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Corticosterone *in ovo* modifies aggressive behaviors and reproductive performances through alterations of the hypothalamic-pituitary-gonadal axis in the chicken

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ABSTRACT

Exposure to excess glucocorticoids during embryonic development affects offspring reproduction and suppresses the hypothalamic-pituitary-gonadal (HPG) axis in mammals. However, whether corticosterone (CORT) causes similar effects in the chicken remains unclear. In the present study, we injected low (0.2 μg) and high (1 μg) doses of CORT *in ovo* before incubation and detected changes in aggressive behavior, tonic immobility (TI), reproductive performances, and HPG axis gene expression in posthatch chickens of different ages. High dose of CORT suppressed growth rate from 3 weeks of age, increased the frequency of aggressive behaviors, which was associated with elevated plasma CORT concentration. High-dose CORT significantly ($P < 0.05$) down-regulated arginine vasotocin (AVT), corticotropin-releasing hormone (CRH), 11β-hydroxysteroid dehydrogenase type 2 (11β-HSD2) and gonadotropin-releasing hormone 1 (GnRH1), while significantly ($P < 0.05$) up-regulated gonadotropin-inhibitory hormone (GnIH) and 11β-HSD1 mRNA expression in the hypothalamus. Glucocorticoid receptor (GR) and 20-hydroxysteroid dehydrogenase (20-HSD) mRNA levels were not affected by CORT treatment. High-dose CORT significantly ($P < 0.05$) reduced egg production and egg quality, which was associated with decreased ovary and oviduct weight. Moreover, CORT exposure significantly decreased ($P < 0.05$) luteinizing hormone (LH) receptor and follicle-stimulating hormone (FSH) receptor mRNA abundance in theca cells of ovarian follicles 1 (F1), F2 and F3. In addition, yolk CORT concentration was significantly higher in eggs laid by hens prenatally exposed to high-dose CORT. Our findings suggest that *in ovo* administration of CORT programs the aggressive behaviors and reproductive functions in the chicken through alterations of HPG axis.

1. Introduction

The phenotype of an organism is influenced not only by the genetic factor, but also by environmental factors that play critical roles in shaping the morphology (Nijhout, 2003) and the reproductive capacity (Alfred et al., 2002). In avian species, maternal influences have aroused much attention after the discovery that avian eggs contain a variety of maternally derived steroid hormones (Groothuis et al., 2005; Schwabl, 1993). Corticosterone (CORT), the predominant glucocorticoid (GC) in birds, has been reported to deposit in the chicken eggs (Rettenbacher et al., 2009). CORT concentration in eggs is modulated by several factors including stressful environments (Hayward

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and Wingfield, 2004), housing conditions (Lay et al., 2011) and the physiological status of the hen (Saino et al., 2005).

Maternal GC can be transferred to the developing embryo/fetus via the placenta in mammals (Seckl, 2004) or the egg in birds (Saino et al., 2005). Prenatal GC exposure is known to have both short- and long-term consequences (Love and Williams, 2008; Seckl, 2004), such as decreased hatch weight (Janczak et al., 2006) and compromised immunity (Rubolini et al., 2005). Prenatal GC exposure reprograms the function of hypothalamic-pituitary-adrenal (HPA) axis and behavior (Seckl and Meaney, 2004). In addition, embryonic exposure to CORT enhances flight performance (Chin et al., 2009) or the recall of a passive avoidance task (Sui et al., 1997), increases fearfulness behavior (Janczak et al., 2006) or the rate of pecking at grains and pebbles (Freire et al., 2006).

Reproductive functions in mammals can be programmed by prenatal GC exposure (Dunn et al., 2010). In avian species, maternal stress modulates the deposition of steroid hormones in their eggs (Henriksen et al., 2011a) and thereby influences the offspring phenotype (Gil, 2003) and behavior (Riedstra et al., 2013). The majority of studies investigating the effects of elevated CORT in the egg focused on growth (Hayward and Wingfield, 2004) and behavior (Henriksen et al., 2011b). Virtually little is known about the effects of embryonic CORT exposure on reproductive performances in the chicken.

In mammals, cortisol exposure decreased GnRH1 pulsatility (Oakley et al., 2009), and inhibited the release of follicle stimulating hormone (FSH) and luteinizing hormone (LH) from the pituitary in vitro (Breen and Karsch, 2006; Sapolsky et al., 2000). In addition, CORT treatment decreased hypothalamic GnRH1 expression (Gore et al., 2006) and suppressed ovary functions (Inazu et al., 1990) in rats. Moreover, early life stress induces permanent modification in HPG axis in later life in mammals (Davies and Norman, 2002). Yet, the effects of embryonic CORT exposure on aggressive behavior, tonic immobility, as well as the egg production, egg quality and ovary functions in the chicken remain unclear.

Therefore, here we use a model of *in ovo* injection of CORT before incubation to test our hypotheses that aggressive behavior, tonic immobility and plasma CORT concentration may be influenced by CORT treatment, and these phenotypic changes may be associated with decreases in egg production and ovary functions. Moreover, the expression of genes involved in HPG axis and ovarian follicle development may also be modified by embryonic CORT exposure.

2. Materials and methods

2.1. CORT treatment and animal housing

Two hundreds and ten fertilized chicken eggs (63.6 ± 0.43 g, ranging from 60.1 g to 67.2 g) were obtained from 26-week-old Hy-Line Brown breeder hens (Wen's group, Guangdong, China) and were randomly divided into three groups (70 in each group). The time line of the experiment is shown in Fig. 1A. Before incubation, CORT (Sigma–Aldrich, USA) was dissolved in absolute alcohol, diluted in PBS thereafter to give doses of 0.2 μg and 1 μg in the volume of 100 μL. Eggs were injected with PBS (control), 0.2 μg (low) or 1 μg (high) dose of CORT. The doses were determined according to the previous publications (Heiblum et al., 2001; Janczak et al., 2006; Haussmann et al., 2012), taking into consideration the CORT concentrations detected in the yolk (3–4 ng/g) and the albumen (0.5 ng/g) of the chicken eggs (Ahmed et al., 2013a). Injection was performed as described by Haussmann et al., 2012. Eggs were chosen randomly and injected by advancing a Hamilton syringe into a hole in the middle of the long axis until the yolk membrane was penetrated (approximately 20 mm below the surface). The incubator was set according to our previous publication (Su et al., 2012). Chicks were hatched inside the incubator and were left to dry completely (up to 12 h) before they were removed. The hatchability of the eggs ranged from 70% to 75% and no obvious differences in hatchability or hatching time were observed among three groups. One-day-old chicks were individually weighed, wing banded, and placed into battery cages with continuous fluorescent lighting. The temperature was adjusted to 32–35 °C during the first week, and reduced approximately 3 °C per week until 21 °C. At 3 weeks of age, females were selected and transferred to floor pens covered with sawdust litter. The stock density was 20–25 kg/m^2. The relative humidity was maintained at 40–60%, and the lighting, ventilation, as well as the feeding and management procedures complied with the Feeding Management Regulations of Yellow-feathered Chicken (NY/T 1871-2010).

Growth performance was recorded weekly from hatching to 10 weeks of age. Behavior test was performed on posthatch day 28 (D28). On D133, hens started to lay eggs. On D175, 6 birds from each group were randomly selected for blood sampling. Blood samples were harvested from the jugular vein into heparinized plastic tubes. Plasma samples, obtained by centrifugation at $3000 \times g$ for 10 min, were stored at −20 °C until CORT measurement. On D182, tonic immobility test was performed. On D245, all chickens were killed by rapid decapitation. The hypothalami were removed within 3 min after killing and the thecal layers of the preovulatory follicles (F1, F2 and F3) were isolated and kept at −80 °C for further analysis. The experiment procedures were approved by the Animal Ethics Committee of Nanjing Agricultural University.

2.2. Aggressive behaviors test

The behavior test was performed on D28 as described previously (Kitaysky et al., 2003). Briefly, 6 chickens from each group were placed in an experimental arena (similar in size and structure to their home pens where chickens were raised) established in a room familiar to chickens. The room was visually and acoustically isolated from the aviary with other chickens. For visual identification, chickens were marked (with different colors) on either the head, the body, or the tail. Chicken's behavior was videotaped for a 60-min period. The aggression was defined as a chicken pecking, grabbing, and twisting skin on the head or the nape of the other chicken. A person

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