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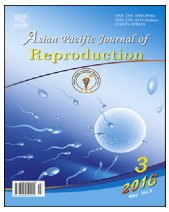

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Prenatal corticosterone altered glucocorticoid receptor and glucocorticoid metabolic enzyme gene expression in chicken ovary

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ABSTRACT

Objective: The acute stress response is an adaptive physiological mechanism which allows an organism to respond and survive deleterious stimuli in the surrounding environment. In mammals, prenatal glucocorticoids exposure (GCs) reprograms offspring phenotype and reproductive performance. In the present study, we investigated potential prenatal GC exposure on the glucocorticoid receptor (GR), mineralocorticoid receptor (MR) and GC metabolic genes mRNA expression in the ovary of chickens.

Methods: We injected low (0.2 μg) and high (1.0 μg) doses of corticosterone (CORT) *in ovo* before incubation and measured the changes in GCs metabolic enzymes genes in ovarian follicles 1 (F1), F2 and F3 post hatching.

Results: The high dose CORT treatment significantly ($P < 0.0$) decreased 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1) mRNA expression in F1, F2, F3 and in the ovary compared to the control and low groups. However, the high dose CORT treatment significantly ($P < 0.0$) increased 11β-hydroxysteroid dehydrogenase type 2 (11β-HSD2) mRNA expressions in F1, F2, F3 and in the ovary compared to control and low groups. Likewise, *in ovo* injection of high dose CORT significantly ($P < 0.0$) decreased 20-hydroxysteroid dehydrogenase (20-HSD) mRNA expression in F2, F3 and ovary compared to the control and low groups. Moreover, CORT treatment reduced GR mRNA expression in F1, F2 and F3 but not ovary. CORT treatment decreased MR mRNA only in F2.

Conclusions: Prenatal CORT exposure modified GR, MR and GC metabolic enzymes gene expression in ovarian follicles, thus it may reprogram reproductive function.

1. Introduction

Not only genetic factor controls the phenotype and reproductive function of organisms but also environmental factors can play a critical role in shaping offspring morphology [1] and reprogram reproductive performance [2]. In avian species, maternal influences have attracted much attention after the discovery that avian eggs contain a variety of steroid hormones [3,4]. Maternal derived steroid hormones are considered as a tool to adjust offspring phenotype [5]. Corticosterone (CORT) is considered the predominant glucocorticoid (GC) in the plasma of avian species, and it has been reported to deposit in the eggs of domestic chickens [6]. Eggs CORT concentrations is modulated by several factors including physiological status of the hen [7], the environment [8] such as housing conditions [9] and artificial elevation of egg CORT [10].

In chickens, reproductive capacity was found to be reprogrammed by prenatal CORT exposure [11]. In birds, maternal stress modulates reproductive hormone concentrations in the eggs [12] and therefore affects offspring phenotype [13] and behavior [14]. The majority of studies

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investigating the effect of elevated CORT in the eggs have focused on growth rate [8] and behavior [15]. However, little is known about the effects of embryonic CORT exposure on reproductive capacity in avian species. Moreover, it is still unknown the effect of prenatal CORT exposure on stress related gene expression in the ovary. The action of GCs on cells is mediated via glucocorticoid receptor (GR), and mineralocorticoid receptor (MR) [16]. The intracellular concentrations of active GC are under control of a number of metabolizing enzymes which is called pre-receptor modulation [17]. The 11β-hydroxysteroid dehydrogenase (11β-HSD1) activates, while 11β-hydroxysteroid dehydrogenase (11β-HSD2) deactivates GCs [18–22]. In avian species, 20-hydroxysteroid dehydrogenase (20-HSD) is an abundantly and ubiquitously expressed enzyme, which transforms GCs to inactive 20-dihydrocorticosterone [23]. The ovary is well equipped with local regulatory mechanisms of GC action [24]. The major regulatory system consists of changes in the expression of the two isoforms of 11β-HSDs that catalyze the inter-conversion of GCs in the ovary [25,26]. Yet, the effects of prenatal CORT exposure on GCs metabolic enzymes gene expression in chicken ovary remain less clear. Therefore, here we used a model of *in ovo* injection of CORT prior to incubation to examine our hypothesis that expression of genes involved in GC metabolism in the ovaries may be modified by embryonic CORT exposure.

2. Materials and methods

2.1. Egg incubation and CORT injection

Two hundred and ten fertilized chicken eggs were selected from eggs laid by hens and divided into three groups (70 in each group). CORT (Sigma–Aldrich, USA) was dissolved in sesame oil and diluted in PBS to make doses of 0.2 μg and 1.0 μg in a volume of 100 μL solution. The high and low CORT dose was selected based on earlier publications [27,28]. Before incubation, the eggs were injected with PBS (control) and a 0.2 μg (low) or a 1.0 μg (high) dose of CORT under aseptic conditions. Eggs were injected by advancing a Hamilton syringe into a hole in the middle of the long axis until the yolk membrane was penetrated. The incubation condition was set based on our previous publication [29]. Chicks hatched inside the incubator and were left to dry completely (up to 12 h) before they were removed. Day-old chicks were individually wing banded, and placed into battery cages with 12 h fluorescent lighting and 12 h dark. The temperature was adjusted to 32–35 °C during the first week, and reduced approximately 3 °C per week until 21 °C. Both sexes were transferred to floor pens covered with sawdust litter. The stocking density was 20–25 kg/m^2. The relative humidity was maintained at 40%–60%, and the lighting, ventilation, as well as the feeding procedures complied. On week 35, all chickens were killed by rapid decapitation. The ovaries were collected and weighed. The ovarian follicles were collected, washed with PBS then put in liquid nitrogen and later kept at −80 °C for further analysis. The experiment procedures were approved by the Animal Ethics Committee of Nanjing Agricultural University.

2.2. RNA extraction and mRNA quantification with real-time PCR

Ovary and ovarian follicle were ground with pestle and mortar in liquid N2 and a portion of approximately 100 mg was used for the RNA extraction using the TRIzol total RNA kit (Invitrogen, Biotechnology Co, Ltd, Carlsbad, CA, USA) according to the manufacturer's instructions, and reverse transcript to cDNA using 0.5 μg/μL (4 μL contains 4 μg) of RNA with the PrimeScript RT reagent kit according to the manufacturers instruction (RNase Free, D2215, Takara, Japan). To investigate the effect of the *in ovo* injection of CORT on the expression of hypothalamic genes, real-time PCR was performed in an Mx3000P (Stratagene, USA) according to published methods [29]. Mock RT and No Template Controls (NTC) were included to monitor the possible contamination of genomic and environmental DNA at the RT and PCR steps. A pooled sample made by mixing equal quantities of the RT products (cDNA) from all the samples was used for optimizing the PCR conditions and tailoring the standard curves for each target gene, and melting curves were performed to insure a single specific PCR product for each gene. The PCR products were sequenced to validate the identity of the amplicons. Primers specific for the 11β-HSD1, 11β-HSD2, 20-HSD, GR, and MR (Table 1) were synthesized by Geneary, Shanghai, China. Chicken β-actin was used as a reference gene for normalization purposes. The method of $2^{-\Delta\Delta Ct}$ was used to analyze the real-time PCR data [30].

2.3. Statistical analysis

Descriptive statistics was performed to check the normality and homogeneity of variances before using parametric analyses.

Table 1
Real-time PCR primers.

Target genes	GenBank accession number	PCR products (bp)	Primer sequences
β-actin	L08165	300	F: 5′-TGCGTGACATCAAGGAGAAG-3′ R: 5′-TGCCAGGGTACATTGTGGTA-3′
GR	DQ227738	102	F: 5′-CTTCCATCCGCCCTTCA-3′ R: 5′-TCGCATCTGTTTCACCC-3′
MR	NM_001159345.1	150	F: 5′-ACGCAGGATATGACAGCTCG-3′ R: 5′-AGTACAGGGGCTTGGCATTC-3′
11β-HSD1	XM_417988.2	229	F: 5′-GGTGGTGAAAGAGGCTGAGAAC-3′ R: 5′-GGAGGCGACTTTACCTGAAACAG-3′
11β-HSD2	XM_003209680.1	229	F: 5′-GGTGGTGAAAGAGGCTGAGAACA-3′ R: 5′-GGAGGCGACTTTACCTGAAACAG-3′
20-HSD	NM_001030795.1	220	F: 5′-CATCCTGAGAAGATAATGTCCAACG-3′ R: 5′-TGCTTTGCAGATCATCAATATCCAG-3′