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Sexual maturation, serum steroid concentrations, and mRNA expression of IGF-1, luteinizing and progesterone hormone receptors and survivin gene in Japanese quail hens

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ABSTRACT

In avian species, sexual maturation represents the evidence of start laying, which is a consequence of the development of ovarian follicles. These follicles are the functional reproductive unit whose maturation and viability critically depends on endocrine, paracrine, and autocrine factors beyond the signals from the central nervous system. The present study was undertaken to investigate the correlation of sexual maturity with tissue growth, mRNA expression of certain genes, and serum steroid concentrations in Japanese quail hens. To carry out the present study, a total of forty Japanese quail hens (5 weeks) were housed individually under uniform husbandry condition with ad libitum quail layer ration and water at 14-hour photo schedule. On sixth week onwards, four birds were sacrificed at each time on 1, 3, 7, 10, 13, 16, 19, 22, 25, and 28 days. Serum was extracted aseptically to analyze the gonadal steroid hormones (estrogen and progesterone) and corticosterone to investigate the liaison with sexual maturation of the species. Expression analyses of four genes i.e., insulin-like growth factor-1, luteinizing hormone receptor, progesterone receptor, and survivin were carried out in the three largest ovarian yellow follicles. A significant ($P < 0.05$) increase in body weight gain and oviduct weight was recorded during the phase of sexual maturation. Smaller follicles revealed higher insulin-like growth factor-1 and survivin gene expression, whereas the reverse result was manifested in both the luteinizing and progesterone hormone receptors. In biochemical study, the gonadal steroids (estrogen and progesterone) were recorded higher at the first half of the experiment when a gradual decrease in corticosterone concentration was confirmed from the very beginning of this study. This result substantiated that sexual maturation in Japanese quail may be completed by the time of 8 weeks after its birth in support of the analyzed information studied in the current investigation.

1. Introduction

Japanese quail is the only smallest domesticated avian species [1] and suitable for the diversification of chicken-dominated poultry industry [2]. Considering the anatomy and physiological homology to chicken, Japanese quail (*Coturnix coturnix japonica*) particularly for their fast-growing genetic makeup, early sexual maturity, and short generation interval has been introduced as a model animal in heavy expensive biomedical studies. In avian species, sexual maturation not only depends on body weight gain but also on follicular development in the ovary. The maturation and viability of ovarian follicles within a breeding season is critically dependent on intraovarian endocrine,

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paracrine, and autocrine factors beyond the signals from the central nervous system [3]. The attainment of sexual maturity is governed by body weight, body fat, lean body mass, photoperiod, age, and modulation of neuroendocrine system [4–6]. In sexually matured birds, the elevated level of estrogen indirectly regulates the rapid growth of oocytes by potentiating the synthesis of yolk precursor proteins in the liver [7]. Meanwhile, the distinct changes in the messenger RNA (mRNA) expression of gonadal steroid hormones synthesizing genes result in increase hormonal level, which synergizes the follicular growth through receptor-mediated mechanism [8]. Insulin-like growth factors (IGFs) play a crucial role in avian reproduction as they hasten dose-dependent gonadal steroid hormone synthesis, cell proliferation, selection and inhibition of follicular apoptosis by inhibiting oligonucleosome formation [9–11]. Metayer, et al. [12] suggest theca as the epicenter of IGFs production in the hierarchical follicles. Activation of nitric oxide synthase-nitric oxide cyclic pathway potentiates an excitatory signal that elicits hypothalamic–pituitary–gonadal (HPG) axis, which induces gonadotropin secretion and subsequently gonadal steroid synthesis from the ovarian cells [13–15].

However, limited information is available so far to correlate the sexual maturation with tissue growth and expression profile of certain genes in Japanese quail. Therefore, the present study was an attempt to investigate the association of sexual maturation with tissue growth, mRNA expression of IGF-1, luteinizing and progesterone receptors, survivin gene, and serum steroid concentrations in Japanese quail hens.

2. Materials and methods

2.1. Experimental birds

A total of 40 healthy Japanese quail pullets (aged 5 weeks) used for this study were randomly selected from the institute experimental quail farm and the experiment was conducted in accordance with institutional practice and within the revised framework of animals (Scientific Procedures) Act of 2002 of Government of India on animal welfare. They were transferred to individual laying cages ($20 \times 20 \times 20 \times \mathrm{cm}^3$) to acclimatize for 1 week and reared under standard managerial condition at 14-hour photo schedule with ad libitum drinking water and quail layer ration (ME-2716 Kcal/kg, CP-20.03%, Ca-3.06%, P-0.33%, Lysin-1.09, and Methionine-0.45%).

2.2. Sample collection

At the beginning of the sixth week, individual birds were weighed in an electronic weighing balance (0.01 g specificity) and four of them were sacrificed by decapitation in each time on Days 1, 3, 7, 10, 13, 16, 19, 22, 25, and 28. Immediately before slaughter, body weight gain was accounted to compute the percent of weight gain. Biochemical study was carried out on sampling of 3 mL blood into sterile centrifuge tube during bleeding. Serum was separated following standard protocol and stored at −80 °C until used. Ovary weight, total oviduct weight, and the number of yellow follicles were also taken into consideration to appreciate the development during sexual maturation. The level of gene expression was studied in the ovarian follicles. Three largest follicles (F1, F2, and F3) were excised from the ovary and cut open transversely along the stigma to drain the yolk material. The follicular membranes were washed three times with ice-cold sterile normal saline (0.9%) to devoid of adhering yolk material.

2.3. RNA isolation and reverse transcription

Total RNA from each follicle was extracted by “RNAgents total RNA isolation system” (Promega, Madison, WI, USA) according to the manufacturer’s instructions. The RNA integrity was checked by 1% denaturing agarose gel electrophoresis. The concentration and purity of RNA preparations were measured through NanoDrop (Thermo2000) and the ratio of 260/280 was more than 2.0 for all the samples. The possible traces of genomic DNA were removed by treating 5 μg of each RNA samples with 5 U of RNase-free DNase (Biogene) at 37 °C for 1 hour. The DNase was subsequently inactivated by incubation at 65 °C for 10 minutes. Each DNase-treated total RNA sample (1 μg) was reverse transcribed using the “RevertAid First strand cDNA synthesis kit” (MBI Fermentas, Hanover, MD, USA) according to the manufacturer’s instructions. The resultant cDNA was stored frozen at −20 °C till used. Negative controls were performed using all components except reverse transcriptase.

2.4. Estimation of IGF-1, LH-R, PG-R, and survivin by RT-qPCR

After reverse transcription, the target mRNA into cDNA, it was quantified using real-time quantitative polymerase chain reaction (RT-qPCR) using Syber Green master mix in IQ5 cycler (Bio-Rad). The amplification of mRNAs was carried out by gene-specific primers designed by Beacon designer software (Premier Biosoft International). Primers were designed from coding region of chicken mRNA sequences available in Gene Bank considering the close relationship between chicken and quail in phylogeny and sequences. The sequence of forward and reverse primer for IGF-1, luteinizing hormone receptor (LH-R), progesterone hormone receptor (PG-R), survivin, and beta-actin (as reference gene) are shown in Table 1. For RT-qPCR assay, the reaction was set in triplicate with each cDNA sample. The amplification was carried out in 25 mL volume containing 1× QuantiTect SYBR Green PCR master mix (SyberGreen1dye, Hot Start Taq DNA polymerase and dNTPs in optimized buffer components; QIAGEN GmBH), a 0.2-μM concentration of each gene-specific primer and 1 μL of cDNA template. The cycling conditions of RT-qPCR include initial denaturation of 95 °C for 10 minutes followed by 40 cycles of denaturation of 95 °C for 30 seconds, annealing for 30 seconds and extension of 72 °C for 45 seconds. For each gene of interest, negative and positive controls were included. Negative controls were samples in which cDNA was not added. A melting curve was performed for each sample after completion of amplification and analyzed in comparison with negative and positive controls, to determine the specificity of polymerase chain reaction reaction. To generate gene-specific standard curves, plasmids containing each of the different genes were serially diluted from 10^{-1} to 10^{-5}.