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ScienceDirect
Theriogenology 71 (2009) 939–946
Theriogenology
www.theriojournal.com

Transcriptome analysis of in vivo and in vitro matured bovine MII oocytes

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Received 22 July 2008; received in revised form 7 October 2008; accepted 19 October 2008

Abstract

In vitro maturation (IVM) of mammalian oocytes does not support the same rates of embryo development or pregnancy when compared to oocytes that have matured in vivo. Therefore, environment has a significant influence on the oocyte's ability to complete maturation and acquire the mRNA and proteins required for successful fertilization and normal embryonic development. The aim of this study was to analyze the MII oocyte transcriptome between in vivo and in vitro conditions. Total RNA was extracted, processed and hybridized to the Affymetrix GeneChip® Bovine Genome Array. Following normalization of the microarray data, analysis revealed 10 differentially expressed genes after IVM compared to in vivo matured controls, including *Aqp3*, *Sept7*, *Abhd4* and *Siah2* ($P < 0.05$). K-means cluster analysis coupled with associated gene ontology, identified several biological processes affected by IVM, including metabolism, energy pathways, cell organization and biogenesis, and cell growth and maintenance. Quantitative real-time PCR validated the microarray data and also revealed altered expression levels after IVM of specific putatively imprinted genes, *Igf2r*, *Peg3* and *Snrpn* ($P < 0.05$). Distinct IVM transcription patterns reflected the oocyte's response to its surrounding environment. Monitoring transcription levels of key oocyte maturation genes may subsequently assist in improving IVM success.
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Keywords: Transcriptome; Bovine oocyte; In vivo; In vitro maturation

1. Introduction

The mammalian oocyte contributes more than just DNA to the subsequent embryo. During oogenesis, maternal transcripts are produced and accumulated to facilitate the completion of meiosis, fertilization, decondense the sperm head, carry out the early embryonic mitotic divisions and activate the embryonic genome.

The follicle and its environment strongly influence the oocyte's ability to complete maturation and acquire developmental competence. Consequently the quality of the oocyte is directly related to the developmental potential of the resulting embryo [1]. Oocyte developmental competence has been positively associated with both the size of the antral follicle [2,3] and stage of follicular wave [3,4]. Oocyte growth coincides with the synthesis of maternal mRNAs and proteins in order to sustain mitotic cleavage divisions during early embryonic development, prior to activation of the embryonic genome [5]. Several studies have shown specific maternal transcripts that are associated with oocyte potential [6–8].

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0093-691X/$ – see front matter © 2009 Elsevier Inc. All rights reserved.
doi:10.1016/j.theriogenology.2008.10.024

Examination of developmentally competent and incompetent oocytes has revealed a significant relationship with follicle size. Candidate genes showing an association with developmental competence included the transcription factor Oct4, H2A which contributes to chromatin support, as well as CKS1B, a component of the CDC28–cyclin complex [6,8]. Bovine oocytes that are incapable of completing this process and acquiring the complete synthesis of mRNAs and proteins have been shown to have lower developmental competence [9].

The efficiency of blastocyst production in cattle using in vitro matured oocytes remains relatively low in comparison to oocytes matured in vivo [10,11]. Ongoing research to develop more efficient in vitro maturation (IVM) procedures has focused on the effects of specific exogenous substrates and factors and then determining specific endpoints, such as MII formation, fertilization and subsequent embryo development [12,13]. Although such endpoints are very useful, they provide little insight into the molecular or physiological impact of the variable under investigation. Gene expression analysis provides valuable information regarding the transcriptional activity of the bovine oocyte genome [14–17]. Even though mRNA transcription ceases after germinal vesicle breakdown (GVBD), differences in the global transcriptome between GV and MII stage bovine oocytes have been reported, representing stability, utilization and degradation of mRNA transcripts during the completion of oogenesis [17].

The aim of this study was to assess global gene expression in IVM and in vivo matured bovine oocytes as a tool towards improving IVM procedures. This work may also provide further insight into the action of oocyte maturation and evaluate bovine oocyte quality on a molecular level.

2. Materials and methods

2.1. In vivo bovine cumulus enclosed oocyte complexes (COCs)

Cumulus enclosed immature oocytes were collected from Angus hybrid cows after six FSH treatments (50 mg i.m., twice daily for 3 d), with transvaginal aspiration (TVA) performed 48 h after the last treatment. In vivo matured COCs (controls) were collected via TVA after the administration of six treatments (50 mg i.m., twice daily for 3 d), followed by 25 mg prostaglandin with the last FSH injection and 50 μg GnRH 37 h later. Procedures with animals were approved by the Colorado State University animal care and use committee.

2.2. In vitro maturation

After TVA, immature COCs were randomly allocated to one of two treatment groups. Maturation occurred in groups of 10 COCs in 50 μL of a defined medium (G-Mat + 100 ng/mL EGF) [18] supplemented with either 2.5 mg/mL recombinant albumin (IVM + G-MM) or 20% serum (IVM + serum) for 23 h at 38.5 °C in 6% CO_2 in air under paraffin oil. After maturation, cumulus cells were removed and discarded. Only MII oocytes, confirmed through the extrusion of the polar body, were processed for microarray analysis.

2.3. RNA extraction

Total RNA was isolated from groups of oocytes ($n = 10$) with three replicates per group, using PicoPure™ RNA Isolation Kit with modifications (Arcturus Bioscience, Mountain View, CA, USA). Briefly, samples were lysed and bound to a membrane where they were treated with RNase-free DNase I (Qiagen, Valencia, CA, USA), washed several times, and eluted in 20 μL. The extracted RNA concentration was evaluated using the NanoDrop spectrophotometer (NanoDrop, Wilmington, DE, USA) with an average of 20 ng.

2.4. RNA amplification, labelling and hybridization

Following isolation, two rounds of linear RNA amplification incorporating a T7 polymerase promoter were performed using the MessageAmp™ II and MessageAmp™ II—Biotin Enhanced Kit (Ambion, Foster City, CA, USA). On average, samples had a starting concentration of 20 ng and a final concentration of 140 μg after two rounds of amplification. RNA integrity was evaluated with the Agilent 2100 Bioanalyzer and RNA 6000 Nano Assay Kit (Agilent Technologies, Palo Alto, CA, USA) with concentration determined using the NanoDrop spectrophotometer. Poor quality RNA samples were excluded from further analysis. Samples were then fragmented and hybridized to the Affymetrix GeneChip® Bovine Genome Array according to the manufacturer's instructions. This microarray platform contains over 23,000 bovine transcripts. A complete list of the annotated genes can be found on the Affymetrix website (http://www.affymetrix.com/products/arrays/specific/bovine.affx). Each Bovine GeneChip Array was scanned using the GenePix 4000B laser scanner (Axon Instruments).

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