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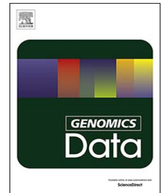

Data in Brief

Oviductal transcriptional profiling of a bovine fertility model by next-generation sequencing

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ABSTRACT

In cattle, the oviduct plays a fundamental role in the reproductive process. Oviductal functions are controlled by the ovarian sex steroids: estradiol and progesterone. Here, we tested the hypothesis that the exposure to contrasting sex steroid milieus differentially impacts the oviductal transcriptional profile. We manipulated growth of the pre-ovulatory follicle to obtain cows that ovulated a larger (LF group) or a smaller (SF group) follicle. The LF group presented greater proestrus/estrus concentrations of estradiol and metaestrus concentrations of progesterone (Gonella-Diaza et al. 2015 [1], Mesquita et al. 2014 [2]). Also, the LF group was associated with greater fertility in timed-artificial insemination programs (Pugliesi et al. 2016 [3]). Cows were slaughtered on day 4 of the estrous cycle and total RNA was extracted from ampulla and isthmus fragments and analyzed by RNAseq. The resulting reads were mapped to the bovine genome (*Bos taurus* UMD 3.1, NCBI). The differential expression analyses revealed that 325 and 367 genes in ampulla and 274 and 316 genes in the isthmus were up-regulated and down-regulated in LF samples, respectively. To validate the RNAseq results, transcript abundance of 23 genes was assessed by qPCR and expression patterns were consistent between the two techniques. A functional enrichment analysis was performed using Database for Annotation, Visualization and Integrated Discovery (DAVID) software. Processes enriched in the LF group included tissue morphology changes (extracellular matrix remodeling), cellular changes (proliferation), and secretion changes (growth factors, ions and metal transporters). An overview of the gene expression data was deposited in the NCBI's Gene Expression Omnibus (GEO) and is accessible through the accession number GSE65681. In conclusion, differences in the peri-ovulatory sex steroid milieu modify the oviductal gene expression profiles. Such differences may be associated with the greater fertility of the LF cows. This dataset is useful for further investigations of the oviductal biology and the impact of sex-steroid on the female reproductive tract.

Specifications

Organism/cell line/tissue	*Bos taurus indicus*, Nelore Breed, oviducts ipsilateral to corpus luteum: ampulla and isthmus
Sex	Female
Sequencer or array type	Illumina HiSeq 2000 (ampulla samples) and Illumina HiSeq 2500 (isthmus samples).
Data format	Analyzed
Experimental factors	Cows were submitted to hormonal manipulation in order to ovulate large or small follicles, causing a different peri-ovulatory sex-steroid milieu.
Experimental features	Ampulla and isthmus samples were collected from cows submitted to endocrine manipulations resulting in the ovulation of smaller or larger follicles.
Consent	N/A

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Sample source location	Experiment was performed in Pirassununga, Brazil, at a Campus of the University of São Paulo; Latitude - 21.953833; Longitude - 47.453143.

Direct link to deposited data

http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE65679

1. Experimental design, materials and methods

1.1. Animals, reproductive management and collection of samples

All animal procedures were approved by the Ethics and Animal Handling Committee of the Faculdade de Medicina Veterinária e Zootecnia — Universidade de São Paulo (CEUA-FMVZ/USP, N° 2287/2011). Animal and reproductive management was performed as described previously [1,2,4]. Non-lactating, multiparous, and cyclic Nelore cows were used in this study. Cows were pre-synchronized (day -27) by intramuscular (IM) injection of GnRH agonist (1 µg of buserelin acetate; Sincroforte, Ouro Fino, Cravinhos, Brazil) and, 7 days later (day -20) an injection of Prostaglandin F2 alpha analog (PGF; 0.5 mg of sodium cloprostenol; Sincrocio, Ouro Fino). On day -20, animals received a Heat detector patch (ESTROTECT, Rockway, Inc. Spring Valley, WI, USA). Animals showing estrus from D-20 to D-15 received an intravaginal progesterone releasing device (1 g; Sincrogest, Ouro Fino) and an IM injection of 2 mg estradiol benzoate (Sincrodiol, Ouro Fino) on D-10. Also, cows in the LF group received an IM injection of PGF. Progesterone devices were removed 8 days later and all animals received a PGF injection at removal and a second PGF injection 6 h later. The progesterone device removal occurred 42 or 30 h before the induction of ovulation in the LF and the SF groups, respectively. GnRH agonist was used to induce ovulation on day 0. Transrectal ultrasound exams were performed in all animals to evaluate growth and ovulation of the pre-ovulatory follicle (POF) and CL area and blood flow. Blood samples were collected from day -2 to day 4 in order to evaluate estradiol and progesterone concentrations by radioimmunoassay. On day 4, the reproductive tracts were collected and the oviduct ipsilateral to the ovary containing the CL was dissected. Samples of ampulla and isthmus were frozen in liquid nitrogen.

2. Animal ranking and selection for RNAseq

In order to be included in the study, animals must have complied with minimal premises that were established prior to the beginning of the study. Animals were excluded if: the preovulatoy follicle was smaller than 8 mm on day 0, ovulation was detected on day 0 or before (i.e. early ovulation) or after day 3 (i.e., late ovulation), ovulation was not detected, or follicular or luteal cysts were detected at any moment during the experiment. Thirteen animals of LF group and 8 animals of SF group matched the premises and were immediately submitted to the induction of ovulation by GnRH administration. Of all slaughtered animals, samples of six (three/group) were submitted to RNAseq analysis. RNAseq of the two regions was performed separately (n = 3 samples per group and per region). In order to select the animals for RNAseq analysis, animals were ranked according to the following variables: maximum diameter of the POF and estradiol concentration at D-1, and CL area and progesterone concentrations at D4. Samples of the top-ranked animals of the LF group and the bottom ranked animals of the SF group were used.

3. RNAseq and bioinformatics

Total RNA was extracted using a commercial kit (All Prep ® DNA/RNA/Protein Mini kit, No. 80004, Qiagen, São Paulo, São Paulo, Brazil)

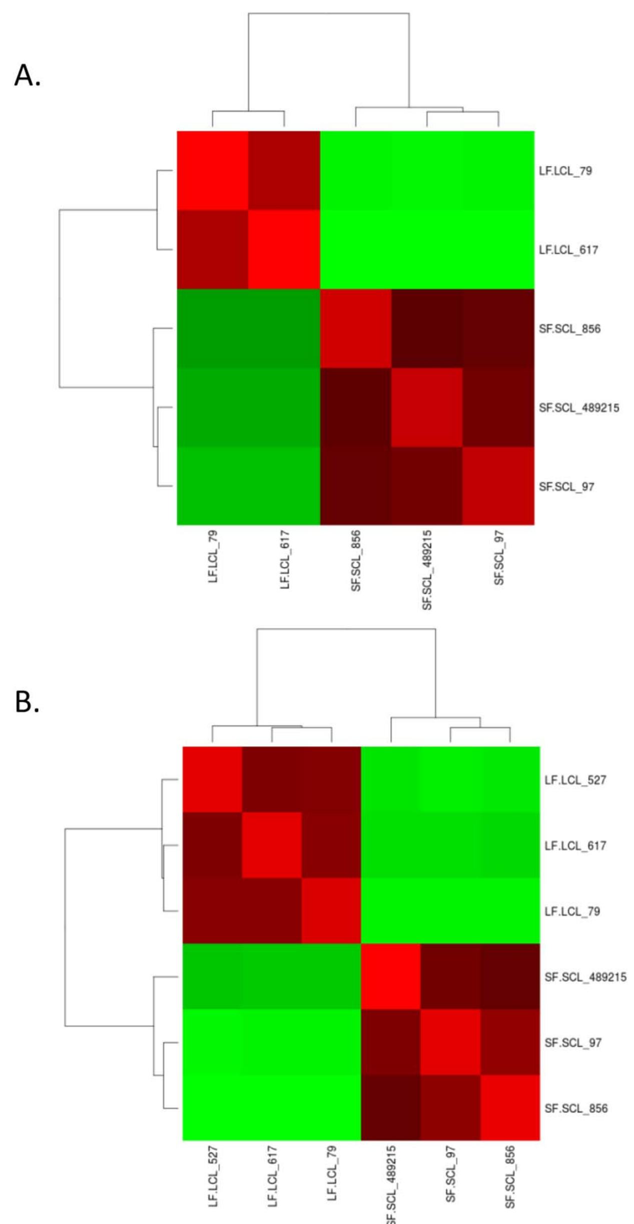

Fig. 1. Clustered heatmap showing the Euclidean distances between the ampulla (Panel A) and isthmus (Panel B) samples submitted to RNAseq, as calculated from the variance-stabilizing transformation of the count data. Each column represents one sample and shows the correlation to all samples with red for the lowest (0) distance and light green for the highest observed distance. Normalized count values were used.(For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

by following the manufacturer's instructions. Total RNA concentration and purity (260/280 and 260/230 nm ratios) was measured using the NanoVue spectrophotometer (GE Healthcare). RNA integrity was assessed using the Agilent Bioanalyzer (Agilent Technologies, Palo Alto, USA). Only samples with an RNA integrity number (RIN) ≥ 8.0 were used for RNAseq analysis. Libraries were generated using the TruSeq protocol for RNAlibraries (Illumina Technologies, San Diego, CA), with 1 µg of total RNA as input. After adapter ligation, end-repair steps, and cDNA synthesis, the cDNA libraries were purified and validated using the Bioanalyzer. Paired-end sequencing of 101 bp reads was performed using the Illumina HiSeq 2000 (ampulla samples) and Illumina HiSeq 2500 (isthmus samples) platforms. Using seqyClean v1.3.12. (https://bitbucket.org/izhbannikov/seqyclean/get/stable.zip) the quality filtering was performed. In order to generate the mapping file, accepted

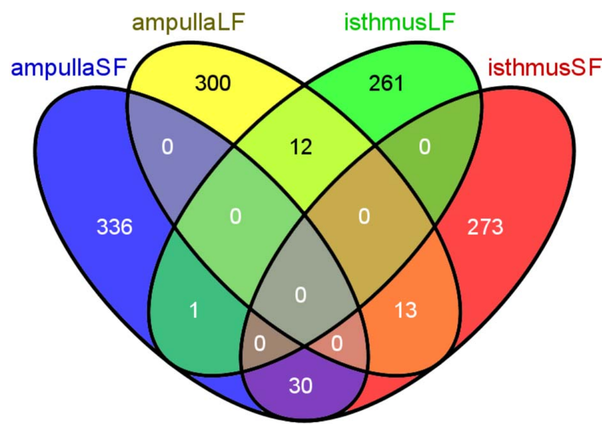

Fig. 2. Venn diagram of differentially expressed genes in ampulla and isthmus samples of LF and SF cows. This diagram shows that there are few common differentially expressed genes between regions and between treatments.

reads were mapped with Tophat v.2.0.8 [5] and Bowtie2 v2.1.0 [6] on the masked bovine genome assembly (*Bos taurus* UMD 3.1, NCBI). Then, the mapping file was sorted with SAMTools v 0.1.18 [7], and using the script from HTSeq-count v0.5.4p2 (http://www-huber.embl.de/users/anders/HTSeq/doc/count.html) the read counts were obtained. Differential expression analysis was performed with package DESEq v1.12.1 [8] from R/Bioconductor [9]. Clustering analysis revealed that gene expression of LF and SF groups differ significantly (Fig. 1). A Venn diagram was constructed using the list of differentially expressed genes (Fig. 2). This diagram shows that there are few common differentially expressed genes between regions. Finally, gene enrichment analysis was performed separately for each oviduct region, using the Database for Annotation, Visualization, and Integrated Discovery (DAVID) [10]. Processes enriched in the LF group included extracellular matrix remodeling, cell proliferation, and secretion. A detailed description of these results is available in Gonella et al. [1]. All reads sequences are available in the Sequence Read Archive (SRA) of the NCBI and an overview of the gene expression results is also available in NCBI's Gene Expression Omnibus (GEO) under the accession number GSE65681 (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE65681).

4. RNAseq validation by qPCR

In order to validate the RNAseq results, the abundance of 23 transcripts was assessed by qPCR. Genes from different functional categories, relevant to oviductal biology, were analyzed (i.e. sex-steroid receptors, angiogenesis, cellular proliferation, extracellular matrix, and synthesis of glycoproteins). For that purpose 1 μg of total RNA was reverse transcribed using a commercial Kit (High Capacity cDNA Reverse Transcription Kit, Life Technologies). The qPCR was performed using the Step-One Plus apparatus (Life Technologies, Carlsbad, CA) and SYBR Green chemistry. Primers details are available in Gonella et al. [1]. Quantitative PCR products were purified and submitted to agarose gel electrophoresis and SANGER sequencing for identification of the PCR products, and identity of target transcripts was confirmed. Quantitative PCR expression data showed agreement with expression patterns obtained by RNAseq analysis, therefore validating global gene expression data [1].

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