REPRODUCTION
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Gene expression of porcine blastocysts from gilts fed organic or inorganic selenium and pyridoxine

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Abstract

In this study, we determined how maternal dietary supplementation with pyridoxine combined with different sources of selenium (Se) affected global gene expression of porcine expanded blastocysts (PEB) during pregnancy. Eighteen gilts were randomly assigned to one of the three experimental diets ($n=6$ per treatment): i) basal diet without supplemental Se or pyridoxine (CONT); ii) CONT + 0.3 mg/kg of Na-selenite and 10 mg/kg of HCl-pyridoxine ($MSeB_6$10); and iii) CONT + 0.3 mg/kg of Se-enriched yeast and 10 mg/kg of HCl-pyridoxine ($OSeB_6$10). All gilts were inseminated at their fifth post-pubertal estrus and killed 5 days later for embryo harvesting. A porcine embryo-specific microarray was used to detect differentially gene expression between $MSeB_6$10 vs CONT, $OSeB_6$10 vs CONT, and $OSeB_6$10 vs $MSeB_6$10. CONT gilts had lower whole blood Se and erythrocyte pyridoxal-5-P concentrations than supplemented gilts ($P<0.05$). No treatment effect was observed on blood plasma Se-glutathione peroxidase activity ($P=0.57$). There were 10, 247, and 96 differentially expressed genes for $MSeB_6$10 vs CONT, $OSeB_6$10 vs CONT, and $OSeB_6$10 vs $MSeB_6$10 respectively. No specific biological process was associated with $MSeB_6$10 vs CONT. However, for $OSeB_6$10 vs CONT, upregulated genes were related with global protein synthesis but not to selenoproteins. The stimulation of some genes related with monooxygenase and thioredoxin families was confirmed by quantitative real-time RT-PCR. In conclusion, $OSeB_6$10 affects PEB metabolism more markedly than $MSeB_6$10. Neither Se sources with pyridoxine influenced the Se-glutathione peroxidase metabolic pathway in the PEB, but $OSeB_6$10 selectively stimulated genes involved with antioxidant defense.

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Introduction

During the last decades, marked increases in ovulation rate in pigs have been associated with reduced embryo survival (Freking *et al*. 2007, Patterson *et al*. 2008), possibly due to the poor quality of oocytes shed by these supplemental follicles (Driancourt *et al*. 1998).

The process of ovulation induces the production of reactive oxygen species (ROS) in the ovary after the luteinizing hormone (LH) surge (Brännström *et al*. 1993). Peri-estrus oxidative stress has been associated with reduced fertility and perturbation of oocytes (Guerin *et al*. 2001) and embryonic development (Ufer & Wang 2011) in many species. Despite this cytotoxicity, a basal oxidation stress appears necessary for the ovulation process (Van der Hoek *et al*. 2000) and subsequent embryo development (Orsi & Leese 2001). Thus, understanding how embryo metabolism regulates the intracellular redox potential is of fundamental importance.

Selenium (Se) is an essential trace element derived from inorganic or organic sources. Organic Se (OSe), the natural form present in feed, is deposited into proteins as selenomethionine (SeMet) following the methionine metabolism (Schrauzer 2003), whereas inorganic Se (MSe), commonly used as a dietary supplement, is readily transformed into selenide and incorporated into some selenoproteins (Windisch 2002). Roughly half of the selenoproteins confer cellular protection against oxidative stress. Both sources of Se can result in Se-dependent glutathione peroxidase (SeGPX) biosynthesis, but the OSe pathway is more complex and responsive to oxidative stress (Gonzalez-Flores *et al*. 2013). Several reactions of OSe to SeGPX pathway are pyridoxine dependent (Yasumoto *et al*. 1979). Roy *et al*. (2011) have shown the importance of pyridoxine for an adequate flow of OSe toward the SeGPX system in response to oxidative stress induced during the peri-estrus period in gilts.

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Although limited, the information on the effects of sources of supplemental Se for sows on developing porcine embryos has shown an increased Se transfer of 60% to embryos from OSe dams along with an enhanced morphological and physiological development at 30 days of gestation. This was observed in spite of a lack of effect on embryo SeGPX activity (Fortier *et al.* 2012). The metabolic and physiological mechanisms involved in such responses deserve further investigation. Genomic approaches such as microarray technologies, which have been developed to study gene expression of the whole genome of developing mammalian embryos (Niemann *et al.* 2007), can be powerful tools for that purpose. Recently, a gene expression microarray specific for porcine embryos has been developed and validated (Tsoi *et al.* 2012), allowing an efficient analysis of large numbers of different pre-determined transcripts in several samples.

This study aimed to determine the effect of maternal OSe or MSe dietary supplementation combined with pyridoxine on gene expression of the whole genome of porcine expanded blastocysts (PEB) via microarray analysis. In addition, biological processes and some key enzymes/proteins related with embryo Se metabolism, in particular SeGPX, were also investigated.

Material and methods

Animals and treatments

Experimental procedures were carried out following the guidelines of the Canadian Council on Animal Care (2009) and were approved by the Institutional Animal Care Committee of the Dairy and Swine Research and Development Centre of Sherbrooke, Québec, Canada (#400). All animals were cared for and slaughtered according to the recommended code of practice of Agriculture Canada (1993).

Eighteen Yorkshire–Landrace gilts were selected for this study at 96.1±4.6 kg BW and aged 135–170 days and six to seven animals were grouped in pens (1.5×2.5 m, half-slatted concrete flooring) until the first estrus was detected. For at least 14 days, they were fed a basal breeding/gestation diet *ad libitum* (Table 1), without Se and pyridoxine supplements but in excess of the recommended NRC (1998) requirements for all other ingredients. Estrus detection was initially performed by introducing a young boar (aged 8–12 months) into the pen once daily (10 min), but was increased to twice daily (10 min each; between 0800 and 0900 h and from 1600 to 1700 h) for the detection of the fifth estrus. From the onset of the first estrus, the gilts were kept in individual stalls (0.6×2.2 m, half-slatted concrete flooring), their daily feed allowance was limited to 2.8 kg, and on the basis of comparable BW and blood concentrations of Se, they were assigned randomly to one of three experimental diets ($n=6$ per treatment): i) basal diet (Table 1) containing 0.3 and 2.4 mg/kg of natural Se and pyridoxine, respectively, top-dressed with 50 g of ground corn without supplemental Se or pyridoxine (CONT); ii) the basal diet top-dressed with 50 g of ground corn with supplemental Se and pyridoxine, providing an equivalent of 0.3 mg/kg of feed of MSe as sodium selenite, and 10 mg/kg of feed of pyridoxine, as hydro-chloride pyridoxine (P9755, Sigma–Aldrich; $MSeB_6 10$); and iii) the basal diet top-dressed with 50 g of ground corn with supplemental Se and pyridoxine, providing an equivalent of 0.3 mg/kg of feed of OSe as Se-enriched yeast, and 10 mg/kg of feed of pyridoxine, as hydro-chloride pyridoxine ($OSeB_6 10$). At the fifth estrus, all gilts were inseminated with 85 ml of semen (3×10^9 live sperm cells pooled from the same three Duroc boars) provided by a local AI center (CIPQ, Inc., St-Lambert, QC, Canada). When estrus was detected in the morning, the gilts were inseminated twice, 8 and 24 h later. When estrus was detected in the afternoon, two inseminations were done 16 and 24 h later. The gilts were killed 5 days after the first insemination. Average BW was 138.5±6.3 and 181.3±6.3 kg at the initiation of treatment and at the end of the experiment respectively.

Table 1 Composition of the basal diet (as-fed basis)[a,b].

Ingredients	Amount (%)
Corn	52.6
Wheat shorts	20.0
Distillers dried grain with solubles	10.0
Canola meal	9.7
Soybean hulls	4.0
Limestone	2.0
Salt	0.6
Biofos	0.5
L-lysine *P*	0.1
Chlorure choline	0.1
Feed curb	0.1
Mineral and vitamin premix[c]	0.3

[a]The calculated compositions for ME, CP, lysine, Ca, and P of the basal diet were 2702 kcal/kg, 14.0, 0.6, 1.0, and 0.6% respectively. [b]The basal Se and pyridoxine contents of the diet were 0.3 and 1.7 mg/kg respectively (analytical values determined according to Matte *et al.* (2001) and Giguère *et al.* (2005) respectively). [c]Provided per kilogram of diet: Mn as manganous oxide, 40 mg; Zn as zinc oxide, 150 mg; Fe as ferrous sulfate, 140 mg; Cu as copper sulfate, 21 mg; I as calcium iodate, 2.0 mg; vitamin A, 14 580 IU; vitamin D, 1500 IU; vitamin E, 44 IU; vitamin K, 2.6 mg; thiamine, 2.7 mg; riboflavin, 4.9 mg; niacin, 31 mg; pantothenic acid, 21 mg; folic acid, 10 mg; biotin, 400 µg; and vitamin B12, 25 µg.

Sampling

Blood samples were collected into EDTA-containing tubes (10 ml; Becton Dickinson and Co., Rutherford, NJ, USA) by jugular vein venipuncture from all gilts at arrival to the research center, on the day after onset of each estrus, and at slaughter. The blood samples were stored at −20 °C for the determination of Se concentrations. Blood plasma and erythrocyte samples were obtained after centrifugation of blood at 1800 ***g*** for 12 min at 4 °C and stored at −20 °C and at −80 °C for the determination of pyridoxal-5-phosphate (P-5-P) and SeGPX activity respectively.

At slaughter, the reproductive tract, liver, and kidneys were collected from all gilts (six per treatment). Liver weight and ovulation rate (number of corpora lutea (CL)) were recorded. Samples of liver and kidney tissue were collected and frozen in liquid nitrogen.

Embryo (day 5) collection and storage

Both uterine horns were immediately flushed twice with 20 ml of PBS/BSA (37 °C) and the flushing samples collected into 50 ml Falcon tubes and kept at 37 °C. In laboratory, flushing samples were transferred to a Petri dish (37 °C) and embryos were harvested using a dissection microscope. Each embryo was transferred to another Petri dish (37 °C) previously prepared with microdroplets of mDPBS/BSA (modified Dulbecco PBS: PBS solution-added D-glucose, sodium pyruvate, magnesium chloride ($MgCl_2 \cdot 6H_2O$), and calcium chloride ($CaCl_2 \cdot 2H_2O$)). The embryos were classified according to their developmental stage as two to four cells, four to eight cells, morula, compact morula, early blastocyst, blastocyst, or expanded blastocyst. The groups of three to six similar stage embryos were transferred to a Petri dish previously containing mDPBS droplets without BSA under mineral oil (37 °C). Then, each group of embryos was transferred to a corresponding microcentrifuge tube with not more than 20 µl of mDPBS without BSA and frozen immediately in dry ice and kept at −80 °C until analysis. The samples of the first flushing of each uterine horn were frozen at −20 °C for the determination of Se and P-5-P concentrations. All gilts were pregnant and available for embryo harvesting, but as the microarray used was specific for PEB, litters had to be selected based on the developmental stage of embryos for suitable biomolecular analysis. The pools of three to six expanded blastocysts from the same gilt could be finally constituted from 11 gilts. They were distributed as follow: 4, 4, and 3 in CONT, $MSeB_6$10, and $OSeB_6$10 fed gilts respectively.

Laboratory analysis

Measurements of Se were done using a fluorimetric method adapted by Giguère *et al*. (2005) from the technique of Sheehan & Gao (1990) and P-5-P was determined using a fluorimetric method adapted by Matte *et al*. (1997) from the technique of Srivastava & Beutler (1973). SeGPX activity was determined in blood plasma by the spectrophotometric method described by Gunzler & Flohe (1985). The activities are reported as milliunits (mU) per mg of protein with 1 unit = 1 µmol NADPH oxidized/min.

Total RNA extraction

The ArcturusW PicoPureW RNA Isolation Kit (Applied Biosystems) was used for RNA extraction on each individual sample tube containing three to six embryos. High-quality total RNA was obtained from each sample after DNase treatment using the RNase-Free DNase Kit according to the protocol from Qiagen. Total RNA quality was evaluated with an Agilent 2100 Bioanalyzer using the RNA 6000 Pico Kit (Agilent Technologies, Mississauga, ON, Canada). RNA integrity number (RIN) index was used as a numerical assessment of the integrity of RNA and ranged between 7.8 and 9.7. RNA amplification (RiboAmp HSPlus Kit; Applied Biosystems) was used to amplify the low quantities of total RNA isolated from the samples, with only 0.6–3.6 ng of total RNA available for amplification. Antisense RNA (aRNA) was generated from each sample and the quality of each amplified aRNA sample was checked using an RNA 6000 Nano Kit (Agilent Technologies). For all good quality samples, Nanodrop ND-1000 (NanoDrop Technologies, Wilmington, DE, USA) was used to determine the aRNA concentration.

Microarray procedure and analysis

The porcine embryo-specific microarray platform generated by Agilent (Tsoi *et al*. 2012) was used in this study. The platform information has been previously deposited and is available from NCBI the Gene Expression Omnibus (GEO) repository with the GEO ID: GPL14925.

A two-color microarray with a dye-swap replicate was performed for the $MSeB_6$10 vs CONT and $OSeB_6$10 vs CONT comparison to identify all the differentially expressed (DE) genes. Four biological replicates for the $MSeB_6$10 vs CONT and three biological and one technical replicate for the $OSeB_6$10 vs CONT comparisons were used in the experimental design. In addition, a reference design (Konig *et al*. 2004) was chosen using the same CONT group used in the previous comparisons as a reference for a reliable indirect comparison of gene expression for $MSeB_6$10 vs $OSeB_6$10.

All the details regarding probe labeling, hybridization, and washing were previously described by Tsoi *et al*. (2012) and Zhou *et al*. (2014). In general, concentrations of the labeled probes and their degrees of labelling (DOL) were determined by Nanodrop ND-1000 with an average of 125.08 ng/ml and 1.2 Cy3 molecules into the anti-sense RNA respectively.

Immediately after the final washing and drying procedures, the arrays were scanned at 5 µm resolution with an Axon 4200AL scanner (635 nm for Cy5 and at 532 nm for Cy3) using the autoscan feature from the default setting, and the images were analyzed with the Gene Pix Pro 6.0 Software (Molecular Device, Sunnyvale, CA, USA). The analyzed images were manually edited for any spots with hybridization artefacts and flagged for exclusion. The data from spot intensity, background subtraction, and normalization were saved in the GenePix Results (GPR) format for further data analysis.

The updated annotation with all the DE genes (adjusted *P* value ≤ 0.05 and log2 fold change ≥ 1.7 or ≤ -1.7), matching with the original probe ID and sequences, was performed first through NCBI Basic Local Alignment Search Tool (BLAST) with the porcine RefSeq RNA (51 160 sequences) and ESTs (1 676 424 sequences) database. Similar search was performed by using the pig accession numbers with the human RefSeq RNA (91 603 sequences) to obtain the gene symbols (GSs) (Supplementary Table 1, see section on supplementary data given at the end of this article) for further Gene Ontology Enrichment Analysis.

Bioinformatics tools and analysis

The Venny interactive tool to associate DE gene lists with Venn diagram (http://bioinfogp.cnb.csic.es/tools/venny/) was used to identify unique GSs from each comparison. The list of unique human GSs obtained from Venny analysis was further uploaded to the GORILLA Classification System (http://cbl-gorilla.cs.technion.ac.il/) to identify biological processes most related to each comparison (Eden *et al*. 2009).

A list of 15 760 unique GSs from re-annotated EMPV1 was used as background when using two unranked lists of genes (target and background lists) as a running mode during the search. The differences were considered significant at false discovery rate (FDR) q-value <0.05.

Quantitative real-time RT-PCR and analysis

A two-step quantitative real-time RT-PCR (RT-qPCR) was performed on the same aRNA samples used for the microarray experiment. Two micrograms of aRNA was obtained from each individual sample with three biological samples from each group (CONT, $n=3$ and $OSeB_6 10$, $n=3$) to synthesise cDNA using the SuperScript VILO cDNA Synthesis Kit (Life Technologies, Inc.). Three technical replicates were performed for each biological sample using the KAPASYBFAST qPCR Kit (Kapa Biosystems, Inc., Wilmington, MA, USA) with diluted cDNA as a template from a range of 100, 50, 25, 5, 1, and 0.25 ng. The RT-qPCR conditions strictly followed the manual provided by the kit manufacturer. A RT without reverse transcriptase (negative control) was also performed together from the same plate with the ABI 7900HT PCR system. Melting curves and primer efficiencies were obtained from SDS2.3 Software (Applied Biosystems) installed in the system by performing auto-setting for threshold cycle (*C*t) and baseline calculation.

Two control genes, hypoxanthine phosphoribosyltransferase 1 (*HPRT1*) and peptidylprolyl isomerase A (*PPIA*), were selected according to RT^2 ProfilerPCR Array Pig Housekeeping Genes (Qiagen, Inc.). Of the two identified genes, *PPIA* was selected as the reference gene due to the lower stability measurement value (Vandesompele *et al.* 2002) compared with *HPRT1*. Based on the microarray data, four DE genes related with ROS disposal systems: coenzyme Q6 monooxygenase (*COQ6*), glutaredoxin-3 (*GLRX3*), peroxiredoxin-4 (*PRDX4*), and thioredoxin (*TXN*) and two genes involved in B_6 (pyridoxine kinase (*PDXK*)) or Se metabolism (Sep (*O*-phosphoserine) tRNA:selenocysteine tRNA synthase (*SEPSECS*)) were chosen for RT-qPCR validation. The mRNA expressions of these genes were normalized with *PPIA*. The factor of upregulation is equal to the given value in the Randomization Data Output Box and the downregulation factor is illustrated as a reciprocal value (Pfaffl *et al.* 2002).

High RT-qPCR efficiencies (99.00–99.99%) and a single peak after melting curve analysis for all housekeeping genes and genes of interest were confirmed. The sequence information of the primers is given in Table 2. The total expression ratio of the six genes of interest was tested for significance between CONT and $OSeB_6 10$.

Statistical analysis

Data were analyzed using the SAS procedure for mixed models (SAS Inst., Inc., Cary, NC, USA) according to a randomized arrangement of treatments in blocks, with the three dietary treatments as the main independent variables. The model was as follows: $Yij=\mu+Fj+eij$, where Yij = dependent variable, Fj = dietary treatments, and eij = residual error. The gilt was considered to be the experimental unit. The residual error term was used to test the treatment effects. For the analysis of blood Se, blood plasma SeGPX activity and blood erythrocyte P-5-P concentration sampling times were added to the model as a second factor and were analyzed using repeated option of the MIXED procedure of SAS. The sampling times were considered during the whole experiment (six samples, from the first estrus to slaughter). A priori comparisons were done between CONT and MSe + OSe groups (SUPPL) and also between MSe and OSe groups (Se source). The differences were considered significant at $P\leq 0.05$ and tendencies at $0.05<P\leq 0.10$, and all results are expressed as adjusted means ± S.E.M.

Microarray data statistical analysis was performed using FlexArray (version 1.6.2, http://genomequebec.mcgill.ca/FlexArray), as described previously by Zhou *et al.* (2014) using a default setting of P value = 0.05 and fold-change threshold = 2.0 respectively. In general, after executing the correct background algorithm using the simple subtraction method, further normalizations within (loess) and between arrays were performed. The normalized log2 ratio of all three comparisons was submitted to NCBI GEO Database (GSE51249). Further statistical analysis using the 'limma' package (Smyth 2005), the Benjamini and Hochberg FDR (BH-FDR), and the multiple comparison

Table 2 Primer sequences used for RT-qPCR amplifications of reference gene and genes encoding antioxidant enzymes in porcine expanded blastocysts.

Genes	Primer sequences (5′→3′)	GenBank accession no.	Product size (bp)	Amplification efficiency (%)
HPRT1	(F) CAGTGACAGCACTTAGAGGTATT (R) GACAACAACTCACCGGAAATAATC	NM_001032376.2	126	99.6
PPIA	(F) GGTGACTTCACACGCCATAA (R) GACCCGTATGCTTCAGGATAAA	NM_214353.1	91	99.1
PRDX4	(F) GTGGCCAAGAGAAGAGCTATAA (R) GCATCTTGACCTGAG GAAGTAT	XM_005673496.1	94	99.8
COQ6	(F) CCAGCCCTTGGGTTCATATT (R) GACTGGTCATAGTTCCAGCTAAC	XM_001929106.3	136	99.3
GLRX3	(F) GGAAACAAACAGGAAGCCAAG (R) GTATGTCGAACGTCTCGTAGTC	XM_005671576.1	91	99.9
PDXK	(F) CAGAGCAAGAGGGACATTGAG (R) CACAAGGACGGAAACAGACA	NM_213943.1	113	99.1
SEPSEC	(F) CAAGGTATCTGGAGCTGACAAT (R) ACCCTTCACATGTCATCAAGAA	XM_003356876.2	124	99.0
TXN	(F) CTCGTAGTGGTCGATTTCTCAG	NM_214313.2	100	99.9

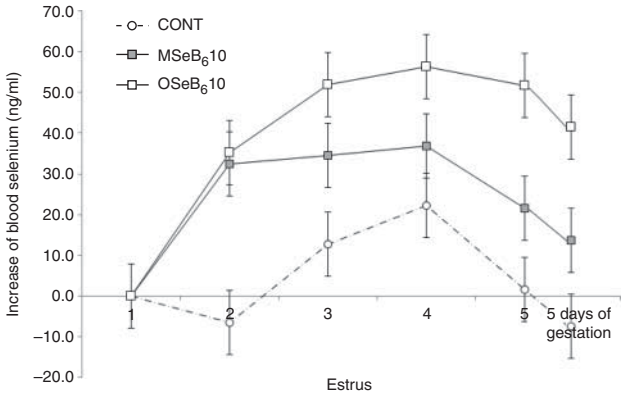

Figure 1 Increase in blood selenium concentration (ng/ml) of gilts on each estrus, presented as LS means±S.E.M. CONT, basal diet containing 0.3 and 2.4 mg/kg of natural Se and pyridoxine respectively; $MSeB_6 10$, basal diet supplemented with 0.3 mg/kg of sodium selenite (Sigma–Aldrich) and 10 mg/kg of hydro-chloride pyridoxine (P9755, Sigma–Aldrich); and $OSeB_6 10$, basal diet supplemented with 0.3 mg/kg of Se-enriched yeast (Alltech, Inc., Nicholasville, KY, USA) and 10 mg/kg of hydro-chloride pyridoxine (P9755, Sigma–Aldrich).

correction condition (Benjamini & Hochberg 1995) along with dye effect correction was performed to identify DE genes.

For RT-qPCR analysis, the Relative Expression Software Tool 2009 (REST; http://rest.gene-quantification.info/) was used to implement a randomized test (Pfaffl *et al.* 2002) and to assess statistical significance of the up- or down-regulation of the target genes after normalization to the reference gene. Statistical analyses were considered significant at $P \leq 0.05$.

Results

Blood measurements

Whole blood Se concentrations expressed as increases relative to pre-treatment values at estrus 1 were lower in CONT than in supplemented gilts (CONT vs SUPPL, $P<0.01$), and greater in OSe gilts than in MSe gilts (Se source, $P<0.05$; Fig. 1). Globally, from the first estrus to 5 days of gestation, whole blood Se decreased by 2.7% for CONT and increased by 4.9 and 15.4% for $MSeB_6 10$ and $OSeB_6 10$ respectively (treatment×time interaction, $P<0.05$). During the complete experimental period, lower concentrations of P-5-P in blood erythrocytes were found in CONT than in supplemented gilts (CONT vs SUPPL, $P<0.05$; Fig. 2).

SeGPX activity in blood plasma tended to increase during the experimental period (time effect, $P<0.06$) but no treatment effect was found ($P=0.57$). Average values were 39.36±2.29, 43.84±4.50, and 42.54±2.33 mU/g protein at first and fifth estrus, and at slaughter respectively.

Physiological and reproduction measurements at slaughter

There was no treatment effects (Table 3) on liver weight ($P \geq 0.17$), liver Se concentration ($P \geq 0.21$), total liver Se ($P \geq 0.24$), kidney Se concentration ($P \geq 0.24$), ovulation rate ($P \geq 0.24$), embryos recovery rate ($P \geq 0.19$), viable embryos ($P \geq 0.11$), degenerated embryos ($P \geq 0.14$), early-stage embryos (from two to four cell to compact morula) ($P \geq 0.81$), advanced-stage embryos (from early blastocyst to expanded blastocyst) ($P \geq 0.18$), and total number of embryos ($P \geq 0.25$). In the uterine flushes, P-5-P concentrations were similar among treatments ($P \geq 0.13$), but Se was not detectable (<10 ng/ml).

Differential gene expression profile

The Venn diagram including the three comparisons is presented in Fig. 3. The comparisons $MSeB_6 10$ vs CONT and $OSeB_6 10$ vs CONT correspond to the synergy of OSe or MSe with B_6, whereas the $OSeB_6 10$ vs $MSeB_6 10$ comparison excludes the B_6 influence and represents a specific effect of OSe vs MSe.

The direct comparison $MSeB_6 10$ vs CONT showed a total of ten DE genes in the PEB, with nine upregulated (90.0%), and one downregulated gene (10.0%) in $MSeB_6 10$. Only two genes had an absolute (− or +) fold change ≥2. All those genes are given in Table 4. For $OSeB_6 10$ vs CONT comparison, a total of 247 genes were DE, with 185 upregulated (75.3%) and 62 down-regulated genes (24.7%) in $OSeB_6 10$. Among these genes, 119 had an absolute fold change ≥2, and eight of them were ≥3. The ten most up- and down-regulated genes are listed in Table 5. Regarding the reference design comparison ($OSeB_6 10$ vs $MSeB_6 10$), there were 96 DE genes. $OSeB_6 10$ had 60 upregulated (62.5%) and

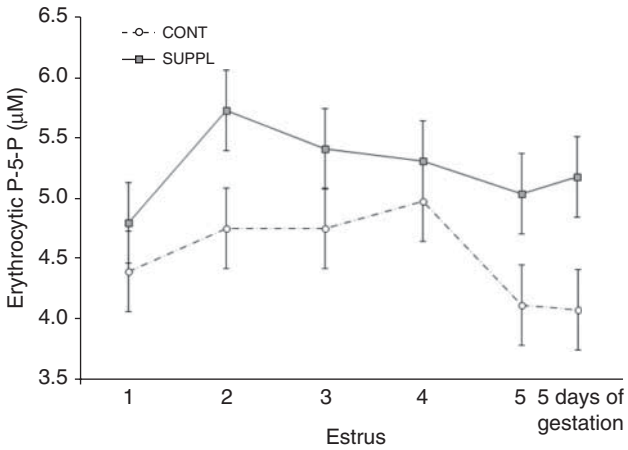

Figure 2 Blood erythrocytic P-5-P concentration (µM) of gilts for each estrus, presented as LS means±S.E.M. CONT, basal diet containing 0.3 and 2.4 mg/kg of natural Se and pyridoxine respectively; $MSeB_6 10$, basal diet supplemented with 0.3 mg/kg of sodium selenite (Sigma–Aldrich) and 10 mg/kg of hydro-chloride pyridoxine (P9755, Sigma–Aldrich); and $OSeB_6 10$, basal diet supplemented with 0.3 mg/kg of Se-enriched yeast (Alltech, Inc.) and 10 mg/kg of hydro-chloride pyridoxine (P9755, Sigma–Aldrich). SUPPL, average values of $MSeB_6 10$+ $OSeB_6 10$.

Table 3 Physiological and reproduction measurements at slaughter.

					A priori comparisons	
Measurements	**CONT**	**MSeB$_6$10**	**OSeB$_6$10**	**S.E.M.**	CONT vs SUPPL	MSeB$_6$10 vs OSeB$_6$10
Uterine flush selenium concentration (ng/ml)	–	–	–	–	–	–
Uterine flush pyridoxal-5-phosphate (μM)	0.33	0.50	0.17	0.16	0.99	0.13
Liver weight (kg)	1.93	1.78	1.91	60.40	0.26	0.17
Liver selenium concentration (μg/g)	1.07	1.14	1.21	0.07	0.21	0.47
Total liver selenium concentration (mg)	2.08	2.04	2.32	0.02	0.60	0.24
Kidney selenium concentration (μg/g)	3.22	3.40	3.63	0.02	0.24	0.44
Ovulation rate/number of corpora lutea (*n*)	18.83	18.83	19.67	1.16	0.86	0.24
Embryo recovery rate (%)	76.92	85.61	92.74	7.17	0.19	0.50
Viable embryos (*n*)	14.17	13.50	18.17	1.88	0.48	0.11
Degenerated embryos (*n*)	0.50	2.00	0.00	0.96	0.65	0.14
Early-stage embryos (*n*)	3.33	4.30	3.67	2.15	0.81	0.83
Advanced-stage embryos (*n*)	10.83	9.17	14.50	2.62	0.76	0.18
Total embryos (*n*)	14.67	15.50	18.17	1.60	0.28	0.25

CONT, basal diet containing 0.3 and 1.7 mg/kg of natural Se and pyridoxine respectively; MSeB$_6$10, basal diet supplemented with 0.3 mg/kg of sodium selenite (Sigma–Aldrich) and 10 mg/kg of hydro-chloride pyridoxine (P9755, Sigma–Aldrich); and OSeB$_6$10, basal diet supplemented with 0.3 mg/kg of Se-enriched yeast (Alltech, Inc.) and 10 mg/kg of hydro-chloride pyridoxine (P9755, Sigma–Aldrich). SUPPL, average values of MSeB$_6$10 + OSeB$_6$10.

36 downregulated genes (37.5%), compared with MSeB$_6$10. A total of 44 genes had an absolute fold change ≥ 2, and four of them were ≥ 3. The ten most up- and down-regulated genes are listed in Table 6. The complete list of genes related with each comparison are shown in Supplementary Table 2, see section on supplementary data given at the end of this article.

Biological process analysis of the unique DE genes

For the analysis of biological processes related with DE genes in PEB after maternal supplementations, the common unique GSs between comparisons were excluded and only the exclusive unique GSs of each comparison were selected (6, 173, and 25 exclusive genes for MSeB$_6$10 vs CONT, OSeB$_6$10 vs CONT, and OSeB$_6$10 vs MSeB$_6$10 comparisons respectively) and uploaded to GORILLA.

Regarding the OSeB$_6$10 vs CONT comparison, four distinct biological processes (gene expression, translation, regulation of translation, and mitotic cell cycle) were stimulated (FDR q-value < 0.05). When comparing MSeB$_6$10 vs CONT and OSeB$_6$10 vs MSeB$_6$10, no biological process was selectively stimulated by MSeB$_6$10 and OSeB$_6$10 respectively (FDR q-value > 0.05).

Expression of genes involved in glutathione and SeGPX metabolism

Although not influenced by treatments, several genes related with the methionine cycle and transsulfuration pathway were identified (Table 7). Also, the genes responsible for the metabolism between forms of pyridoxine (dietary HCl-pyridoxine vs metabolic P-5-P) were identified: pyridoxamine 5′-phosphate oxidase (*PNPO*, fold change ranging from −0.2 to 0.1 and average $P = 0.41$) and *PDXK* (identified by RT-qPCR).

Other ROS disposal-related genes

Treatment effects on the expression of genes related with ROS balance included upregulation of *GLRX3* (1.7 fold change, $P < 0.01$), *PRDX4* (1.7 fold change, $P < 0.01$),

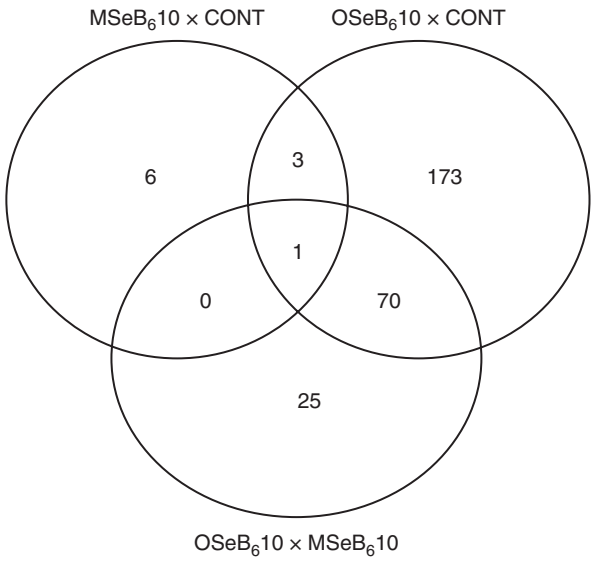

Figure 3 Venn diagram summarizing the microarray analysis of MSeB$_6$10 vs CONT, OSeB$_6$10 vs CONT, and OSeB$_6$10 vs MSeB$_6$10 comparisons.

Table 4 Differentially expressed genes in the expanded blastocysts of gilts supplemented with $MSeB_6$10 compared with CONT group.

Genes	Description	Fold change	*P* value
Up- and down-regulated genes			
PCBP2	Poly(rC)-binding protein 2, transcript variant X6	2.1	1.73×10^{-2}
DALRD3	DALR anticodon-binding domain containing 3	2.0	3.43×10^{-2}
CCT3	Chaperonin-containing TCP1, subunit 3 (gamma)	1.9	1.58×10^{-2}
PAAF1	Proteasomal ATPase-associated factor 1, transcript variant X1	1.9	1.62×10^{-2}
EIF4G2	Eukaryotic translation initiation factor 4 gamma 2	1.8	2.97×10^{-2}
MCL1	Myeloid cell leukemia sequence 1 (BCL2-related)	1.7	2.81×10^{-2}
TUBA1A	Tubulin, alpha 1a	1.7	3.34×10^{-2}
HSD17B14	Hydroxysteroid (17-beta) dehydrogenase 14	1.7	2.49×10^{-2}
TMEM129	Transmembrane protein 129, transcript variant 1	1.7	4.76×10^{-2}
HAAO	3-Hydroxyanthranilate 3,4-dioxygenase	−1.9	8.79×10^{-3}

and *COQ6* (1.7 fold change, $P<0.01$) in the $OSeB_6$10 vs CONT comparison, and downregulation of *TXN* (fold change ranging from −2.4 to −1.9, $P<0.01$) and microsomal glutathione transferase 2 (*MGST2*; fold change ranging from −2.6 to −2.0, and $P<0.05$) in the $OSeB_6$10 vs CONT and $OSeB_6$10 vs $MSeB_6$10 comparisons. Although not Se-dependent, it is noteworthy to mention that major antioxidant enzymes such as superoxide dismutases and catalase were not influenced by treatments.

Validation of microarray data by RT-qPCR

RT-qPCR confirmed the previous findings for *COQ6*, *GLRX3*, *PRDX4*, and *TXN*, with expression trends similar to the microarray study. In contrast, RT-qPCR analysis indicated no difference in relative *PDXK* expression among treatments ($P=0.38$) and that *SEPSECS* was upregulated in the $OSeB_6$10 group compared with CONT ($P<0.01$) (Fig. 4).

Discussion

Blood Se concentrations slightly decreased from the first estrus until 5 days of gestation in CONT gilts fed the basal diet containing a natural level of 0.3 mg/kg of feed of Se. Such response occurred despite the fact that the present basal dietary Se level was largely higher than the basal level found in cereals grown in Se-deficient soils (Mahan *et al*. 2005) and also higher than the NRC (1998, 2012) recommendations of 0.15 mg/kg. The Se source effect on whole blood Se concentration of gilts is consistent with previous reports (Schrauzer 2000, Svoboda *et al*. 2008, Fortier *et al*. 2012). The absorbed MSe is converted into functional selenoproteins containing selenocysteine (Sec), with slight storage in organs (Windisch 2002). The organic source is either metabolized to reactive forms of Se or stored as SeMet during protein synthesis (Schrauzer 2003).

The enrichment analysis indicated a marked difference of DE genes among $MSeB_6$10 vs CONT,

Table 5 Top ten up- and down-regulated genes in the expanded blastocysts of gilts supplemented with $OSeB_6$10 compared with CONT group.

Genes	Description	Fold change	*P* value
Upregulated genes			
ETNPPL	Ethanolamine-phosphate phospho-lyase	3.9	2.13×10^{-5}
AK4	Adenylate kinase 3-like 1	3.1	1.62×10^{-4}
EEF1A2	Eukaryotic translation elongation factor 1 alpha 2	3.0	7.57×10^{-6}
CDV3	Protein CDV3 homolog (LOC100627162)	3.0	1.24×10^{-5}
EEF1A2	Eukaryotic translation elongation factor 1 alpha 2	2.9	1.01×10^{-4}
IL6	Interleukin 6 (interferon, beta 2)	2.9	9.14×10^{-4}
HENMT1	Small RNA 2′-*O*-methyltransferase-like (LOC100152743)	2.9	1.48×10^{-4}
EEF1A2	Eukaryotic translation elongation factor 1 alpha 2	2.8	2.91×10^{-6}
ST3GAL5	Lactosylceramide alpha-2,3-sialyltransferase-like (LOC100522988)	2.8	3.05×10^{-5}
ATP6V0D2	ATPase, H+ transporting, lysosomal, V0 subunit d2	2.7	1.35×10^{-5}
Downregulated genes			
CHAC1	ChaC, cation transport regulator homolog 1	−5.0	2.38×10^{-3}
STC2	Stanniocalcin 2	−3.6	5.31×10^{-4}
MT1L	Metallothionein-1C-like (LOC100739663)	−3.0	3.18×10^{-5}
NLN	Neurolysin (metallopeptidase M3 family)	−3.0	4.16×10^{-6}
ASNS	Asparagine synthetase (glutamine-hydrolyzing)	−2.9	3.24×10^{-6}
RGN	Regucalcin-like (LOC100523295)	−2.9	2.32×10^{-4}
CYP51A1	Cytochrome P450, family 51, subfamily A, polypeptide 1	−2.8	2.87×10^{-6}
MGST2	Microsomal glutathione *S*-transferase 2	−2.6	2.21×10^{-5}
AIF1	Allograft inflammatory factor 1	−2.6	1.36×10^{-3}
MGST2	Microsomal glutathione *S*-transferase 2	−2.5	4.26×10^{-5}

Table 6 Top ten up- and down-regulated genes in the expanded blastocysts of gilts supplemented with $OSeB_6$10 compared with $MSeB_6$10 group.

Genes	Description	Fold change	*P* value
Upregulated $OSeB_6$10 genes			
ETNPPL	Ethanolamine-phosphate phospho-lyase	3.4	2.18×10^{-3}
AK4	Adenylate kinase 3-like 1	3.0	2.01×10^{-3}
IL6	Interleukin 6 (interferon, beta 2)	2.8	2.21×10^{-3}
ATP6V0D2	ATPase, H+ transporting, lysosomal, V0 subunit d2	2.7	1.50×10^{-3}
MYL6	Myosin, light chain 6, alkali, smooth muscle and non-muscle	2.5	4.82×10^{-2}
RUVBL2	RuvB-like 2, transcript variant X3	2.4	2.10×10^{-2}
EEF1A2	Eukaryotic translation elongation factor 1 alpha 2	2.4	1.46×10^{-2}
ST3GAL5	Lactosylceramide alpha-2,3-sialyltransferase-like (LOC100522988)	2.4	5.25×10^{-3}
KPNA3	Karyopherin alpha 3	2.3	1.13×10^{-3}
EEF1A2	Eukaryotic translation elongation factor 1 alpha 2	2.2	1.16×10^{-2}
Downregulated $OSeB_6$10 genes			
CHAC1	ChaC, cation transport regulator homolog 1	−4.0	6.72×10^{-3}
STC2	Stanniocalcin 2	−3.4	8.99×10^{-3}
ASNS	Asparagine synthetase (glutamine-hydrolyzing)	−2.9	8.15×10^{-3}
RGN	Regucalcin transcript variant 1	−2.8	1.74×10^{-2}
NLN	Neurolysin	−2.4	1.44×10^{-3}
SLC7A9	Solute carrier family 7 (amino acid transporter light chain), member 9	−2.4	4.36×10^{-2}
MT1L	Metallothionein 1L	−2.3	9.89×10^{-3}
IDI1	Isopentenyl-diphosphate delta isomerase 1	−2.3	1.20×10^{-3}
RGN	Regucalcin, transcript variant 2	−2.2	4.30×10^{-2}
MINOS1	Mitochondrial inner membrane organizing system 1	−2.2	1.39×10^{-3}

$OSeB_6$10 vs CONT, and $OSeB_6$10 vs $MSeB_6$10 comparisons. Globally, $OSeB_6$10 supplementation to gilts affected PEB transcriptome more drastically than $MSeB_6$10, suggesting an additive effect of B_6 with OSe but not with MSe. Such responses might be related to the fact that the organic source of Se can become an integral part of many proteins, not only antioxidant enzymes, whereas MSe is largely directed toward antioxidant metabolism, as mentioned above. Moreover, the complexity of the metabolic interaction between B_6 and OSe compared with MSe (Le Floc'h *et al*. 2012) could also explain the higher number of genes affected.

Although it cannot be ruled out that feeding a chemical compound or yeast extracts as supplements may have conferred differences in genes expression, metabolic indicators such as circulating Se showed that the Se status was clearly altered and modulated by sources and levels of dietary Se. It appears unlikely that such major systemic changes on the metabolism of sows were due to the yeast fraction of dietary organic Se. The marked DE differences (especially from $OSeB_6$10) would require such type of systemic route for metabolism because they were observed on organisms (expanded blastocysts) that are self-regulated and genetically dissimilar (half allogenic) from those (dams) receiving directly the treatments. In terms of Se transfer from dams to embryos, whatever the treatment, Se was undetectable in the uterine flushing. Therefore, although the pre-implantation embryo is nourished by the uterine fluids, it appears that this route of Se transfer is negligible for embryonic metabolism. However, before conception, the Se content of pre-ovulatory oocytes might have been influenced by the systemic effect of different blood Se concentrations, considering that the follicular fluid in which the oocyte matures is a product of the transfer of blood constituents (Fortune 1994). In such way, more Se could have been available to embryos obtained from $OSeB_6$10-treated gilts. Unfortunately, our Se determination technique does not allow measurements at the embryonic level.

Brennan *et al*. (2011), studying the effects of OSe and MSe dietary supplementation on gene expression profiles in oviduct tissue from broiler-breeder hens, also found that, even without differences in tissue Se concentration, gene expression differed between the respective treatments. This would suggest that Se concentrations may not be always a reliable indicator of the impact of Se on physiological processes.

Although treatment-dependent DE was not observed, several genes of interest were identified in the methionine cycle and transsulfuration pathway, suggesting that PEBs are capable of synthesizing SeGPX. However, surprisingly, at this stage of development neither $MSeB_6$10 nor $OSeB_6$10 was able to over-stimulate SeGPX synthesis. These observations are consistent with Fortier *et al*. (2012), who showed that the SeGPX content in 30-day porcine embryos did not differ between MSe and OSe maternal supplementation. These intriguing results raise many hypothesis regarding Sec evolutionary events, Se metabolism, and selenoproteins synthesis.

Leinfelder *et al*. (1988) and Bock *et al*. (1991) proposed that during the evolution of Sec within the genetic code, UGA was initially a sense codon for this amino acid and that Sec was used not only for synthesis of SeGPX but widely for several enzymes. Later in the evolutionary process, oxygen was introduced into the atmosphere which selected against the use of Sec as this amino acid is oxygen-labile. Consequently, the global utilization of Sec decreased during evolution and became limited to anaerobic environments only. As the expanded blastocyst

Table 7 Sulfur and Se antioxidation-related genes expressed in the porcine expanded blastocyst.

GS	Description	*A*-value[a]	S.D.
Common metabolism for Se and sulfur			
MAT1A	*S*-adenosylmethionine synthetase isoform type 1	6.1	0.3
MAT2A	*S*-adenosylmethionine synthetase isoform type 2	9.1	1.8
AHCY	*S*-adenosylhomocysteine hydrolase	9.5	1.0
AHCYL1	*S*-adenosylhomocysteine hydrolase-like 1	6.3	0.5
AHCYL2	*S*-adenosylhomocysteine hydrolase-like 2	6.4	0.4
CTH	Cystathionine gamma-lyase	7.7	2.9
GSH synthesis, recycling, and utilization			
GCLC	Glutamate–cysteine ligase catalytic subunit	8.0	3.8
GCLM	Gamma-glutamylcysteine synthetase regulatory subunit	8.9	1.7
GSS	Glutathione synthase	6.9	0.6
GSR	Glutathione reductase	7.0	0.8
GGT1	Gamma-glutamyltransferase 1	6.5	1.0
GGT5	Gamma-glutamyltransferase 5	6.5	0.6
GGT6	Gamma-glutamyltransferase 6	6.2	0.4
GGT7	Gamma-glutamyltransferase 7	6.2	0.3
GPX5	Glutathione peroxidase 5	6.2	0.3
GPX8	Glutathione peroxidase 8	6.2	0.5
GSTA1	Glutathione *S*-transferase alpha 1	6.1	0.4
GSTA4	Glutathione *S*-transferase alpha 4	7.3	0.9
GSTA5	Glutathione *S*-transferase alpha 5	6.7	0.5
GSTCD	Glutathione *S*-transferase, C-terminal containing domain	7.1	2.2
GSTK1	Glutathione *S*-transferase kappa 1	7.1	0.5
GSTM1	Glutathione *S*-transferase mu 1	6.6	0.8
GSTM3	Glutathione *S*-transferase mu 3	7.7	1.7
GSTO1	Glutathione *S*-transferase omega 1	9.4	1.8
GSTO2	Glutathione *S*-transferase omega 2	7.6	0.8
GSTP1	Glutathione *S*-transferase pi 1	9.8	2.4
GSTT1	Glutathione *S*-transferase theta 1	6.2	1.0
GSTT4	Glutathione *S*-transferase theta 4	6.0	0.3
GSTZ1	Glutathione transferase zeta 1	6.3	0.3
MGST2	Microsomal glutathione transferase 2	8.4	2.9
MGST3	Microsomal glutathione transferase 3	7.7	0.7
PTGES	Prostaglandin E synthase	9.5	2.4
PTGES2	Prostaglandin E synthase 2	6.7	0.8
PTGES3	Prostaglandin E synthase 3	9.8	2.9
Exclusive metabolism of SeGPX			
SCLY	Selenocysteine lyase	6.0	0.4
SEPHS2	Selenophosphate synthetase 2	6.4	0.4
SARS	Seryl tRNA ligase	6.9	0.9
SARS2	Seryl tRNA ligase 2	6.1	0.5
PSTK	Phosphoseryl-tRNA kinase	6.7	0.3
SEPSECS	Sep (*O*-phosphoserine) tRNA:SectRNA synthase	6.2	0.3
EEFSEC	Eukaryotic elongation factor selenocysteine-tRNA specific	6.2	0.2
SECISBP2	Selenocysteine insertion sequence-binding protein 2	6.6	0.6
SECISBP2L	Selenocysteine insertion sequence-binding protein 2 like	6.3	0.5
Selenoproteins			
SEPN1	Selenoprotein N, variant 1	6.1	0.2
SEPP1	Selenoprotein P, variant 1	6.1	0.4
DIO3	Iodothyronine 3	6.4	1.3
SELK	Selenoprotein K	8.8	2.2
SELM	Selenoprotein M	7.1	1.8
SELO	Selenoprotein O	8.1	2.5
SELS	VCP-interacting membrane protein, variant 2	8.8	1.9
SELT	Selenoprotein T	6.4	0.4
SELV	Selenoprotein V	6.5	0.2
TXNRD1	Thioredoxin reductase 1	6.6	0.6
TXNRD2	Thioredoxin reductase 2	6.1	0.2
TXNRD3	Thioredoxin reductase 3	7.0	0.9
GPX2	Glutathione peroxidase 2	6.2	0.3
GPX3	Glutathione peroxidase 3	7.0	1.2
GPX6	Glutathione peroxidase 6	7.4	1.7

[a]The level of gene expression was indicated by the average *A*-value reflecting spot intensity higher than the background intensity. Average *A*-value from dark-corner spot intensity was consider to be background (mean *A*-value = 6.0 and S.D. = 0.3).

develops under hypoxic and even almost anaerobic conditions (Fischer & Bavister 1993), the possibility exists that Sec was required and used for global protein synthesis in preference to selenoproteins synthesis, as suggested by Gladyshev & Kryukov (2001), in anaerobic organisms.

The enzymatic activity of many antioxidative enzymes in embryos is much lower than in adults, and thus,

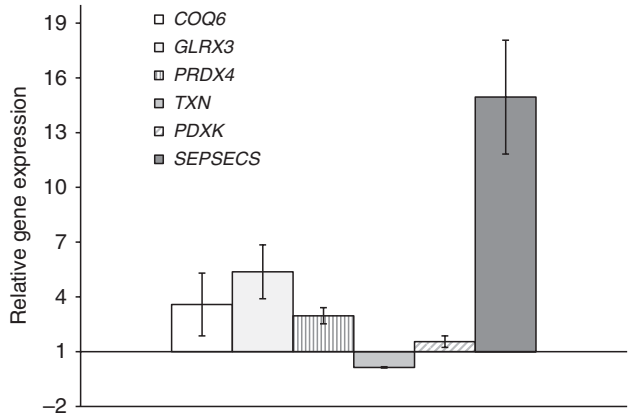

Figure 4 RT-qPCR expression trends for *COQ6*, *GLRX3*, *PRDX4*, *TXN*, *PDXK*, and *SEPSECS* in porcine expanded blastocysts recovered from $OSeB_6$10 supplemented gilts, shown as relative gene expression to CONT (±S.E.M.). *PPIA* was used to normalize the mRNA expression levels. $OSeB_6$10=basal diet supplemented with 0.3 mg/kg of Se-enriched yeast (Alltech, Inc.) and 10 mg/kg of hydro-chloride pyridoxine (P9755, Sigma–Aldrich).

embryos are particularly sensitive to oxidative damage (Parman *et al*. 1999, Winn & Wells 1999). The SeGPX system is a major antioxidant complex in the organism, and is responsive to Se and B_6 status (Roy *et al*. 2011), because B_6 catalyzes the conversion of SeMet to Sec, which is further incorporated into SeGPX and other selenoproteins. The RT-qPCR analysis revealed that *SEPSECS* (the gene that encodes a protein responsible for the last step in the synthesis of selenocysteinyl-tRNA (Sec)) was upregulated in $OSeB_6$10 gilts. However, as this was the only SeGPX-related gene to be upregulated, it cannot be stated that this overall metabolic pathway was in fact over-stimulated.

In addition, Gardiner & Reed (1995), studying the recovery of glutathione (GSH) in mouse pre-implantation embryos, found that the blastocyst cannot convert sulfur–methionine into sulfur–cysteine via the cystathionine transsulfuration pathway. This is a relevant and critical finding for Se metabolism, because SeMet follows the sulfur–methionine biochemical pathway toward Sec (Sunde 1984). Thus, SeMet would not produce Sec and, therefore, might direct Se toward general protein synthesis or methylation. Se compounds methylation enzymes cystathionine gamma-lyase (CTH) and indolethylamine *N*-methyltransferase (INMT), along with other general protein methylation enzymes glutamic-oxaloacetic transaminase 1 and 2 (*GOT1* and *GOT2*), glutamic-pyruvate transaminase (*GPT*), betaine-homocysteine *S*-methyltransferase (*BHMT*), and protein-L-isoaspartate *O*-methyltransferase (*PCMT1*) were not DE, whereas several genes related with general elongation factors were upregulated and biological process related with translation, regulation of translation, and mitotic cell cycle was stimulated.

This finding reinforces the concept that, at this stage of development, Se is likely directed to the global protein synthesis in the PEB. The contribution of B_6 to this effect appeared important because when the comparison was made between $OSeB_6$10 vs $MSeB_6$10, few genes related with general elongation factors were upregulated and no biological process was selectively stimulated by $OSeB_6$10.

Se-glutathione peroxidase not only depends on Se and B_6 but also on the availability of GSH for its enzymatic activity (Ufer & Wang 2011). According to Yoshida *et al*. (1993), the amount of GSH in the porcine oocyte exceeds the needs for fertilization, but during pre-implantation development, embryonic GSH continuously decreases and reaches a nadir at the blastocyst stage, whereas endogenous ROSs are produced at their highest level at this time. These low GSH and high ROS levels are possibly required for normal differentiation (Pierce *et al*. 1991, Parchment 1993), in which ROS may act as an important regulatory system for apoptosis in the mouse blastocyst (Pierce *et al*. 1991). According to Gardiner & Reed (1994), hydrogen peroxide in mouse blastocoel fluid causes apoptosis of pre-trophectodermal cells of the blastocyst, whereas the inner-cell-mass cells, destined to become the embryo, are protected from the toxic effects of hydrogen peroxide. The same authors suggest that this protection is performed via mechanisms that utilize GSH. This apparent control of embryo GSH production is illustrated by the present microarray and RT-qPCR data showing that *TXN* and *GLRX* (genes encoding proteins involved in recycling of GSH) were respectively down- and up-regulated in $OSeB_6$10 gilts.

Despite the lack of upregulated genes related with the transsulfuration pathway of methionine toward the SeGPX system, $OSeB_6$10 maternal supplementation differently expressed other genes related with cell antioxidant defense such as those of the thioredoxin (*GLRX3*, *PRDX4*, and *TXN*) and monooxygenase (*COQ6*) families. For the thioredoxin family, these genes are involved with key regulators of redox signaling and consequently of the intracellular effects of ROS (Fisher *et al*. 1999, Nordberg & Arnér 2001, Ahsan *et al*. 2009). They are also critical for the control of DNA damage, cell proliferation, and differentiation (Laurent *et al*. 1964, Schenk *et al*. 1996, Saitoh *et al*. 1998) in PEB. For the monooxygenase family, *COQ6* is required for the biosynthesis of ubiquinone, one of the most potent lipophilic antioxidants found to be involved in the protection of cell damage by ROS (Hyun *et al*. 2006).

The identification of these DE genes is coherent with the previously described stimulation of mitotic cell cycle as a biological process in $OSeB_6$10 gilts. In this way, it is noteworthy to mention that, although not statistically significant, the greatest number of viable embryos was collected from these gilts and this in absence of degenerated embryos (Table 3). Therefore, considering

previous results reported by Fortier *et al*. (2012) in which enhanced 30-day embryos development was observed in OSe gilts without effect on embryo SeGPX activity, it can be hypothesized that these antioxidative defenses from the thioredoxin and monooxygenase families could act as supplementary mechanisms to the SeGPX system in PEB from $OSeB_6$10-supplemented sows and this may persist thereafter for at least the first third of gestation.

In conclusion, maternal dietary OSe supplementation with pyridoxine considerably stimulated the transcriptome of PEB as compared with unsupplemented gilts. For the comparison between sources of Se with equivalent supplemental pyridoxine, there was also an important response in terms of DE genes, but no specific biological process was identified. Porcine expanded blastocysts are potentially capable of synthesizing selenoproteins, including SeGPX; however, both Se sources with pyridoxine did not influence the SeGPX metabolic pathway at this stage.

In PEB from $OSeB_6$10 supplemented gilts, other members of the thioredoxin family, as well as ubiquinones, appear to complement the antioxidant defense and the regulation of cell proliferation. However, the mechanisms by which OSe combined with pyridoxine controls these processes remain to be explained.

Supplementary data

This is linked to the online version of the paper at http://dx.doi.org/10.1530/REP-14-0408.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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