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Expression of genes for enzymes synthesizing lysophosphatidic acid, its receptors and follicle developmental factors derived from the cumulus-oocyte complex is dependent on the ovarian follicle type in cows

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ABSTRACT

Cumulus-oocyte complexes (COCs) release factors potentially involved in follicular growth and development, such as growth and differentiation factor 9 (GDF9), bone-morphogenetic protein 15 (BMP15), follistatin (FST) and cathepsins (CTSs). Moreover, the quality of the oocytes and follicles may be related to both the lipid composition of the follicle cells and follicular fluid. One of the lipids, locally regulating the reproductive functions in ovaries of cattle, is lysophosphatidic acid (LPA). In this study, the expression was investigated of the genes for LPA and other factors in COCs of follicles at different stages of development and regression. The relative abundances of mRNA were determined by real-time PCR for receptors for LPA (LPARs), enzymes synthesizing LPA (autotaxin (AX) and phospholipase A2 (PLA2)), BMP15, GDF9, CTSZ, CTSB and FST in COCs isolated from healthy, transitional and atretic follicles. The expression of genes for the LPARs, AX, PLA2 and the factors involved in follicular development in cattle COCs is follicle-type dependent. Greater expression of LPAR1-3 and AX genes were detected in the healthy follicles compared to the atretic and transitional follicles ($P < 0.05$). The relative abundance of GDF9, BMP15, CTSZ and CTSB was also greater in COCs from healthy follicles than from transitional and atretic follicles ($P < 0.05$). It is postulated that the greater expression of LPARs and AX genes in healthy follicles compared with atretic follicles indicates an enhanced role of LPA in follicular development. Results of the present study also suggest the regulatory role of factors derived from the COCs in the growth and development of follicles.

1. Introduction

The ovarian follicle is the functional unit of the ovary, in which the somatic (theca and granulosal cells) and germ (oocyte) components are in close proximity to each other and have interdependent functions (Moenter et al., 1992; Aerts and Bols, 2010). The ovarian follicular microenvironment and maternal signals, which are mediated primarily through granulosal and cumulus cells, are

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responsible for nurturing the oocyte growth and gradual acquisition of developmental competence (Gilchrist et al., 2008). Additionally, the cumulus-oocyte complexes (COCs) release factors that are involved in follicular growth and development, such as members of the transforming growth factor beta (TGFβ) superfamily (Bodensteiner et al., 1999). Furthermore, the oocyte quality may be closely related to both oocyte and the follicular fluid lipid composition (Lapa et al., 2011; Prates et al., 2013). Among the lipids that regulate female reproductive functions is lysophosphatidic acid (LPA). It has been documented that LPA is responsible for modulation of prostaglandin (PG) synthesis and secretions in the endometrium and progesterone from the corpus luteum (Ye et al., 2005; Woclawek-Potocka et al., 2010). The endogenous LPA also positively affected conception rates of cattle (Woclawek-Potocka et al., 2010). In the granulosal and thecal cells of cattle, expression of genes for the receptors of LPA and enzymes regulating the production of LPA correlate with factors involved in follicular growth and development (Sinderewicz et al., 2018). *In vitro* studies have been conducted that indicate LPA increased oocyte maturation rates and cumulus cell expansion and decreased apoptosis in COCs of cattle (Boruszewska et al., 2015; Zhang et al., 2015). The expression of genes for LPA in COC's derived from the different follicle types have not previously been investigated. Considering the regulatory role of LPA in the reproductive tract of cattle, the follicle-type-dependent gene expression and role of LPA in granulosal and thecal cells and the interdependence of all follicular components, the aim of the present study was to investigate the expression of LPA genes in COCs of cattle originating from different follicle types (healthy, transitional and atretic). In this study, there was also assessment of whether oocyte quality markers (follistatin (FST), growth and differentiation factor 9 (GDF9), bone morphogenetic protein 15 (BMP15), and the cysteine proteinases-cathepsins (CTSB and *CTSZ*)) gene expression is follicle-type-dependent. Furthermore, it was hypothesized that expression of the genes for the enzymes regulating LPA production, receptors for LPA and factors derived from cumulus-oocyte complexes is follicle-type-dependent.

2. Materials and methods

2.1. Collection of the ovaries

All experimental procedures were approved by the Local Animal Care and Use Committee in Olsztyn, Poland (Agreement No. 34/2012/N). Ovaries of cattle, without consideration of the stage of the estrous cycle, were collected at a local abattoir and transported to the laboratory in sterile phosphate buffered saline (PBS buffer (137 mM NaCl, 2.7 mM KCl, 10 mM Na2KPO4, 1.8 mM KH2PO4, pH = 7.4)), supplemented with 0.4% gentamycin (Sigma Aldrich, Saint Louis, MO, USA; #G-1397), within 40 min.

2.2. Collection of the experimental material

The follicular fluid was aspirated using a syringe from a single, subordinate ovarian follicles (diameter < 5 mm). Based on the intra-follicular estradiol:progesterone (E2:P4) ratio (according to Grimes and Ireland, 1986), the ovarian follicles were divided into three categories: healthy (E2:P4 > 1), transitional (0.01 < E2:P4 < 1) and atretic (E2:P4 < 0.01). The E2 and P4 concentrations were measured in the follicular fluid using the RIA method (with, respectively, the DIAsource E2–RIA–CT Kit (sensitivity: 2.7 pg/mL, intra-assay precision: CV = 5.6%, inter-assay precision: CV = 10.4%), KIP0629, Diasource and the DIAsource PROG–RIA–CT Kit (sensitivity: 0.05 ng/mL, intra-assay precision: CV = 5.2%, inter-assay precision: CV = 8.6%), KIP1458, Diasource, Ottignies-Louvain-la-Neuve, Belgium). After collection of the follicular fluid, the antral cavity of each follicle was flushed with cold PBS to recover the COCs under the stereoscopy microscope (SZX10, Olympus, Poland). The COCs, obtained from single follicles, categorized as healthy (n = 12), transitional (n = 12) or atretic (n = 12) were used for the study. The samples were collected in Extraction Buffer (#KIT0204, Arcturus PicoPure RNA Isolation Kit, Applied Biosystems, Life Technologies, Carlsbad, CA, USA) and stored at −80 °C until the RNA extraction process.

2.3. Total RNA extraction, reverse transcription (RT) and real-time PCR

The samples were processed for RNA isolation according to manufacturer's instructions (#KIT0204, Arcturus PicoPure RNA Isolation Kit, Applied Biosystems, Life Technologies, Carlsbad, CA, USA). DNase treatment was performed to remove genomic DNA contamination using RNase-free DNase Set (#79254, Qiagen, Hilden, Germany). Samples were stored at −80 °C until reverse transcription procedures were conducted. Before use, the RNA content and quality were evaluated by spectrophotometric measurements. Reverse transcription (RT) was performed using oligo (dT)12–18 primers (#18418-012, Thermo Scientific, Carlsbad, CA, USA) by Super Script III reverse transcriptase (#18080-044, Thermo Scientific, Carlsbad, CA, USA) in a total volume of 20 µL. The RT products were stored at −20 °C until real-time PCR amplification.

The relative abundance of mRNA for the enzymes responsible for LPA synthesis (phospholipase A2 – PLA2 and autotaxin – AX), receptors for LPA (LPAR1–LPAR4) and oocyte quality markers (BMP15, GDF9, FST, CTSB, CTSZ) was measured by real-time PCR using specific TaqMan probes. Real-time PCR was performed with an ABI Prism 7900 (Applied Biosystems, Life Technologies, Carlsbad, CA, USA) sequence detection system using Maxima Probe/ROX qPCR Master Mix (#K0231, Thermo Scientific, Carlsbad, CA, USA). The PCR reactions were performed in 384-well plates. The mRNA transcription results were normalized to the β-actin (ACTB, an internal control) amount of mRNA and expressed as arbitrary units. The housekeeping gene was chosen using the NormFinder software by comparing the following two candidate genes: GAPDH and β-actin (Andersen et al., 2004). The primers and probes were designed using an online software package (http://frodo.wi.mit.edu/primer3/input.htm). The data for primer sequences and the sizes of the amplified fragments of all of the transcripts are included in Table 1. For the relative abundance quantification of mRNA, the Miner software was used, based on the kinetics of individual PCR reactions. The Miner algorithm allows direct calculation