Data in Brief

Identification of differentially expressed genes in sexed pig embryos during post-hatching development in primiparous sows exposed to differing intermittent suckling and breeding strategies

Stephen Tsoi,⁎, Milena Blanes, Tai Yuan Chen, Pieter Langendijk, Rebecca Athon, George Foxcroft, Michael Dyck

Abstract

The aim of commercial pig breeding programs is to maximize the number of pigs produced per sow per year. Given that sows exhibit an estrus during lactation is a potential means of increasing productivity of a pig breeding herd without reducing in lactation length, conventionally, weaning of piglets at a relatively young age is often related to post-weaning piglet performance which compromises piglet welfare. Therefore, intermittent suckling (IS) is a management technique in which lactating sows are separated from their piglets for a fixed period of the days and allowing sows to continue nursing piglets while exhibiting estrus and being breed during lactation, thereby promoting both piglet well-being and sow reproductive performance [1]. For this study, primiparous sows (PP) were exposed to 28 day (D28) lactation with intermittent suckling (IS) during the final week prior to weaning. The sows detected to be in estrus during lactation were either bred at this first estrus (FE) during lactation (IS21FE), or were “skipped” and bred at their second estrus which occurred after final weaning at D28 (IS21SE). Despite the benefits of IS, the effects of the maternal physiology related to breeding during lactation on embryonic transcriptome are largely unknown. Recent advances in the ability to assess embryonic gene expression in both sexes have made these analyses possible. Here, we describe the experimental procedures of two color microarray analyses and annotation of differentially expressed (DE) genes in detail corresponding to data deposited at NCBI in the Gene Expression Omnibus under accession number GSE53576 and GSE73020 for day 9 embryos (D9E) and day 30 embryos (D30E) respectively. Although only a few DE genes were discovered between IS21FE and IS21SE in both sexes from D9E or D30E, the raw data are still valuable for future use to understand the gene expression profiling from two different developmental stages.

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Keywords: Development, Differential gene expression, Embryo, Microarray, Pig, PCR sex typing

Specifications

| Organism/cell line/tissue | Sex | Sequencer or array type | Data format | Experimental factors |
|---------------------------|-----|-------------------------|-------------|----------------------|
| Day 9 and day 30 embryos from primiparous sows exposed to differing intermittent suckling and breeding strategies | Male and female embryos | Agilent custom made array-031068, EMP1V1 (GPL17779) | Raw data in gpr files and LOWESS normalized log2 ratio using without or with C28 as a reference group when comparing IS21FE versus IS21SE from pre-sexed day 9 or day 30 embryos respectively |

(continued)

1. Direct link to deposited data

http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE53576.
Microarray design & performance

A single band size corresponding to 850 bp amplicon appeared in the gel for both sexes with an additional smaller amplicon of 670 bp observed. A dye-swap method was used to perform PCR with an initial denaturation at 98 °C for 10 s, and then with extension temperature at 72 °C for 30 s, followed by setting the PCR program for 30 cycles (Fig. 1A). Dyes labeling and arrays hybridization were performed under Ozone Free Box™ (BioTray, Villeurbanne, France) inside a dark room with light control system.

2. Microarray analyses

Microarray data analyses were performed using FlexArray software version 1.6.3 (http://genomequebec.mcgill.ca/FlexArray) for data normalization methods using simple background subtraction, LOWESS normalization within and between arrays (Fig. 2). Further analysis to detect DE genes was performed using embedded programs in the software such as limma[7] and the Benjamini and Hochberg false discovery rate (BH-FDR) [8] multiple comparison correction condition with additional switching on the calculation setting for false positives due to the dye effect. In analyzing D30E, C28 was set as a reference during the analysis in order to detect DE genes between IS21FE and IS21SE (Fig. 3). Threshold parameters setting for DE genes were considered to be significant when a fold change (FC) was ≥2 (or ≤0.5) with a BH-FDR adjusted P value (B-H P-value) ≤0.05 in both studies. Under Volcano plot view of P-values from Flexarray analyses between IS21FE and IS21SE treatment, more spots were identified to be statistically significantly in female (27 spots) than male (4 spots) of D30E (Fig. 4). A similar trend was found in D9E study with a total of 26 and 2 spots detected to be significant in female and male respectively.

2.1. Embryos collection and PCR sexing

First parity sows were submitted to an ovulation-induction protocol, intermittent suckling (IS), during lactation [2]. Sows were humanely euthanized at day 9 (D9E) and day 30 (D30E) of gestation for embryo collection. An additional control group of day 30 embryos (IS28) was also collected from control sows bred after weaning at day 28 of lactation. All embryos were stored at −80 °C before further usage. A modified HotSHOT method [4] was used to obtain DNA for sex typing. PCR sexing was performed using a single pair of primers (Table 1) redesigned based on the pig amelogenin (AMEL) genes located on X and Y chromosomes [5,6], Phire Hot Start II DNA Polymerase (F-122S, ThermoFisher Scientific) was used to perform PCR with an initial denaturation at 98 °C for 30 s, followed by setting the PCR program for 30 cycles first at 98 °C for 5 s, annealing temperature at 51.8 °C for 5 s and extension at 72 °C for 10 s, and then with final extension temperature at 72 °C for 1 min. A single band size corresponding to 850 bp amplicon appeared in the gel for both sexes with an additional smaller amplicon of 670 bp observed for male embryos due to 180 bp of deletion in Y chromosome.

2.2. Microarray design & performance

Agilent custom made array-031068 referring to porcine embryo-specific microarray (EMPV1) was used in this study [3]. A dye-swatched (Cy3 & Cy5 fluorescent dyes) direct comparison design with 3 biological replicates was used in both D9E (GSE53576) and D30E (GSE73020) studies for both sexes as shown in Fig. 1. In D9E study, no control group was used when comparing between IS21FE and IS21SE directly (Fig. 1A), however, for the D30E study (GSE73020) a control group (C28) was dye-swapped either with IS21FE or IS21SE (Fig. 1B). Dyes labeling and arrays hybridization were performed under Ozone Free Box™ (BioTray, Villeurbanne, France) inside a dark room with light control system.

http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE73020 See Fig. 1.

2. Experimental design, materials and methods

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2.4. Gene annotation

Gene annotation was performed using probe sequences from NCBI BLAST program http://blast.ncbi.nlm.nih.gov/Blast.cgi?PAGE_TYPE=BlastSearch&BLAST_SPEC=OGP__9823__10718 by selecting two different pig nucleotide databases: Annotated RNAs (Annotation Release 105) or Genome (Scrofa10.2 reference Annotation Release 105) to maximize the search with positive hits. Sequences were considered to be significant alignments when the identity was more than a 98% match with the bit score ranging from 56.5 to 111. After extensive re-annotation, only 23 of the DE genes from both studies (Table 2) received a match with the bit score ranging from 56.5 to 111. Extensive re-annotation, only 23 of the DE genes from both studies (Table 2) received the same gene symbols in pig with their human orthologs, and it was found that only two genes, GTPBP2 [9] and MIR9-3 [10], could be regulated by the reproductive hormone estrogen after extensive PubMed literature search.

In conclusion, only a few DE genes were identified in D9E or D30E between IS21FE and IS21SE and more DE genes were found in females than males in response to the unique physiological condition present in IS treated PP sows.

Conflict of interest

The authors declare no conflict of interest.

Table 1

| Primer names | Sequence | Length | GC content | Melt temp | GenBank accession |
|--------------|----------|--------|------------|-----------|------------------|
| AMELF        | 5′-CCTGCATCAGAGCATAGAC-3′ | 21     | 43%        | 58.9 °C   | AB091791.1       |
| AMELR        | 5′-CTCAGTATCCACTACTAGCC-3′| 23     | 47.83%     | 59.6 °C   | AB091792.1       |

* Melting temperature calculation according to the Tm requirement of Phire hot Start II DNA Polymerase from thermo-scientific-web-tools/tm-calculator.
Fig. 2. Box plot of M-values of expression before and after the normalization process using simple background subtraction, LOWESS normalization within and between arrays.

Fig. 3. Experimental design settings for D30E from FlexArray analyses in (A) female and (B) male embryos using a control group as a reference when comparing between IS21FE and IS21SE to identify DE genes.
Fig. 4. Volcano plots from Flexarray analyses between IS21FE and IS21SE treatments in (A) female and (B) male D30E. The large red diamonds = significant spots, FC = fold change threshold, Adj P-val = Adjusted P-value threshold and black spots influenced by dye effect.

Table 2
Gene annotation data.

| Query id     | Subject ids (human) | % Identity | Alignment length, | Mismatches | Gap opens, | q. start | q. end | s. start | s. end | evaluate | Bit score | Gene symbol (human) | Description |
|--------------|---------------------|------------|-------------------|------------|------------|----------|--------|----------|--------|-----------|-----------|---------------------|--------------|
| NM_001185169.1 | NM_005698.3         | 88.776     | 1577              | 148        | 7          | 1        | 1574   | 24       | 1574   | 0         | 2040      | SCAMP3             | Homo sapiens secretory carrier membrane protein 3 (SCAMP3), transcript variant 1, mRNA |
| NM_001244237.1 | NM_022731.4         | 93.41      | 956               | 62         | 1          | 1        | 955    | 90       | 1045   | 0         | 1436      | NUCKS1            | Homo sapiens nuclear casein kinase and cyclin-dependent kinase substrate 1 (NUCKS1), mRNA |
| NM_001244939.1 | NM_000849.4         | 87.065     | 804               | 97         | 5          | 2        | 804    | 282      | 1079   | 0         | 966       | GSTM3              | Homo sapiens glutathione 5-transferase mu 3 (brain) (GSTM3), transcript variant 1, mRNA |
| NM_001246214.1 | NM_021034.2         | 87.338     | 308               | 38         | 1          | 46       | 353    | 151      | 457    | 3.42E-102 | 376       | IFITM2             | Homo sapiens interferon induced transmembrane protein 3 (IFITM3), transcript variant 1, mRNA |
| NM_214420.1   | NM_000772.2         | 81.368     | 1900              | 341        | 8          | 47       | 1937   | 256      | 2151   | 0         | 1806      | CYP2C49            | Homo sapiens cytochrome P450, family 2, subfamily C, polypeptide 18 (CYP2C18), transcript variant 1, mRNA |
| NR_035366.1   | NR_029525.1         | 93.506     | 77                | 5          | 0          | 1        | 77     | 3        | 79     | 5.09E-24  | 116       | MIR16-2           | Homo sapiens microRNA 16-2 (MIR16-2), microRNA |
| NR_128410.1   | NR_029692.1         | 100        | 79                | 0          | 0          | 1        | 79     | 6        | 84     | 3.66E-32  | 143       | MIR9-3            | Homo sapiens microRNA 9-3 (MIR9-3), microRNA |
| XM_001927622.6 | NM_006699.3         | 83.103     | 5492              | 690        | 75         | 51       | 5412   | 1        | 5384   | 0         | 5597      | MAN1A2             | Homo sapiens mannosidase, alpha, class 1A, member 2 (MAN1A2), mRNA |
| XM_003124162.1 | NM_001005213.1      | 84.875     | 919               | 134        | 3          | 1        | 915    | 1        | 918    | 0         | 1021      | OR5G1              | Homo sapiens olfactory receptor, family 9, subfamily G, member 1 (OR5G1), mRNA |
| XM_003125012.3 | NM_006062.2         | 80.803     | 2615              | 344        | 49         | 7        | 2512   | 1        | 2566   | 0         | 2374      | SMYD5              | Homo sapiens SMYD family member 5 (SMYD5), mRNA |
| XM_003128412.5 | NM_019096.4         | 86.846     | 3018              | 288        | 34         | 1        | 2962   | 49       | 3013   | 0         | 3599      | GTPBP2             | Homo sapiens GTP binding protein 2 (GTPBP2), transcript variant 1, mRNA |
| XM_003353380.3 | NM_014793.4         | 84.317     | 2219              | 330        | 5          | 5        | 2214   | 35       | 2244   | 0         | 2426      | LCMT2              | Homo sapiens leucine carboxyl methyltransferase 2 (LCMT2), mRNA |
| XM_003357386.4 | NM_001684.4         | 87.621     | 2690              | 317        | 7          | 14       | 2700   | 23       | 2699   | 0         | 3333      | ATP2B4             | Homo sapiens ATPase, Ca++ + transporting, plasma membrane 4 (ATP2B4), transcript variant 2, mRNA |
| XM_005667703.2 | NM_001127358.1      | 91.055     | 4874              | 344        | 34         | 11       | 4862   | 4        | 4807   | 0         | 6754      | PHTF2              | Homo sapiens putative homeodomain transcription factor 2 (PHTF2), transcript variant 3, mRNA |

(continued on next page)
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