Data in brief

Endometrial transcriptional profiling of a bovine fertility model by Next-Generation Sequencing

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2. Experimental design, materials and methods

2.1. Animals, reproductive management and experimental design

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). Experiments were performed at the research farm of the University of São Paulo in Pirassununga, São Paulo, Brazil. Non-lactating, multiparous, cyclic (presenting a corpus luteum) Nelore (Bos indicus) cows, presenting no gross reproductive abnormalities by gynecological examination, ranging from 4 to 10 years old, weighing 461.6 ± 50.1 kg and presenting body condition score of 3.67 ± 0.33, participated in the study. Animals were maintained on Brachiaria pasture, and received supplementation based on sugar cane and/or corn silage, concentrate and minerals, according to their maintenance requirements, as well as water ad libitum. The pre-synchronization step consisted of two intramuscular injections of sodium cloprostenol (PGF; 0.5 mg; Sincrocio, Ouro Fino, Cravinhos, Brazil), 14 days apart. At the second PGF administration (D-20) an ESTROTECT Heat detector patch was placed between the hip and the tail head of eighty-three cows (Rockway, Inc. Spring Valley, WI, USA). Animals were observed for signs of estrus behavior twice daily from D-19 to D-16 and once daily from D-15 to D-10. Only animals that had a recent (current cycle) and PGF-responsive CL (at least 5 days old) on D-10 stayed in the experiment. After the pre-synchronization, cows that had P4 <1.0 ng/mL or were not detected in estrus were removed from the experiment (N = 9). The remaining cows (N = 74) were implanted with an intravaginal P4-releasing device (1 g; Sincrogest, Ourofino) on D-10 and received an intramuscular injection of 2 mg estradiol benzoate (Sincrodio1, Ourofino). Simultaneously, approximately half of the cows received an intramuscular injection of PGF. Progesterone-releasing devices were removed, prior to GnRH injection, from cows that received (large follicle group; LF; N = 35) and cows that did not receive PGF at device insertion (small follicle group; SF; N = 39). At device removal, all animals received two PGF injections, 6 h apart. Ovulation was induced by an injection of 10 μg Buserelin on D0 (Sincroforte, Ourofino). According to Mesquita et al. 2014, by design, animals were excluded from data analyses if: progesterone concentration on D-10 was less than 1 ng/mL, progesterone concentration on D-2 was greater than 3 ng/mL in the LF group, progesterone concentration on D-2 was less than 2 ng/mL in the SF group, dominant follicle diameter on D-0 was less than 8 mm, ovulation was detected at the D-0 ultrasound examination or before (i.e., early ovulation), ovulation was detected at the D-3 ultrasound examination (i.e., late ovulation), ovulation was not detected, or follicular, luteal cysts or lesions impairing animal well-being were detected at any moment during the experiment. Animals that responded to treatments as defined by design were slaughtered on D7 post-induction of ovulation (N = 60). Transrectal ultrasound examinations were performed on day 10, daily from day −2 to day 0 and from day 3 to day 7, and every 12 h from day 1 to day 2. Examinations were performed with the aid of a duplex B-mode and pulsed-wave color Doppler ultrasound instrument (MyLab30 Vet Gold; Esaote Healthcare, São Paulo, SP, Brazil) equipped with a multi-frequency linear transducer. Ovulation was defined as the disappearance of the preovulatory follicle previously identified followed by the identification of the development of a corpus luteum on the same approximate topographical location on the ovary. The diameter of follicles and CLs was calculated as the average between measurements of two perpendicular axes of each structure. Progesterone concentrations for days −10, −6 and −2, and from day 1 until day 7 were determined by a solid-phase radioimmunoassay (Coat-a-count, DPC, Los Angeles, USA), whereas plasma E2 concentrations for days −2, −1 and 0 were determined using a commercial RIA kit (Double Antibody Estradiol, DPC, Los Angeles, USA) as validated previously [2,3].

2.2. Animal ranking and selection for transcriptional profiling

Of all slaughtered animals, endometrial fragments of six (three/group) were selected to be submitted to RNA-Seq. A multivariate analysis approach was taken to identify the most representative samples within each group, based on the following variables: follicle diameter from day −2 to day 0, maximal diameter of the preovulatory follicle, volume of the corpus luteum from days 3 to 7, CL weight and progesterone concentration from days 3 to 7. The Orthogonal Projections to Latent Structures Discriminant Analysis (OPLS-DA) was carried out using SIMCA-P+ version 12.0 (Umetrics, Umea, Sweden), and included all animals that matched the criteria described above and that ovulated within 48 h of the induction of ovulation. Final model explained 75% of the data variability.

2.3. Tissue processing

On day 7, cows were slaughtered by use of a captive bolt followed by jugular exsanguination. Reproductive tracts were processed on ice and dissected within 15 min of slaughter. Inter-caruncular endometrial tissue fragments were dissected, with the aid of scissors and forceps, from the anterior, medial and posterior segments of the uterine horn ipsilateral to the ovary containing the CL. Fragments from different uterine horn segments were pooled, snap-frozen and stored at −80 °C for later processing.

2.4. RNA isolation and sample quality control

Approximately 30 mg of endometrial tissue were ground in liquid nitrogen using a stainless steel mortar and pestle system, and immediately mixed with buffer RLT from the RNeasy Mini columns kit (Qiagen, São Paulo, SP, Brazil), as per manufacturer’s instructions. To maximize lysis, tissue suspension was passed at least ten times through a 21 G needle. Tissue extract in RLT buffer was centrifuged at 13,000 × g for 3 min, and supernatant was loaded on RNAeasy columns. Column-based RNA isolation was conducted according to instructions and RNA was eluted with 40 μl of RNase free water. Elution step was repeated using the resultant 40 μl from the first elution to increase RNA concentration. Concentration of total RNA on extracts was determined by a spectrophotometer (NanoDrop, Thermo Scientific, Wilmington, USA). Prior to reverse-transcription, 1 μg of total RNA was treated with DNase I (Life Technologies, São Paulo, SP, Brazil) for 15 min at room temperature in a 10 μl reaction, followed by addition of 1 μl of EDTA (25 mM) and heating at 65 °C for 10 min to inactivate DNase I. Integrity of total RNA extracts, based on RNA Integrity Number (RIN), ranged from 8.3 to 8.7 (Agilent RNA 6000 Nano chip; Bioanalyzer, Agilent Technologies).

2.5. mRNA libraries and sequencing

Four micrograms of RNA were used with the TruSeq RNA Sample Preparation kit (Illumina, San Diego, CA) to prepare the libraries for RNA-Seq. The insert sizes were estimated through the Agilent DNA 1000 chip (Agilent Technologies) and the libraries concentration was measured through Quantitative Real-Time PCR with a KAPA Library Quantification kit (KAPA Biosystems). Samples were diluted, pooled in equimolar amounts and then sequenced using a HiScanSQ sequencer (Illumina, San Diego, CA).

2.6. Bioinformatics

Following sequencing, the 100 bp paired end (PE) reads were filtered using a Perl script which removed all reads with a mean quality
under 26. The reads were mapped with Bowtie2 v2.1.0 [4] on the masked bovine genome assembly (Bos taurus UMD 3.1, http://www.ncbi.nlm.nih.gov/genome/guide/cow/index.html). The mapping file was sorted using SAMTools v 0.1.18 [5] and read counts were obtained using the script from HTSeq-count v0.5.4p2 (http://www-huber.embl.de/users/anders/HTSeq/doc/count.html; [6]). The differential expression analysis was performed with package DESeq v1.12.1 [7], from R [8]. Using the function estimateSizeFactors, the normalized counts were obtained (baseMean values, which are the number of reads divided by the size factor or normalization constant). The standard deviation along the baseMean values was also calculated for each transcript. In order to avoid artifacts caused by low expression profiles and high expression variance, only transcripts that had an average of baseMean >5 and the mean greater than the standard variation were analyzed. The threshold for evaluating significance was obtained by applying a p-value of 0.05 FDR-Benjamini–Hochberg [9]. Integrated analysis of different functional databases was done using the functional annotation tool of the Database for Annotation, Visualization, and Integrated Discovery (DAVID) [10] using as background the set of genes that passed through the differential expression analysis filter.

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