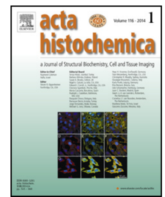
Acta Histochemica 118 (2016) 553–559

Contents lists available at ScienceDirect

Acta Histochemica

journal homepage: www.elsevier.de/acthis



Lipid droplets in cultured luteal cells in non-pregnant sheep fed different planes of nutrition

Vilaivan Khanthusaeng [b], Jiratti Thammasiri [b], Casie S. Bass [a], Chainarong Navanukraw [b], Pawel Borowicz [a], Dale A. Redmer [a], Anna T. Grazul-Bilska [a,*]

[a] Department of Animal Sciences, North Dakota State University, Fargo, ND 58108, USA
[b] Agricultural Biotechnology Research Center for Sustainable Economy (ABRCSE), Department of Animal Science, Faculty of Agriculture, Khon Kaen University, Khon Kaen 40002, Thailand

ARTICLE INFO

Article history:
Received 28 January 2016
Received in revised form 4 May 2016
Accepted 23 May 2016

Keywords:
Lipid droplets
BODIPY
Luteal cells
Ovary
Sheep

ABSTRACT

Accumulation of lipid droplets (LD) in luteal cells likely is important for energy storage and steroidogenesis in the highly metabolically active corpus luteum (CL). The objective of this study was to determine the effect of plane of nutrition on progesterone (P4) secretion, and lipid droplet number and size in cultured ovine luteal cells. Ewes were randomly assigned to one of three nutritional groups: control (C; 100% NRC requirements, n = 9), overfed (O; 2 × C, n = 12), or underfed (U; 0.6 × C, n = 10). Superovulation was induced by follicle stimulating hormone injections. At the early and mid-luteal phases of the estrous cycle, CL were dissected from ovaries, and luteal cells isolated enzymatically. Luteal cells were incubated overnight in medium containing serum in chamber slides. Media were then changed to serum-free and after 24 h incubation, media were collected for P4 analysis, and cells were fixed in formalin and stained with BODIPY followed by DAPI staining. Z-stacks of optical sections of large and small luteal cells (LLC and SLC, respectively) were obtained using a laser-scanning microscope. Rendered 3D images of individual LLC and SLC were analyzed for cell volume, and total and individual LD volume, number and percentage of cellular volume occupied by LD by using Imaris software. Concentrations of P4 in serum and media were greater ($P < 0.05$) at the mid than early-luteal phase, and were not affected by nutritional plane. LD total volume and number were greater ($P < 0.001$) in LLC than SLC; however, mean volume of individual LD was greater ($P < 0.02$) in SLC than LLC. In LLC, total LD volume was greater ($P < 0.02$) in O than C and U ewes. In SLC, total LD volume and number was greater ($P < 0.003$) at the mid than early-luteal phase, and percentage of cell volume occupied by LD was greater ($P < 0.002$) in U than C and O ewes. These data demonstrate that both stage of luteal development and nutritional plane affect selected LD measurements and thus may affect luteal functions. Furthermore, these data confirm that LD dynamics differ among parenchymal steroidogenic luteal cell types.

© 2016 Elsevier GmbH. All rights reserved.

1. Introduction

Through secretion of progesterone (P4), corpora lutea (CL) control reproductive cyclicity and preparation of the uterus for potential pregnancy in mammalian species (Niswender et al., 1994; Niswender, 2002; Miyamoto et al., 2009; Shirasuna et al., 2012; Stouffer et al., 2013). The CL consists of several cell types including parenchymal steroidogenic large and small luteal cells (LLC and SLC, respectively), and non-parenchymal cells including endothelial cells, pericytes, fibroblast, blood cells and others (Milvae et al., 1996; Vonnahme et al., 2006; Devoto et al., 2009). Parenchymal LLC and SLC have been well characterized for several species demonstrating different steroidogenic activity, responsiveness to luteinizing hormone (LH), morphology and other features in ruminants and other species (O'Shea et al., 1986, 1989; Niswender and Nett, 1994). Furthermore, it has been shown that luteal cells contain large numbers of lipid droplets (LD; O'Shea et al., 1986, 1989, 1990; Fields et al., 1992; Fields and Fields, 1996; Towns et al., 1999); but the function of LDs in luteal cells has not been investigated in detail. Moreover, several studies demonstrated that selected functions of CL, including P4 secretion, can be affected by nutritional plane (O'Callaghan and Boland, 1999; Kaminski et al., 2015). However, the effects of diet on LDs in the CL have not been investigated for any species.

* Corresponding author.
E-mail address: Anna.Grazul-Bilska@ndsu.edu (A.T. Grazul-Bilska).

http://dx.doi.org/10.1016/j.acthis.2016.05.007
0065-1281/© 2016 Elsevier GmbH. All rights reserved.

Lipid droplets are cellular organelles serving as an energy reservoir as well as mediators in physiological and pathological conditions (Farese and Walther, 2009; Murphy et al., 2009; Beller et al., 2010; Digel et al., 2010; Greenberg and Coleman, 2011; Greenberg et al., 2011; Zechner et al., 2012; Konige et al., 2014; Ohsaki et al., 2014; Previs et al., 2014; Wilfling et al., 2014). Lipid droplets contain numerous molecules including fatty acids as reservoirs for energy substrates, and cholesterol esters and cholesterol for membrane biosynthesis and steroid production (Beller et al., 2010; Brasaemle and Wolins, 2012). In addition, LDs have an ability to protect cells from lipotoxicity (Farese and Walther, 2009). Associated with LDs are numerous proteins including the family of perilipins (PLIN), cell death–inducing DNA fragmentation factor 45-like effector (CIDE), hormone-sensitive lipase (HSL) and others that play regulatory roles in steroidogenesis and lipid metabolism (Londos et al., 1995; Beller et al., 2010; Zechner et al., 2012; Yang et al., 2012; Konige et al., 2014; Manna et al., 2015). Thus, it has been postulated that LDs are highly dynamic, heterogeneous cellular organelles interacting with other organelles including endoplasmic reticuli, mitochondria and peroxisomes (Murphy et al., 2009; Beller et al., 2010; Digel et al., 2010; Walther and Farese, 2012).

A variety of methodologies have been used to identify LDs in cells and tissues including electron, confocal or light microscopy, histological binding methods (e.g., oil red O, Nile red, BODIPY), and/or immunohistochemistry to detect proteins associated with LDs (e.g., PLIN) in different organs (Straub et al., 2008, 2013; Digel et al., 2010; Ohsaki et al., 2010, 2014; Walther and Farese, 2012; Crunk et al., 2013; Fujimoto et al., 2013; Suzuki et al., 2013; Klymchenko and Kreder, 2014). Application of image analysis allows for quantitative evaluation of LDs in a cell or tissue (Straub et al., 2008; Prats et al., 2013). In this study, we generated confocal 3D images of cultured luteal cells stained using BODIPY followed by image analysis to determine several measurements of a cell and corresponding LDs.

Although luteal functions have been studied intensively, very little is known about importance of LDs in the CL for any species. We hypothesized that selected measurements of LDs will differ in LLC and SLC, and will be affected by the stage of the estrous cycle and plane of nutrition. The objectives of this study were to optimize and validate novel method of LD expression in cultured luteal cells, and determine the total and individual volume and number of LDs in cultured LLC and SLC from the early and mid-luteal phases of the estrous cycle from ewes fed control, excess and restricted diets.

2. Materials and methods

2.1. Animal and experimental design

All animal procedures were approved by the North Dakota State University (NDSU) Institutional Animal Care and Use Committee (#A12013). The study was initiated during the normal breeding season in August and finished in December.

Non-pregnant, non-lactating Rambouillet ewes between 3 and 5 years of age and of similar genetic background were individually penned at the Animal Nutrition and Physiology Center on the NDSU campus. Ewes were stratified by weight and randomly assigned into one of three dietary treatments: control (C; n = 9) received a maintenance diet (100% NRC requirements; 2.4 Mcal of metabolizable energy [ME]/kg diet dry matter), overfed (O; n = 12) received 200% NRC requirements, and underfed (U; n = 10) received 60% NRC requirements 60 days prior to the onset of the estrous cycle (day 0). Diet composition is provided in Table 1. Ewes were fed half of their individual diet daily at 0800 and the remaining half at 1500, and all portions were consumed. For the duration of the experiment, ewes were weighed and body condition score (BCS) was determined weekly (Kaminski et al., 2015). Diets were adjusted weekly for each ewe to ensure the proper nutritional plane was achieved at day 0, and maintained throughout the estrous cycle until completion of experiment at the early (day 5) or mid (day 10)-luteal phases of the second estrous cycle. The nutritional treatment has been described in detail before (Grazul-Bilska et al., 2015; Kaminski et al., 2015).

Estrus was synchronized by insertion of a controlled internal drug release (CIDR) device for 14 days. Approximately 36 h after removal of the CIDR ewes were considered in estrus and treated as day 0 of the estrous cycle (Kaminski et al., 2015). Ewes were injected twice daily (morning and evening) with follicle stimulating hormone (FSH-P; Sioux Biochemical, Sioux Center, IA, USA) on days 13, 14 and 15 of the estrous cycle (5, 4 and 3 mg/injection, respectively; Grazul-Bilska et al., 1991, 2001).

2.2. Tissue and blood collection

At the early and mid-luteal phases of the estrous cycle, ovaries were collected, immersed in phosphate buffered saline (PBS) and transported to the laboratory. Then, CL were dissected from ovaries, and minced for enzymatic digestion as previously described (Grazul-Bilska et al., 1991, 2001). Blood samples were also collected at the early and mid-luteal phases, centrifuged (20 min at 1500 g), and serum was stored at -20° C until P4 analysis.

2.3. Dissociation, culture and imaging of luteal cells

Minced luteal tissues were incubated in dissociation medium consisting of Hank's Balanced Salt Solution (HBSS; Gibco, Grand Island, NY, USA) supplemented with collagenase type 4 (0.1% wt/vol; Worthington, Lakewood, NJ, USA), bovine serum albumin (BSA; 2%, wt/vol; Sigma, St. Louis, MO), penicillin/streptomycin (P/S; 100 U/mL/100 μg/mL; Gibco) in a shaking (100 cycles/min) water bath at 37C in a capped 50-mL Erlenmeyer flasks. After initial 15–20 min incubation, medium containing dispersed luteal cells was aspirated, and approximately 3 mL of fresh dissociation medium was added to the remaining tissue. Dissociation medium was then aspirated and replaced every 7–10 min for ~10 incubations. This process was continued for approximately 2–3 h. At the end of collection, medium containing dispersed luteal cells was centrifuged (600g) for 10 min. The pellet was washed three times with HBSS containing P/S (as described above) and then resuspended in the same medium via trituration with a siliconized Pasteur pipette. The cell suspension was filtered through a sterile nylon filter with 50 μm pores (Tetko, New York, NY, USA) to remove pieces of tissue and then centrifuged (600g; 10 min). Cells were resuspended in Dulbecco's Modified Eagle Medium (DMEM; Gibco) plating medium containing 10% (vol/vol) calf serum (CS; Gibco), 10% fetal bovine serum (FBS; Gibco) and P/S. Luteal cells were counted using a hemocytometer and viability was evaluated by trypan blue (Gibco) exclusion. The viability of freshly dispersed luteal cells was $87.1 \pm 2.4\%$ and the proportion of LLC in the total steroidogenic cell population was $8.3 \pm 1.6\%$ for early and mid-luteal phases for all nutrition treatments.

Cells were plated in 8-chamber plastic slides (Ibidi GmbH, Martinsried, Germany) at a concentration of 75,000 cells/well/0.5 mL in DMEM medium containing 10% CS, 10% FBS and P/S. After overnight incubation, medium was changed to serum-free DMEM and cells were cultured for 24 h. Then, medium was collected for P4 analysis and cells were fixed in 3% formalin followed by BODIPY 493/503 staining (1 μg/mL of PBS; Molecular Probes, Eugene, OR) for 20 min (Ohsaki et al., 2010; Spangenburg et al., 2011). Nuclei were stained using DAPI solution (300 mM; Molecular Probes,). Optical sections including whole LLC or SLC were obtained using a confocal laser-scanning microscope (Zeiss LSM700, Zeiss Inc., Thornwood, NY)

Download English Version:

https://daneshyari.com/en/article/1923283

Download Persian Version:

https://daneshyari.com/article/1923283

Daneshyari.com