Animal Reproduction Science 170 (2016) 38–50

Contents lists available at ScienceDirect

Animal Reproduction Science

journal homepage: www.elsevier.com/locate/anireprosci

Ultrastructural changes of goat corpus luteum during the estrous cycle

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ARTICLE INFO

Article history:
Received 3 November 2015
Received in revised form 31 March 2016
Accepted 1 April 2016
Available online 13 April 2016

Keywords:
Electron microscopy
Steroidogenesis
Angiogenesis
Goat
Endoplasmic reticulum
Mitochondria

ABSTRACT

The present study was designed to study the ultrastructure of goat corpora lutea (CL, $n = 10$) and structural changes as related to steroidogenic functions during the estrous cycle. The reproduction status of goats was estimated by analyzing serum progesterone concentrations. The CL at various stages was surgically collected. To characterize ultrastructural features associated with steroidogenesis, tissue and cellular structures were studied. Blood supplies were examined based on features of the endothelial cells and capillary structures in the CL. Activated endothelial cells and developing vessels were observed in the early stage, whereas mature endothelial cells, accumulating extracellular matrix fibers, and stabilized vessels were observed in the middle and late stages of assessment. In the late stage of assessment, shrunken goat luteal cells scattered around the capillaries were detected and formed circular regression areas. Features of autophagy and luteal cell apoptosis were noted. In large luteal cells, steroidogenic organelles were present, including microvillar channels, endoplasmic reticulum, and mitochondria. Conformational changes in the endoplasmic reticulum and increased mitochondria with tubular cristae were observed in the early-middle CL transitions. In contrast, mitochondria swelled and the cristae transformed to the lamellar type in the late stage, suggesting that organelle plasticity could contribute to steroidogenesis in goat CL. In conclusion, results suggest angiogenesis occurs in early developing CL and programmed cell death occurred in the late stage of CL assessment in the present study. Structures and quantiles of steroidogenic organelles are correlated with the steroidogenic functions in goats.

1. Introduction

Corpora lutea (CL) are endocrine tissues with a limited lifespan in mammals. The ovulation from a mature antral follicle during the estrous cycle results in the formation of CL. During this process, granulosa and theca interna cells of the follicle wall proliferate and differentiate into large

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http://dx.doi.org/10.1016/j.anireprosci.2016.04.001
0378-4320/

luteal cells (LLC) and small luteal cells (SLC), respectively (Devoto et al., 2002; Robker et al., 2009). These changes are further supported by tissue remodeling and angiogenesis (Koga et al., 2000; Kaessmeyer and Plendl, 2009). The CL undergo luteolysis when secretions from the embryo do not occur to the extent there is a signal of pregnancy. During this process, immune responses and programmed cell death promote functional and structural regression of CL (Pate et al., 2012). Functional CL supply progesterone (P_4) to maintain the physiological status of the endometrium, which has an important role in the early stages of pregnancy (Gemmell, 1995).

Endocrine functions of CL are controlled by the hypothalamus-pituitary axis. Tropic hormones such as luteinizing hormone from adenohypophysis activate the expression and function of genes of enzymes that regulate steroidogenisis, which control the generation of P_4 from cholesterol (Bates and Bowling, 2013). In steroidogenic cells, various pathways for the utilization of cholesterol have been identified. Depending on the cell type and animal species, de novo synthesis, endocytosis, or selective uptake pathways might be included (Hu et al., 2010). In CL, cholesterol is activated in storage pools and transported into mitochondria by the steroidogenic acute regulatory protein complex (Stocco, 2001). The side chain cleavage enzyme CYP11A1 converts cholesterol into pregnenolone in the mitochondrial inner membrane (Simpson, 1979). The P_4 is subsequently synthesized through pregnenolone hydroxylation by hydroxyl-delta-5-steroid dehydrogenase at the endoplasmic reticulum (ER; Simard et al., 2005). The structural changes in CL during the estrous cycle have been studied in primates and rodents (Del Canto et al., 2007; Hernandez et al., 2009; Choi et al., 2011). Within cells, steroidogenic functions have been correlated with ultrastructural changes in the ER and mitochondria (Reaven et al., 1998; Duarte et al., 2012). However, studies with various species in assessing CL structure and function could contribute to clarifying additional mechanisms and providing further applications for control of CL.

The establishment of ultrastructural image libraries is a fundamental way to explore organisms, where detailed structural changes can be visualized. The ultrastructure of CL has been investigated in many species (Levine et al., 1979; Garcia Iglesias et al., 1992; Fraser et al., 1999; Gajecka et al., 2008). As compared with other farm animals, the onset of breeding seasons in goats occurs during shortening photoperiods with spontaneous and continuous estrous cycles during the breeding season (Fatet et al., 2011). As a small ruminant, goats have one or two functional CL that can be obtained for study during each estrous cycle. In literatures, the ultrasonographic studies have revealed the dynamic changes of goat CL in volume and texture (Arashiro et al., 2010a), and the ultrastructure of luteal cells has been studied (Gemmell et al., 1977; O'Shea, 1987). However, the dynamic changes remain to be explored through ultrastructural resolution. Because the structural changes could occur during the lifespan of CL and be associated with steroidogenesis, the present study was designed to clarify the developments of CL during the estrous cycle with ultrastructural assessments in goats.

2. Material and methods

2.1. Animals

Adult crossbreed does from Saanen and Alpine ($n = 8$, total 10 ovaries were collected) were housed on the Experimental Farm of National Taiwan University. Animals were maintained on natural dark-light cycles, and water and grass were available *ad libitum*. All experiments were conducted in accordance with the Guide for Care and Use of Laboratory Animals and were approved by the Research Ethics Office of National Taiwan University. Blood sample collections began in February and occurred three times per week to monitor estrous cycles. The nadir point of P_4 in the cycle was regarded as Day 0 and goats with consistent serum cycles of P_4 concentrations (at least two continuous cycles) were chosen for the study. Considering the changes of CL could be continuous in the estrous cycle, the ovaries were surgically sampled on specific days including Day 4 ($n = 2$), 8 ($n = 1$), 10 ($n = 1$), 12 ($n = 2$), 14 ($n = 2$), 16 ($n = 1$), and 18 ($n = 1$) of the estrous cycle.

Before surgery, animals were fasted and isolated from other animals for 12 h. Sedation was established by intravenous (IV) xylazine injections. Inhalation anesthesia was maintained using 1% to 3% isoflurane in mixed gas of oxygen and nitric oxide (2:1). The ovaries were bisected and the appearances of CL was assessed for stage classifications. The CL samples were dissected from ovaries and bisected immediately in precooled fixatives. For each sample, one half was immersed in 10% (v/v) formalin (in PBS, pH 7.4) for paraffin sections. The other half was diced into 1 mm^3 cubes and immersed in 2.5% glutaraldehyde (in 0.1 M phosphate buffer, pH 7.3) for electron microscopic preparations. After sampling and cauterization, the subcutis and skin were sutured. For recovery, inhalation anesthesia was removed and the tolazoline (0.5 mg/kg BW) was administered (IV). The Amoxicillin/clavulanic acid (8.75 mg/kg BW, intramuscular) and the Flunixin meglumine (2 mg/kg BW, IV) were given at standing. The antibiotic and analgesic were injected once a day for consecutive 4 days.

2.2. Enzyme immunoassays (EIA)

Serum P_4 concentrations were determined using the direct competitive enzyme immunoassays (EIA, Wu et al., 2000). Briefly, Sample aliquots (50 μl) of diluted buffer and horseradish peroxidase-linked P_4 conjugate (150 μl) were added to microtiter plates coated with 200 μl P_4 antibody (1:40,000 dilution). After incubation for 15 min at room temperature with gentle shaking, plates were washed three times with Tween-20 in 0.01 M PBS (pH 7.0). Color was developed using 200 μl of 3.7 mM o-phenylenediamine in 0.03% H_2O_2 for 20 min. Reactions were stopped by the addition of 50 μl sulfuric acid. Absorbance was determined using a dual wavelength reader at 490/630 nm and compared with P_4 standard curves. Coefficients of variation, within and between assays, were 7% and 12%, respectively. The sensitivity of the assay was 0.3 pg/ml. All standards and samples were assayed in duplicate. The sample dilution ratios were