Genomics Data 9 (2016) 30–34

Contents lists available at ScienceDirect

Genomics Data

journal homepage: www.elsevier.com/locate/gdata

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

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ARTICLE INFO

Article history:
Received 30 May 2016
Accepted 2 June 2016
Available online 4 June 2016

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

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.

Specifications	
Organism/cell line/tissue	Day 9 and day 30 embryos from primiparous sows exposed to differing intermittent suckling and breeding strategies
Sex	Male and female embryos
Sequencer or array type	Agilent custom made array-031068, EMPV1 (GPL17779)
Data format	Raw data in gpr files and LOWESS normalized log2 ratio
Experimental factors	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*)

Specifications	
Experimental features	Transcriptome response of day 9 and day 30 female and male embryos to two different maternal breeding strategies IS21FE and IS21SE
Consent	N/A
Sample source location	Edmonton/Alberta/Canada

1. Direct link to deposited data

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

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http://dx.doi.org/10.1016/j.gdata.2016.06.001

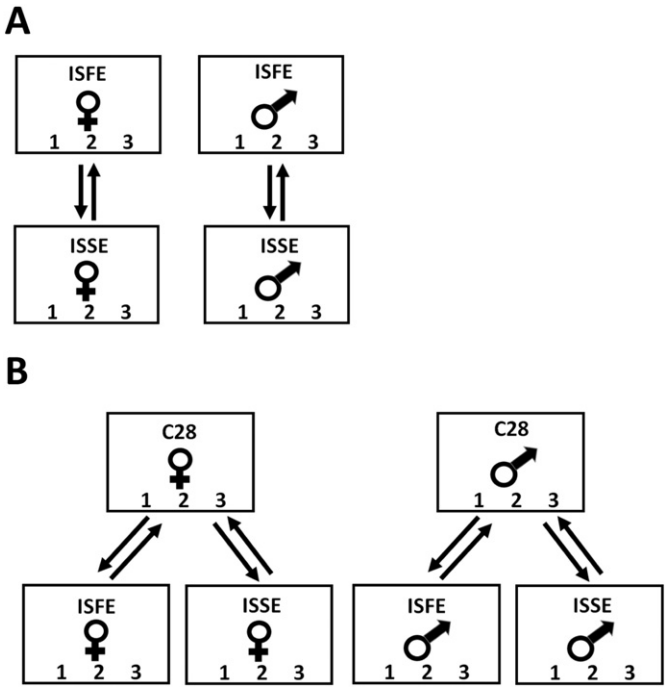

Fig. 1. Microarray design for day 9 and day 30 embryos of both sexes indicated in A and B respectively. The numbers indicate the biological replicates in each group and arrows indicate dye swap between groups. Three embryos with similar weight were pooled in each biological replicate.

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

2. Experimental design, materials and methods

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 (C28) 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 61.8 °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-swapped (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.

2.3. 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 statistical 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.

2.4. 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 (Sscrofa10.2 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 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
PCR primers information.

Primer names	Sequence	Length	GC content	Melt temp[a]	GenBank accession
AMELF	5′-GCTTGCATCAAGAAGATAGAG-3′	21	43%	58.8 °C	AB091791.1
AMELR	5′-GCTCAGTTAATCCTACTCTAGCC-3′	23	47.83%	59.6 °C	AB091792.1

[a] Melting temperature calculation according to the Tm requirement of Phire hot Start II DNA Polymerase from thermo-scientific-web-tools/tm-calculator.

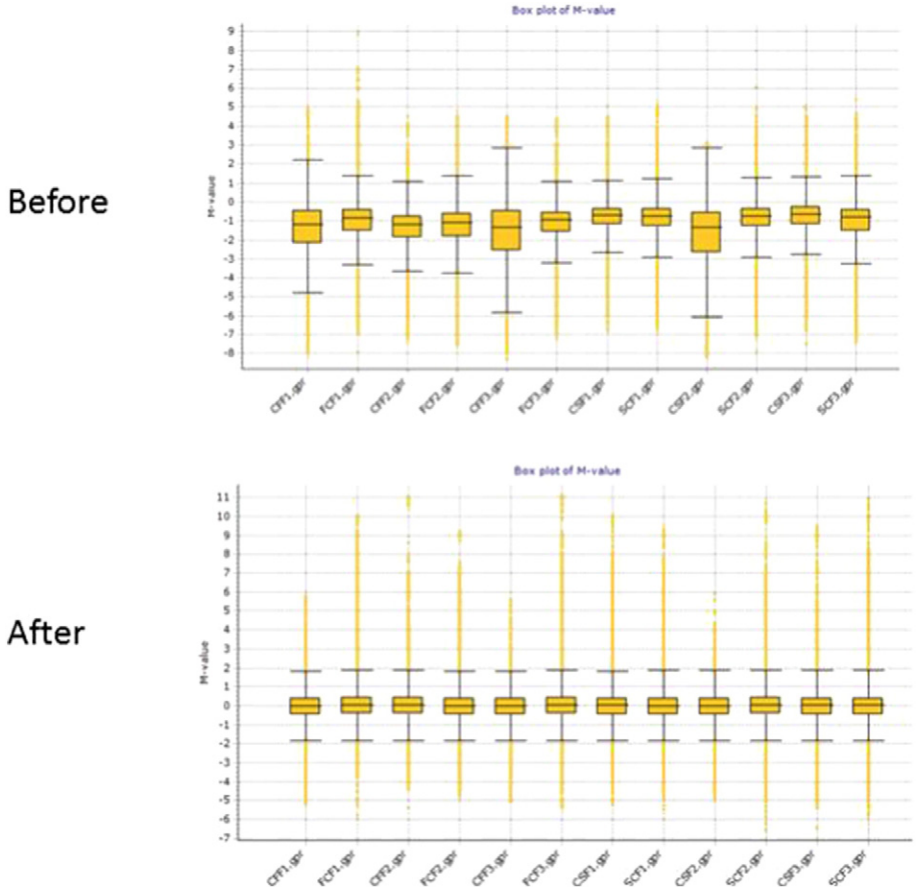

Fig. 2. Box plot of M-values of expression before and after the normalization process using simple background subtraction, LOWESS normalization within and between arrays.

A

Experimental Design

Assign

Sample ID	Comments	FF vs SF	Reference
CFF1.gpr - Red		FF	☐
CFF1.gpr - Green			☑
FCF1.gpr - Red			☑
FCF1.gpr - Green		FF	☐
CFF2.gpr - Red		FF	☐
CFF2.gpr - Green			☑
FCF2.gpr - Red			☑
FCF2.gpr - Green		FF	☐
CFF3.gpr - Red		FF	☐
CFF3.gpr - Green			☑
FCF3.gpr - Red			☑
FCF3.gpr - Green		FF	☐
CSF1.gpr - Red		SF	☐
CSF1.gpr - Green			☑
SCF1.gpr - Red			☑
SCF1.gpr - Green		SF	☐
CSF2.gpr - Red		SF	☐
CSF2.gpr - Green			☑
SCF2.gpr - Red			☑
SCF2.gpr - Green		SF	☐
CSF3.gpr - Red		SF	☐
CSF3.gpr - Green			☑
SCF3.gpr - Red			☑
SCF3.gpr - Green		SF	☐

B

Experimental Design

Assign

Sample ID	Comments	FE vs SE	Reference
SCM3.gpr - Red			☑
SCM3.gpr - Green		SE	☐
SCM2.gpr - Red			☑
SCM2.gpr - Green		SE	☐
SCM1.gpr - Red			☑
SCM1.gpr - Green		SE	☐
FCM3.gpr - Red			☑
FCM3.gpr - Green		FE	☐
FCM2.gpr - Red			☑
FCM2.gpr - Green		FE	☐
FCM1.gpr - Red			☑
FCM1.gpr - Green		FE	☐
CSM3.gpr - Red		SE	☐
CSM3.gpr - Green			☑
CSM2.gpr - Red		SE	☐
CSM2.gpr - Green			☑
CSM1.gpr - Red		SE	☐
CSM1.gpr - Green			☑
CFM3.gpr - Red		FE	☐
CFM3.gpr - Green			☑
CFM2.gpr - Red		FE	☐
CFM2.gpr - Green			☑
CFM1.gpr - Red		FE	☐
CFM1.gpr - Green			☑

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.

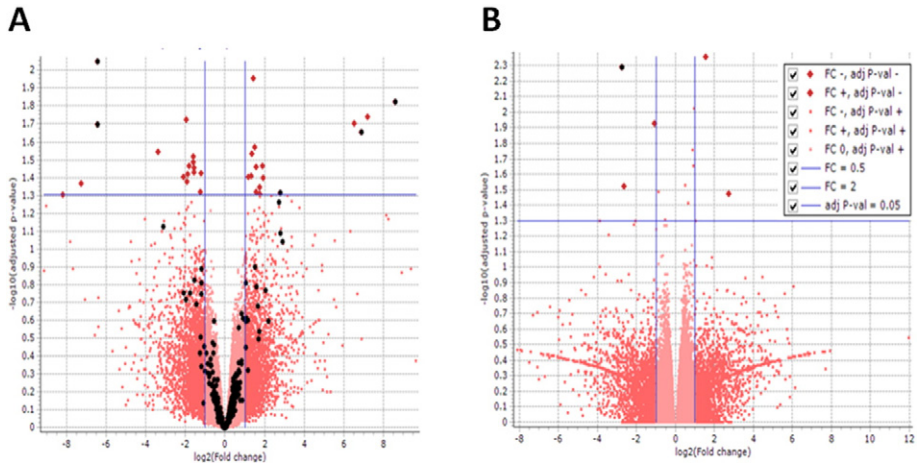

Fig. 4. Volcano plots from Flexarray analyses between IS21FE and IS21SE treatments in (A) female and (B) male D30E. The large red diamonds = significant spots, FC = fold change threshold, Adj P-val = Adjusted P-value threshold and black spots influenced by dye effect.

Table 2
Gene annotation data.

Query id	Subject ids (human)	% Identity	Alignment length,	Mismatches	Gap opens,	q. start	q. end	s. start,	s. end	evalue	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 S-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	OR9G1	*Homo sapiens* olfactory receptor, family 9, subfamily G, member 1 (OR9G1), 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)

Table 2 (*continued*)

Query id	Subject ids (human)	% Identity	Alignment length,	Mismatches	Gap opens,	q. start	q. end	s. start,	s. end	evalue	Bit score	Gene symbol (human)	Description
XM_005667845.2	NM_024420.2	88.309	2874	304	10	40	2907	80	2927	0	3653	PLA2G4A	*Homo sapiens* phospholipase A2, group IVA (cytosolic, calcium-dependent) (PLA2G4A), transcript variant 1, mRNA
XM_005668710.2	NR_003525.2	73.904	2920	700	27	4085	6984	3299	6176	0	1764	LRRC37A6P	*Homo sapiens* leucine rich repeat containing 37, member A6, pseudogene (LRRC37A6P), non-coding RNA
XM_013978437.1	NM_203459.2	75.143	4200	925	24	841	4937	669	4852	0	2866	CAMSAP2	*Homo sapiens* calmodulin regulated spectrin-associated protein family, member 2 (CAMSAP2), transcript variant 2, mRNA
XM_013980250.1	NM_001178123.1	92.962	2103	126	11	1	2094	71	2160	0	3095	ABI1	*Homo sapiens* abl-interactor 1 (ABI1), transcript variant 10, mRNA
XM_013984138.1	NM_052905.3	91.127	3539	297	4	125	3650	2	3536	0	4964	FMNL2	*Homo sapiens* formin-like 2 (FMNL2), mRNA
XM_013986179.1	NM_198510.2	80.555	3245	551	13	1	3208	31	3232	0	3021	ITIH6	*Homo sapiens* inter-alpha-trypsin inhibitor heavy chain family, member 6 (ITIH6), mRNA
XM_013989713.1	NM_016548.3	73.903	2575	442	62	1	2432	33	2520	0	1543	GOLM1	*Homo sapiens* golgi membrane protein 1 (GOLM1), transcript variant 1, mRNA
XM_013991759.1	NM_001195141.1	79.151	1367	231	15	9	1349	24	1362	0	1162	TCOF1	*Homo sapiens* Treacher Collins-Franceschetti syndrome 1 (TCOF1), transcript variant 7, mRNA
XM_013999054.1	NM_001010980.4	79.569	881	143	16	715	1572	102	968	0	738	NCMAP	*Homo sapiens* noncompact myelin associated protein (NCMAP), mRNA

Acknowledgements

This research was funded in part by the CRC for High Integrity Australian Pork and in part by funding for the NSERC Embryogene Strategic Research Network.

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