

## Effect of Exogenous Estrogen Treatment on the Gonadal Estrogen Receptor- $\alpha$ Expression in Male Chickens (*Gallus domesticus*)

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### Abstract

Sexual differentiation in chickens is controlled by both direct genetic and hormonal mechanisms which can cause genetic sex reversal through manipulations. Estrogen is critical for normal ovarian development and *in-ovo* estrogen treatment, can feminize genetically male (ZZ) chicken gonads temporarily although a permanent sex reversal could be observed by blocking the Estrogen synthesis in genetic females. The reason for this temporary nature of male to female sex reversal is still unknown. Although *in-ovo* estrogen treatment triggered the Estrogen Receptor- $\alpha$  (ER- $\alpha$ ) expression in embryonic gonads, the post hatching gonadal expression of ER- $\alpha$  with age which can possibly affect the temporary nature of this phenomenon has not been studied adequately. Therefore, the aim of the current study was to investigate the post-hatching ER- $\alpha$  expression in left gonads when the exogenous estradiol supply is continuous. Commercial Shaver brown chicken eggs were incubated under standard conditions by giving two *in-ovo* injections, 0.1mg/egg Estradiol Cypionate and same volume of sterile Phosphate Buffered Saline to the treatment and control eggs, respectively. The genetic sexing was done using W chromosome specific sex marker HUR0424. A half of treated male chicks were given with a weekly post-hatching injection of 0.1mg/chick Estradiol Cypionate intra-muscularly (T2) and the rest was raised with no post-hatching injection (T1). Three chicks from each group, including control (C) were sacrificed at 1, 4 and 8 weeks intervals. Total RNA was extracted from the left gonads using the TRIzol method followed by reverse transcription of ER- $\alpha$  gene using a pair of gene specific primers. Following the PCR amplification, the relative quantification of the ER- $\alpha$  expression was performed relative to the expression of reference gene Glyceraldehyde-3-Phosphate Dehydrogenase. Data were analyzed using (SAS) version 9.0. The expression of ER- $\alpha$  showed a significant increase with the age ( $P < 0.0001$ ) and difference among treatments ( $P < 0.0001$ ) where the highest expression was observed at T2 followed by T1. However there was no significant interaction effect observed ( $P = 0.1203$ ) among the groups. Accordingly it can be suggested that although the ER- $\alpha$  expression was induced by exogenous estradiol, there is a male specific mechanism to decrease the triggered ER- $\alpha$  expression, with age.

**Keywords:** Estradiol cypionate, *Gallus domesticus*, Gonadal estrogen receptor  $\alpha$ , sex reversal

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