Available online at www.sciencedirect.com

SCIENCE DIRECT®

Theriogenology

Theriogenology 66 (2006) 217–223

www.journals.elsevierhealth.com/periodicals/the

Ovulatory cycle-related alterations in the thecal growth and membrane protein content of thecal tissue of hen preovulatory follicles

Vladimir A. Lebedev[a], Irina Y. Lebedeva[a], Roland Grossmann[a], Tatiana I. Kuzmina[b], Nahid Parvizi[a,*]

[a] *Department of Functional Genomics and Bioregulation, Institute of Animal Science (FAL), Mariensee, 31535 Neustadt, Germany*

[b] *Department of Genetics and Biotechnology, Research Institute for Farm Animal Genetics and Breeding, Pushkin, St. Petersburg 196625, Russia*

Received 6 July 2005; received in revised form 2 November 2005; accepted 4 November 2005

Abstract

In the hen ovary, each preovulatory follicle in the hierarchy, irrespective of its size and the level of its maturity is exposed to the preovulatory LH surge in each ovulatory cycle of an egg laying sequence. In the present study, the thecal weight and membrane protein content of theca layers at different stages of hen ovulatory cycle were assessed. Hens were killed 2 h (stage I), 9 h (stage II), 16 h (stage III), and 23 h (stage IV) after oviposition. The first (F1), second (F2), third (F3), fourth (F4) and fifth (F5) largest yellow follicles were utilized. In all follicles except F1, the thecal weight rose considerably between stages I and III ($P < 0.05$) followed by a slight cessation of the thecal growth at stage IV. The mean content of the theca membrane protein in F1–F5 follicles was lowest at stage III, increasing at stage IV ($P < 0.05$), although, in the case of individual follicles the difference was significant ($P < 0.05$) in F3 follicles only. Estradiol-17β levels in the plasma were lowest (but not significant) at stage III, and a fourfold increase in the plasma progesterone concentration occurred at stage IV. These findings demonstrate for the first time the ovulatory cycle-related alterations in the thecal weight and membrane protein content in the hen preovulatory follicles. Data suggest that the preovulatory rise in ovarian steroid hormones is probably involved in transient termination of the growth and induction of differentiation of the theca in preovulatory follicles as they pass from one category to the next.

Keywords: Hen ovulatory cycle; Preovulatory follicle; Theca layer; Granulosa layer

1. Introduction

The ovary of the domestic hen (*Gallus domesticus*) contains a steadily supplemented pool of 5–7 large yellow follicles (>8 mm in diameter), which vary in size and degree of maturation, permitting them to ovulate successively [1]. Hen ovulatory cycle covers the final 24–27 h of the follicular maturation during which the follicle becomes sensitive to luteinizing hormone (LH) and ovulates after the preovulatory release of LH. Each preovulatory follicle in the hierarchy, however, irrespective of its size and the level of its maturity is exposed to the preovulatory LH surge in each ovulatory cycle of an egg laying sequence [1]. In contrast to mammalian species, a three-cell model for ovarian

---

[*] Corresponding author. Tel.: +49 5034 871163; fax: +49 5034 871247.
E-mail address: nahid.parvizi@fal.de (N. Parvizi).

doi:10.1016/j.theriogenology.2005.11.004

steroidogenesis was established in the hen, with steroidogenic functions of hen follicular structural components being distinct from those in mammals. In hens, granulosa cells do not possess aromatase activity and produce only progesterone [2,3]. Androgen synthesis occurs in interstitial cells of theca interna, and aromatase containing cells, secreting estrogens, are constituents of theca externa [3–5]. Aromatase activity of theca cells declines during hen follicular maturation, while the ability of interstitial cells of the theca to produce androgens decreases only in the two most mature follicles [4,6,7]. In contrast, progesterone secretion is positively correlated to the maturational degree of follicle [8,9]. Furthermore, production of steroid hormones by granulosa and theca layers of preovulatory follicles with different degrees of maturity varies significantly during the ovulatory cycle and correlates with the preovulatory rise in the plasma LH level [7–9].

Previously, we have demonstrated the presence of high-affinity growth hormone (GH)-binding sites in granulosa and theca layers of hen preovulatory follicles, which are regulated in a tissue-specific manner during follicular maturation [10]. We have also shown that the binding capacity and affinity of GH-binding sites in granulosa cells of the first, second and fifth largest yellow follicles increase following the preovulatory LH surge. Binding capacity and affinity of GH-binding underwent, however, no significant changes in the theca layer of preovulatory follicles during the ovulatory cycle.

Therefore, objectives of the present study were (1) to assess changes in the weight and membrane protein content of the thecal tissue of preovulatory follicles during hen ovulatory cycle and (2) to determine relationships between time to expected ovulation of preovulatory follicles and the protein content of thecal tissue and plasma concentrations of estradiol-17β and progesterone.

2. Materials and methods

2.1. Experimental design

White Leghorn hens (LSL) were kept in individual cages under a lighting regimen of 12 h light:12 h dark with free access to food and water. Egg laying was recorded daily for each hen, and a total of 25 birds (41–47-week-old), laying more than seven eggs per sequence were selected for the experiment. Time of oviposition was monitored in 15 min intervals from the beginning of the laying sequence preceding the sequence under study. Time of ovulation was estimated on the basis that it occurs approximately 30 min after oviposition [11]. Under the lighting regimen used, the time lapse between successive ovipositions was 24.4 ± 0.1 h. This time was taken as the duration of the ovulatory cycle, and was divided into four successive stages. Stage I covers processes occurring in preovulatory follicles immediately after ovulation, when the second largest follicle becomes the first follicle, the third largest follicle becomes the second, etc. Stage II spans the time lapse between 8 and 12 h after ovulation, in the course of which the persistent increase in the FSH-binding ability of the theca of all preovulatory follicles proceeds [12]. Stage III directly precedes the preovulatory LH surge, which occurs 4–6 h before ovulation [12]. Stage IV encompasses the period of time between the LH peak and the ovulation. Hens were slaughtered at 2 h (stage I; $n = 6$), 9 h (stage II; $n = 7$), 16 h (stage III; $n = 5$), and 23 h (stage IV; $n = 7$) after oviposition, corresponding to 1.5, 8.5, 15.5, and 22.5 h after assumed ovulation. Hens were killed in the middle of their clutch. All experiments were approved by the regional Animal Ethics Committee. Immediately after slaughtering, blood samples were collected from the heart in heparin-coated tubes. Plasma samples were frozen and stored at −20 °C for estradiol-17β and progesterone assays.

Ovaries were removed and rinsed in saline. The first (F1), second (F2), third (F3), fourth (F4) and fifth (F5) largest yellow follicles were detached from the ovaries. Theca layers were separated as described by Gilbert et al. [13]. The thecal tissues were washed several times in saline and weighed.

2.2. Tissue preparation

Theca tissue was processed as described previously [10]. It was minced with scissors and mechanically homogenized in ice-cold Dulbecco PBS (DPBS; 1:5, w/v) containing 1 mM phenylmethanesulfonyl fluoride. The homogenate was filtered through a screen cup (50 mesh), further homogenized with an ultrasonic cell disruptor (cell disruptor B15; Branson Sonic Power, Schwäbisch Gemünd, Germany), filtered through a screen cup (200 mesh) and centrifuged at 2300 × g for 30 min. The pellet was washed in DPBS and centrifuged again. The pellet volume was brought up to 3 ml by DPBS and an aliquot of the suspension was taken for protein determination by the method of Bradford [14]. All procedures were performed at 4 °C.