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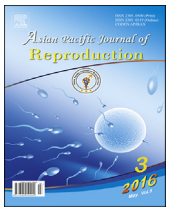

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Prenatal corticosterone altered glucocorticoid receptor and glucocorticoid metabolic enzyme gene expression in chicken ovary

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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′

The relative quantitative data of gene expression were analyzed by one-way ANOVA using SPSS 16.0 for Windows, followed by a least-significant difference (*LSD*) test for individual comparisons. A *P*-value ≤ 0.05 was considered significant.

3. Results

3.1. Ovary weight and oviduct weight

High dose of CORT treatment significantly ($P < 0.05$) decreased ovary weight and oviduct weight compared to low and control groups. Low dose of CORT treatment did not affect ovary weight or oviduct weight compared to low and control groups (Table 2).

Table 2
Effect of *in ovo* injection of CORT on ovary weight and oviduct weight.

Group	Ovary weight	Oviduct weight
Control	13.75 ± 0.56	71.43 ± 3.22
Low	12.48 ± 0.53	63.85 ± 6.75
High	6.65 ± 0.56	33.07 ± 8.19

3.2. Ovarian follicles theca cells GC metabolic enzymes, GR and MR mRNA expression

CORT treatment significantly decreased ($P < 0.05$) ovarian F1, F2, F3 and ovary 11β-HSD1 mRNA expression (Figure 1A). However, low dose of CORT did not alter 11β-HSD1 mRNA expression if F1. In contrast, high dose of CORT treatment significantly increased ($P < 0.05$) 11β-HSD2 mRNA F1, F2, F3 and ovary (Figure 1B), whereas, 20-HSD mRNA was down-regulated in F2, F3 and ovary regarding CORT treatment (Figure 1C). Both low and high doses of CORT treatment significantly decreased ($P < 0.05$) GR mRNA expression in ovarian F2 and F3 but not F1 or ovary (Figure 1D). Likewise, low and high doses of CORT treatment decreased MR mRNA in F2 (Figure 1E).

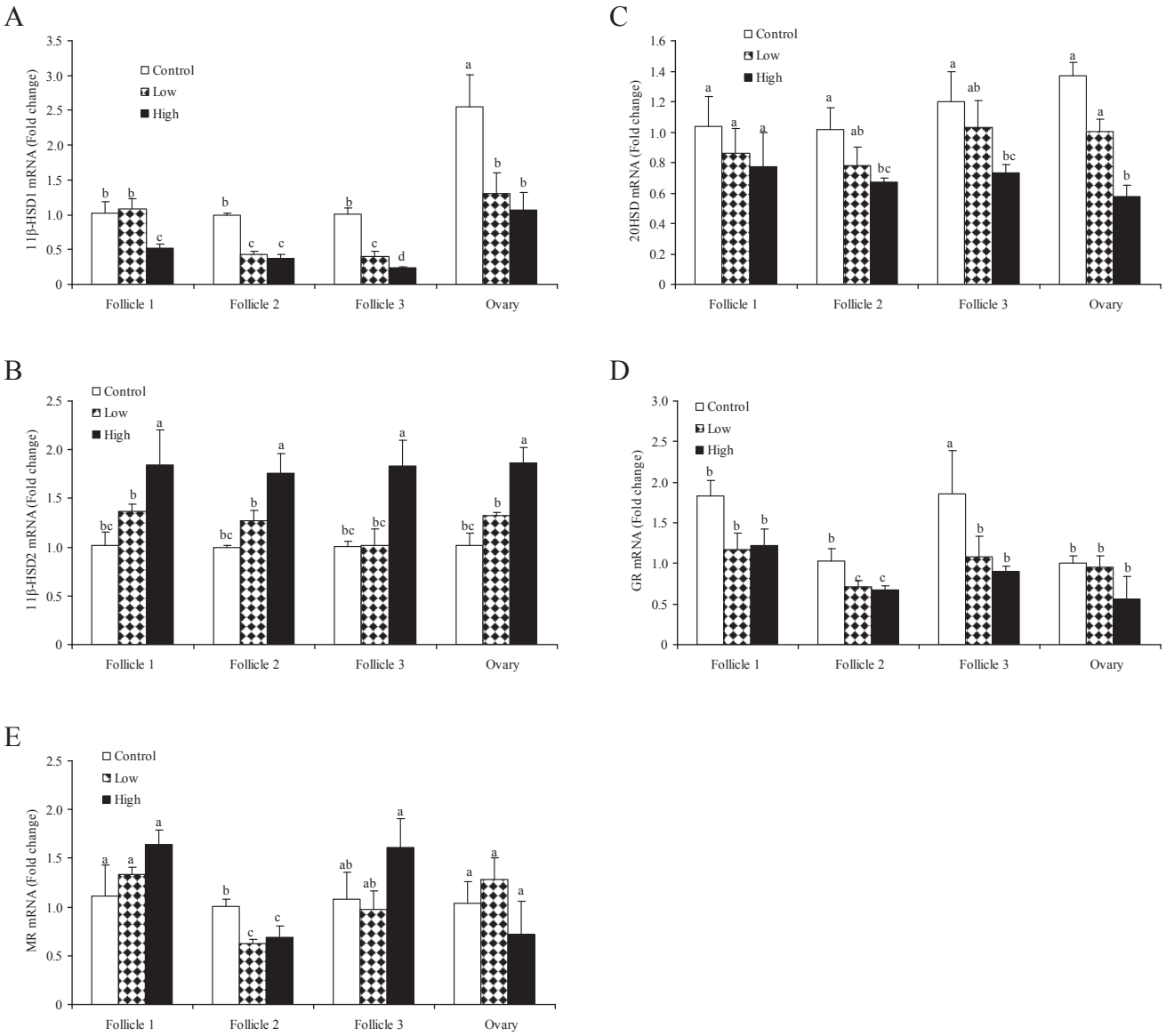

Figure 1. Effect of *in ovo* injection of CORT on ovarian follicles and ovary 11β-HSD1 mRNA (A), 11β-HSD2 mRNA (B), 20-HSD mRNA (C), GR mRNA (D) and MR mRNA (E) expression.
Values are mean ± SEM, $n = 6$/group. Different letters above the bars indicate significantly different mean values at $P < 0.05$.

4. Discussion

In mammals, early life experiences re-program the hypothalamic-pituitary-gonadal (HPG) axis, which is associated with increased reproductive dysfunction later in life (Heim and Nemeroff, 2001). We report here, for the first time, the effect of prenatal CORT exposure on GC metabolic enzymes gene expression in the ovarian follicles in chickens. *In ovo* administration of CORT decreased ovary weight and oviduct weight. These findings are in agreement with previous studies that prenatal stress has been reported to decrease ovary weight in guinea pig offspring [31].

Previous reports indicated that maternal adversity during the prenatal period altered 11β-HSDs expression in humans [32,33]. Here we reported for the first time the effect of CORT *in ovo* on 11β-HSDs in the ovary. CORT treatment decreased ovarian follicles 11β-HSD1 and 20-HSD mRNA expression whereas increased 11β-HSD2 mRNA expression in ovarian follicles. Earlier studies indicated that upregulation of 11β-HSD1 may follow prenatal stress hormone exposure which is considered a novel mechanism for fetal origins of adult metabolic syndrome [34], whereas 11B-HSD2 appears/is involved in fetal programming of behavior in mice [35]. In human ovaries, the simultaneous upregulation of 11β-HSD1 and downregulation of 11β-HSD2 [36] induced luteinized granulose cells to be rich with 11β-reductase activity [37], which most probably explains why both total and free cortisol levels in human follicular fluid become high after the ovulation inducing LH surge [38]. The enzymes 11β-HSDs, are the main GC activation/inactivation enzymes in the placenta of mammals, has also been reported in the oviduct and ovary of chickens [39] and in the ovary of zebra finches [40]. The alteration of these enzymes mRNA might explain the mechanism of low transferring rate of CORT from the maternal plasma to her eggs and thus suggests that mammals and birds have similar strategies to protect their embryos from overexposure to maternal CORT.

In addition, CORT treatment decreased GR receptor mRNA and MR receptor mRNA levels in theca layer of ovarian follicles. These findings are in agreement with previous findings that exposure to dexamethasone decreased GR mRNA levels in hamster ovarian cell line [41]. Because the current study is the first to reveal the effects of prenatal CORT exposure on changes in GR and MR mRNA expression in the ovary of domestic fowl, we will consider the potential implications of these results. In mammals and humans, disruption of GR/MR stability is associated with several health disorders, such as post-traumatic stress disorder [42], depression [43], and anxiety [44]. In general, disturbances in the ratio of GR⁄MR after chronic stress increases the overall 'wear and tear' in the organism, frequently referred to as allostatic load [45]. Such disruption decreases resilience and reduces the ability of an organism to respond appropriately to acute stress [46]. Further studies are required to elucidate whether these transcriptional changes are permanent or transient.

In conclusion, we demonstrate that embryonic exposure to CORT decreased oviduct and ovary weight associated with alterations of GR, MR and glucocorticoid metabolic enzymes genes expression in chicken ovary.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgments

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