



Tamoxifen activates hypothalamic L-dopa synthesis to stimulate ovarian estrogen production in chicken

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ABSTRACT

Estrogen is the primary sex hormone responsible for the development and modulation of the female reproductive system in all vertebrates including avian species. The actions of estrogen are mediated by the estrogen receptor, which could be modulated by the selective estrogen receptor modulator tamoxifen (TAM). In this study, we administered TAM into the actively laying chicken to investigate the ovarian and hypothalamic responses to the estrogen action blockage. The laying was disrupted and the development of the pre-ovulatory hierarchical follicles was arrested. However, the TAM treatment caused an increase of estrogen level in both serum and ovary. Among the main estrogen targeted tissues, the hypothalamus showed specific dopaminergic activation as indicated by gene expression analysis. In the ovary, L-dopa, the precursor of dopamine, could stimulate the estrogen synthesis in undifferentiated follicles but not in the differentiated pre-ovulatory follicles. Thus, we established a feedback loop links ovarian estrogen production with hypothalamic L-dopa synthesis and we propose that the dopamine is involved in estrogen action to regulate the ovarian follicle development and ovulation.

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1. Introduction

Avian species are an important comparative vertebrate model for the research in endocrinology and reproductive biology. The chicken is also one of the most important agricultural animals, producing 1.1 trillion eggs per year [1]. Thus, studying the physiology of chicken reproduction could benefit both the understanding of basic reproductive biology and the improvement of food industry.

As in other vertebrates, the female avian reproductive systems are primarily regulated by the hypothalamus-pituitary-gonadal axis [2]. The hypothalamus produces gonadotropin-releasing hormone (GnRH) that stimulates pituitary production of follicle-stimulating hormone (FSH) and luteinizing hormone (LH), which in turn direct the ovarian function and reproductive activity.

Ovarian derived steroids, predominantly estrogen and progesterone, return to the central nervous system through the bloodstream and provide a feedback to the hypothalamic GnRH production and release. It is well known that in birds, estrogen plays a central role in the female reproduction by stimulating ovary and follicle development [3,4]. The effects of estrogen in the target cells are mediated by estrogen receptors (ER), which are expressed not only in the hypothalamus and pituitary but also in ovary and oviduct of chicken. Once activated by estrogen, the ER is able to translocate into the nucleus and bind to DNA to regulate the activity of different genes [5].

Tamoxifen (TAM) is a synthetic estrogen antagonist that could inhibit the transcription of target genes by binding to ER and has been widely used in the therapy for cancer patients with ER-positive tumours [6]. Previous studies in birds have confirmed that TAM could bind to chicken ER with high affinity and limit the transcription of estrogen-induced genes [7–9]. Therefore, TAM was frequently employed to investigate the roles of ER in different chicken tissues by loss-of-function means.

Here we show that TAM treatment in reproductive active chicken resulted in the disruption of laying, which may mainly

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attribute to the lowered FSH and LH. In contrast, the estrogen levels increased in both ovary and serum. Genome-wide transcriptome analysis of hypothalamus revealed that the hypothalamic l-dopa synthesis was enhanced by TAM treatment. Furthermore, we provide novel evidence that l-dopa could stimulate estrogen production in the undifferentiated but not differentiated follicles in the ovary. We reason that blocking ER leads to enhanced hypothalamic l-dopa synthesis, which in turn stimulates estrogen production in the ovary.

2. Materials and methods

2.1. Animals and reagents

Sexually mature (older than 23 weeks) Hy-Line Brown hens were collected from a local research farm affiliated with Taishan University. All animals had free access to water and feed. The hens were housed in separate cages under a daily light period of 14 h and the laying events were recorded by checking the cage every 2 h during the light period to determine the regular laying sequence. For the tamoxifen (TAM) experiment, the control group ($n = 6$) animals were treated subcutaneously with vehicle (ethanol) and the experimental group ($n = 6$) with TAM at a dose of 50 mg/kg of body weight. Hens were treated daily and all the animals were killed on day 7 of the experiment. For the l-dopa experiment, the

control birds ($n = 5$) were treated subcutaneously with vehicle, the TAM group ($n = 5$) with TAM (50 mg/kg) and the l-dopa group ($n = 5$) with l-dopa (50 mg/kg). The animals were sacrificed 24 h later. The hypothalamus region was rapidly isolated under a dissecting microscope and snap frozen in liquid nitrogen. For the ovarian tissues, the yolk in the pre-ovulatory follicles was carefully removed with a syringe and a 25-gauge needle. Then the stroma tissue containing the pre-hierarchical small white follicles (SWF, 2–4 mm) and the largest pre-ovulatory follicles (F1–F5) were collected and snap frozen. Tissue samples were kept at -80°C until the total RNA isolation. TAM and l-dopa were purchased from Sigma. All animal experiments were approved by the Institutional Animal Care and Use Ethics Committee of Taishan University (Permit Number: 2012002) and performed in accordance with the “Guidelines for Experimental Animals” of the Ministry of Science and Technology of China.

2.2. RNA-seq and realtime PCR

Total RNA was extracted from hypothalamus and RNA-seq libraries were prepared using TruSeq RNA sample preparation kit standard protocols (Illumina). Libraries were then sequenced on an Illumina HiSeq 2000 using paired-end chemistry and 100-bp cycles to an average depth of 32 M read pairs/sample. Reads were aligned to *Gallus_gallus-5.0* (<https://www.ncbi.nlm.nih.gov/grc/chicken/>)

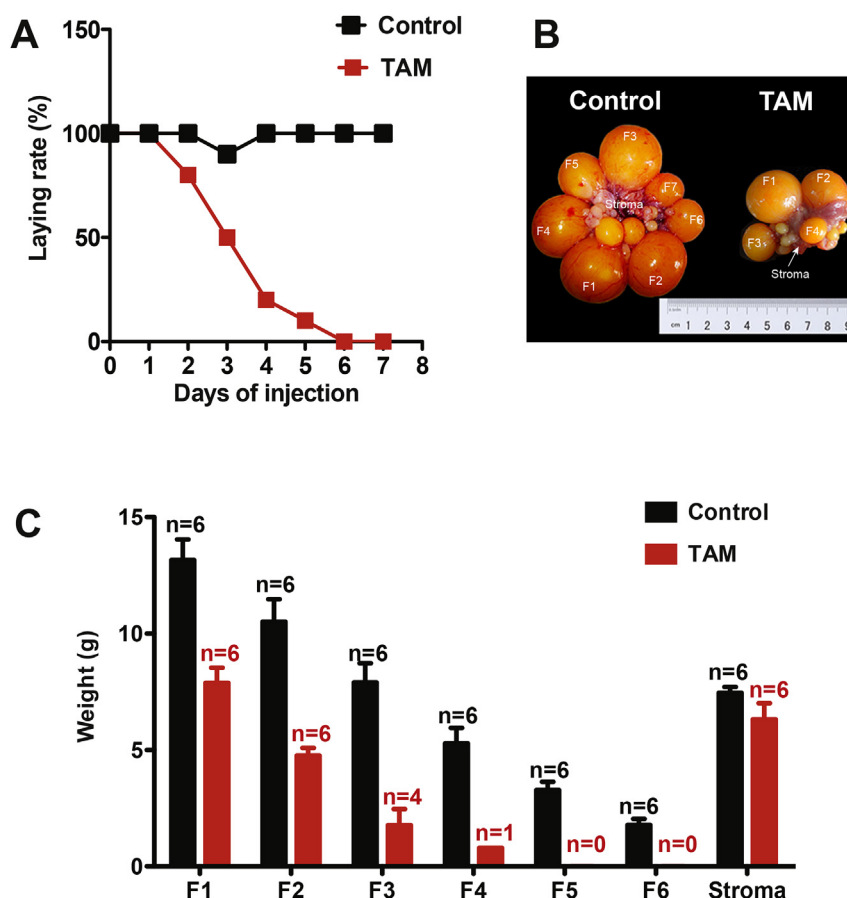


Fig. 1. The effect of tamoxifen on egg-laying rate and follicle development in laying chicken. (A) Tamoxifen (TAM) treatment reduced the egg laying frequency and caused a complete pause at day 6 ($n = 6$). (B) The ovaries from TAM treated and control animals. F1 indicates the largest follicle, F2 the second largest and so on. (C) Both the number and weight (including yolk) of hierarchical follicles were reduced in the ovaries of TAM treated animals. The weight of stroma tissue containing the small white undifferentiated follicles remains unchanged after TAM treatment. “n” indicates the number of each type of follicles (F1–F6) could be identified from all the 6 ovaries of every group. Data were presented as mean $\pm$ SEM.

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