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Detection of genes associated with developmental competence of bovine oocytes

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ABSTRACT

The developmental competence of oocytes is acquired progressively during folliculogenesis and is linked to follicular size. It has been documented that oocytes originating from larger follicles exhibit a greater ability to develop to the blastocyst stage. The differences in cytoplasmic factors such as mRNA transcripts could explain the differences in oocyte developmental potential. We used bovine oligonucleotide microarrays to characterize differences between the gene expression profiles of germinal vesicle stage (GV) oocytes with greater developmental competence from medium follicles (MF) and those with less developmental competence from small follicles (SF). After normalizing the microarray data, our analysis found differences in the level of 60 transcripts (≥1.4 fold), corresponding to 49 upregulated and 11 downregulated transcripts in MF oocytes compared to SF oocytes. The gene expression data were classified according to gene ontology, the majority of the genes were associated with the regulation of transcription, translation, the cell cycle, and mitochondrial activity. A subset of 16 selected genes was validated for GV oocytes by quantitative real-time RT-PCR; significant differences ($P < 0.01$) were found in the level of *TAF1A*, *MTRF1L*, *ATP5C1*, *UBL5* and *MAP3K13* between the MF and SF oocytes. After maturation the transcript level remained stable for *ATP5F1*, *BRD7*, and *UBL5* in both oocyte categories. The transcript level of another 13 genes substantially dropped in the MF and/or SF oocytes. It can be concluded that the developmental competence of bovine oocytes and embryos may be a quantitative trait dependent on small changes in the transcription profiles of many genes.

1. Introduction

Although basic research on the reproduction of domestic ruminants has intensified over the past two decades, the efficiency of embryo in vitro production is still very low, especially in the bovine. Follicular somatic cells and their internal environment significantly affect the ability of the oocyte to complete maturation and acquire developmental competence. It was demonstrated that the meiotic (Fair et al., 1995) competence of oocytes (Lequarre et al., 2005; Machatkova et al., 2004) correlates with the size of

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the oocyte, which is associated with increasing diameter of the follicle (Arlotto et al., 1996).

During oogenesis, oocytes acquire in concordance with folliculogenesis the ability to mature. Almost 80% of fully grown oocytes in antral follicles are able to mature. In contrast, a low percentage of oocytes are developmentally competent. Only 40–60% of recovered oocytes support embryo development to the blastocyst stage (Lonergan and Fair, 2008).

The developmental competence of oocytes is affected by many factors—e.g., follicle size, the phase of follicular growth, the oestrus cycle and the quality of cumulus cells surrounding the oocyte (Hagemann et al., 1999; Hendriksen et al., 2000). Several groups reported that overall, oocytes isolated from small antral follicles are considered to be less developmentally competent than oocytes isolated from large follicles, probably due to the influence of the dominant follicle (Hagemann et al., 1999; Machatkova et al., 2004). Oocytes originating from medium (≥6 mm) or larger follicles have greater developmental competence than oocytes isolated from small follicles (3–4 mm) (Pavlok et al., 1992). Lonergan et al. (1994) obtained twice as many blastocysts by in vitro maturation and culture from oocytes isolated from follicles greater than 6 mm than from oocytes from 2 to 6 mm follicles. Another study of three research teams also confirmed the positive relationship between follicle size and developmental competence (Lequarre et al., 2005).

The transcriptional activity of an oocyte changes during its growth within the follicle. Growing oocytes in early secondary follicles are transcriptionally active, as proved by the synthesis of heterogeneous nuclear and ribosomal RNAs (Fair et al., 1997). During its growth phase, the oocyte accumulates mRNA and proteins essential for the completion of meiosis, fertilization and early embryonic development. At the onset of germinal vesicle break down (GVBD), transcription is reduced to the detection limit and is no longer detectable in MII oocytes (Tomek et al., 2002). The overall transcriptional profile reflects the functional state of the cytoplasm (Kanka et al., 2012).

The vast majority of our knowledge of the genes required during the normal development of oocytes was obtained recently by using microarray technology. Researchers made an effort to find differentially expressed genes between GV and MII and oocytes (Fair et al., 2007; Katz-Jaffe et al., 2009; Mamo et al., 2011), oocytes isolated from small and large antral follicles (Lequarre et al., 2005) or oocytes matured either in vivo or in vitro oocytes (Katz-Jaffe et al., 2009; Mamo et al., 2011).

The aim of our study was to determine if there are differences between diverse meiotically and developmentally competent oocytes. We employed bovine oligonucleotide microarrays to compare gene expression profiles of healthy oocytes isolated either from medium (MF, 6–10 mm) or small (SF, 2–5 mm) follicles at the germinal vesicle stage. Further, we focused on the expression of 16 selected genes to analyze the changes during oocyte maturation. Gene expression profiles during early embryo development were also followed for 9 selected transcripts.

2. Materials and methods

2.1. Microarray experiments

2.1.1. Oocyte collection

Slaughtered Holstein dairy cows, aged 4–6 years, were used as oocyte donors. The oocytes from medium (MF; 6–10 mm) and small (SF; 2–5 mm) follicles were collected by aspiration and slicing, respectively, and categorized according to Blondin and Sirard (1995). Only healthy oocytes (homogenous cytoplasm without dark spots and compact homogenous cumuli) were selected for microarray analysis. Cumulus cells were removed mechanically by gentle pipetting. Oocytes were washed in PBS, frozen and stored at −80 °C.

2.1.2. Microarray analysis

2.1.2.1. Experimental design of microarray experiment. Two samples of MF and SF oocytes, each represented by three independently prepared biological replicates (pools of 20 oocytes) with two dye-swap technical replicates for each biological replicate were hybridized to six bovine oligonucleotide microarrays (Missouri Consortium, http://www.ag.arizona.edu/microarray/BOM.html) as follows: each biological replicate (six independent pooled samples) was labeled with AlexaFluor 647 or AlexaFluor 555 (MF or SF, resp., Invitrogen, CA) and 'dye-swap' method with reversed labeling was performed.

2.1.2.2. Isolation of mRNAs for microarray and antisense RNA amplification. Poly(A)+mRNAs were extracted from oocytes using the Dynabeads mRNA DIRECT Micro Kit (Life Technologies, Norway) according to the manufacturer's instructions. The mRNA was eluted from Dynabeads in 20 μL of 10 mM Tris–HCl after heating the samples at 85 °C for 2 min. Just before analysis the volume was adjusted to 40 μL with RNase free H_2O. The quality and concentration of the RNA was assessed by a NanoDrop 1000 spectrophotometer (NanoDrop Technologies, DE) and RNA integrity was confirmed by bioanalysis on Agilent 2100 Bioanalyzer (Agilent Technologies, Inc., CA). Amplified RNA (aRNA) was prepared with two round amplification protocol using Amino Allyl MessageAmp ™II aRNA Amplification Kit (Ambion, TX). In subsequent labeling reaction, 5 μg of aRNAs were labeled with Alexa Fluor 555 or 647 dyes (Invitrogen, CA). Labeled aRNA were purified using Pico Pure RNA isolation Kit (Arcturus Bioscience, Inc., CA) according to the manufacturer's instructions.

2.1.2.3. Probe fragmentation and hybridization. Microarray slides were rehydrated for 10 s above water bath at 50 °C, dried on the heat block for 5 s at 65 °C and cooled for 1 min at RT. This procedure was repeated 4×. UV cross-linking was performed by exposing rehydrated slides to 180 mJ of UV radiation. After crosslinking the slides were washed in 0.1% SDS with constant gently mixing for 5 min followed by a rinse in ddH_2O and 3 min incubation in 100% ethanol with constant gently mixing, all steps at RT. Slides were dried by centrifugation for 4 min at 2000 × *g*. Just before use the microarray slides were pre-hybridized at 42 °C for