biologic activity and is responsible for interaction with the FSH receptor (Tomas et al., 1990; Simoni et al., 1997). FSHR is essential for integrating the pituitary FSH signal with gonadal response (Heckert, 2001). iii) Both FSHβ and FSHR genes play important roles in control of the hypothalamic-pituitary-gonadal axis. The rare mutations of the FSHβ subunit were shown to produce distinctive phenotypes in reproductive physiology (Themmen and Huhtaniemi, 2000; Chopineau et al., 2001). iv) Coordination of the FSHβ subunit with other factors, such as the alpha-subunit, luteinizing hormone (LH) and steroidogenic factor 1 (SF-1), can promote the response of the gonads to FSH (Heckert, 2001), correspondingly stimulating dramatically dominant follicular growth, and triggering ovulation of the follicle (Chappel and Howles, 1991).

Sequence comparison demonstrated that the deduced amino acids of the FSHβ subunit are highly conserved, whereas different polymorphism patterns of the rare variations of amino acids in the variable sites appear among the various species. It has been reported that within the sequences of vertebrate FSHβ subunit proteins, the region at the 100-110 (Gly-Pro-Ser-Tyr-Cys-Ser-Phe-Gly-Glu-Met-Lys) in humans has an impact on FSH specificity (Moyle et al., 1994). The 100-110 sequence of the FSHβ subunit surrounds the α-subunit forming a ‘seatbelt’ which is fastened by the 26-110 disulfide bond (Chopineau et al., 2001). Of course, for the rat and mouse, the sequence corresponds to the residues of 99-109 amino acids. In chicken and other poultry (Table 1), the corresponding region of the 98-108 residues is more conserved than in the mammals, of which, the amino acid residues, Ser105-His106-Asn107-Gly108, within the FSHβ subunit are unique to the sampled chicken and quail. The amino acid residues Ser105-His106-Asn107-Gly108 in the chicken substitute Gly107-Glu108-Met109-Lys110 in the human; Gly107-Asp108-Met109-Lys110 in the bovine; and Ser107-Asp108-Ile109-Arg110 in the ovine (Figure 3). The variation in this region probably has a strong influence on conformation of the heterodimer, or on binding with the receptor, but the mechanism by which this region has an impact on specificity in mammals is not understood (Chopineau et al., 2001). Taken together, higher conservation of this region in the chicken may be due to the critical role of the normal function of the FSHβ gene in the hormonal specificity and regulation of reproduction. More extensive research on how the sequence functions is ongoing.

It’s well known that the FSHR gene was selectively expressed on granulosa cells of the ovary in the female and sertoli cells of the testis in the male, but relatively little is known about the distribution of FSHR mRNA expression in other tissues of the chicken. In the present study, expression of FSHR mRNA was detected in Dagu hens at 23 weeks of age. It was found that FSHR mRNA was expressed in uterus, oviduct, pancreas and glandular stomach tissues (in addition to ovary), but not in the pituitary gland and kidney out of the sampled tissues in hens. This may not be completely consistent with the report (You et al., 1996) that the chicken FSHR mRNA transcript was not detected in the oviduct, liver and other tissues using Northern blot analysis. The divergence probably results from the following aspects: i) It has been reported that the expression level of non-gonadal gonadotropin (including LH/hCG) receptors is much lower than in the gonads of mammals (Ziecik et al., 2005; Ziecik et al., 2007). The expression level of chicken FSHR mRNA in oviduct tissue sampled at the reproductive phase was relatively lower in this study, so that it was not clearly observed by Northern blot analysis. ii) It is well accepted that the sensitivity of the method to investigate the levels of mRNA expression by Northern blot is much poor than by RT-PCR analysis. Here, the expressions of FSHR mRNA in the non-gonadal tissues (uterus, oviduct, pancreas and glandular stomach) in hens revealed that FSH plays a substantial role as a molecular autocrine-paracrine regulator to its non-gonadal receptors presented extensively in the chicken, which has been previously confirmed in humans and other mammals (Mizrahi, 1999; Shemesh, 2001;
Fields and Shemesh, 2004; Ziecik et al., 2005). To understand the physiological mechanism of FSH receptors in extra-gonadal tissues of the chicken, more research is required.

With the aim of studying the functions of the chicken FSHβ subunit, we constructed the pET-28a-FSHb expression vector, and it effectively expressed in E. coli BL21 (DE3) cells. The recombinant protein with a molecular weight of about 19 kDa was obtained with around 4 kDa of amino acid residues from pET-28a vector included, which corresponds to the target protein of the chicken FSHβ subunit at about 14.74 kDa. Clear active effects of the administrated recombinant FSHβ protein on mRNA expression of the FSHR gene in the sampled uterus, ovary and oviduct tissues of Dagu hens were revealed, whereas no significant increases of FSHR mRNA expression levels in glandular stomach and kidney tissues were demonstrated, thereby confirming that the expressed FSHβ protein possessed biological activity. Although still greater understanding is needed, the present observation provides evidence for an up-regulating effect of the FSHβ protein on the expression of FSHR mRNA within the breed, and for a potential role in regulation of chicken reproductive physiology that may also utilize signaling mechanisms similarly to gonadal receptors as reported in mammals (Garcia-Campayo and Boime, 2001; Stewart, 2001; Fields and Shemesh, 2004).

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