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Peri-conceptional nutritional restriction alters transcriptomic profile in the peri-implantation pig embryos

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ABSTRACT

Restricted nutritional consumption during the peri-conceptional period affects the potential for DNA methylation and alters endometrial transcriptomic profile during the peri-implantation period. The restricted diet fed to females during the peri-conceptional period may affect the transcriptomic profile in peri-implantation embryos. In the present study, the transcriptome of embryos of normal-diet-fed gilts was determined and compared with that in embryos of restricted-diet-fed gilts during the peri-implantation period. The restricted-diet-fed gilts were fed forage, in which the dose of proteins and energy had been reduced by 30% compared to the normal diet (Polish Norms of Nutrition). To clarify the issue Agilent's Porcine (V2) Two-Color Gene Expression Microarray 4 × 44 was used. Analysis of the microarray data revealed that the expression of 787 genes with known biological function were consistently altered (496 up- and 291 down-regulated) in embryos. The accurately annotated genes were organized into five categories and 18 subcategories containing 62 biological pathways. The qPCR analysis of ten selected genes [i.e., 5 acid phosphatase, tartrate resistant (*ACP5*), high mobility group box 2 (*HMGB2*), prostaglandin-endoperoxide synthase 2 (*PTGS2*), arachidonate 12-lipoxygenase (*ALOX12*), adiponectin receptor 2 (*ADIPOR2*), DNA (cytosine-5)-methyltransferase 1 (*DNMT1*), steroidogenic acute regulatory protein (*STAR*), progesterone receptor membrane component 2 (*PGRMC2*), progestin and adipoQ receptor family member 7 (*PAQR7*) and serpin family A member 1 (*SERPINA1*)] confirmed altered gene expression in embryos of restricted-diet-fed gilts. The insight into embryonic transcriptome indicates that female under-nutrition during the peri-conceptional period may create alterations in the pattern of genes expressed in the peri-implantation embryos.

1. Introduction

In female reproduction there are two important periods during which there are particularly profound epigenetic modifications and reprogramming. The first one encompasses ovulation, fertilization and zygote formation and cellular differentiation of embryos during morula-blastocyst stages. The second one occurs during the peri-implantation period (Reik et al., 2001; Dean et al., 2005;

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Tomizawa et al., 2011). Early developmental mammalian embryos, therefore, might be particularly susceptible to epigenetic modifications in the response to environmental perturbations during these specific developmental periods (MacLaughlin and McMillen, 2007; Fleming et al., 2012).

Restricted nutritional consumption by females during the peri-conceptional period affects intrauterine microenvironment and has the potential to affect DNA methylation in the uterus (Tsuma et al., 1996; Prunier and Quesnel, 2000; Franczak et al., 2016a, b). Furthermore, feeding a restricted diet induces alterations in the endometrial transcriptome, modifies the concentration of total cholesterol, phosphorus and calcium in peripheral blood plasma and estradiol-17β (E_2) in uterine flushings of gilts during the peri-implantation period (Franczak et al., 2016a, b; Zglejc et al., 2018). Early developmental pig embryos have a unique hormonal environment in the uterine lumen (Franczak and Bogacki, 2009). It is still not clear if restricted diet that is fed to females during the peri-conceptional period affects the transcriptomic profile of peri-implantation embryos. Hence, the present study was conducted to focus on the alterations in embryonic transcriptome during the peri-implantation period caused by the restricted diet fed to females during the peri-conceptional period. Up- or down-regulated genes with known biological functions, the most significantly altered biological pathways, gene ontology (GO) terms combined with functional annotations and interaction network of the selected set of genes expressed during the peri-implantation period in embryos harvested from restricted-diet-fed gilts, were identified.

2. Material and methods

All experiments were approved by the Animal Ethics Committee, University of Warmia and Mazury in Olsztyn, Poland – decision no. 54/2015/DTN.

2.1. Animals and collection of embryos for RNA extraction

The animals and the detailed protocol of feeding, the composition and nutritive value of the normal and restricted-diet and specification of daily intake of energy, total protein and lysine were described previously (Franczak et al., 2016a; b; Zglejc and Franczak, 2017). Briefly, embryos were harvested during the peri-implantation period (i.e., days 15–16 of pregnancy) from restricted-diet-fed gilts, in which the protein and energy content was reduced by 30% (Fig. 1, $n = 4$) and normal-diet-fed gilts ($n = 4$), with the ratio of feed being consistent with the Norms for Nutrition of Pigs (Poland, 1993) during the peri-conceptional period (Fig. 1). Entire embryos of normal and restricted-diet-fed gilts were flushed from uterine horns with PBS (20 ml). The whole embryos (i.e., inner cell mass and trophoblast) were quickly removed from uterine flushing's, snap-frozen in liquid nitrogen for 24 h and stored at -80 °C until RNA isolation.

2.2. RNA isolation

The RNA was isolated from entire embryos (inner cell mass and trophoblast) with the use of a Qiagen RNesasy Mini Kit (Qiagen, Valencia, CA, USA), according to the manufacturer's protocol. To obtain pure RNA, DNAse (RNase free DNAse Kit, Qiagen Valencia, CA, USA) was added during the RNA isolation procedure. Subsequently, RNA integrity (RIN), quality and quantity were evaluated with microcapillary electrophoresis (2100 Bioanalyzer, Agilent Technologies, Santa Clara, CA, USA). Only samples with RIN of greater than 8 were used for further analysis.

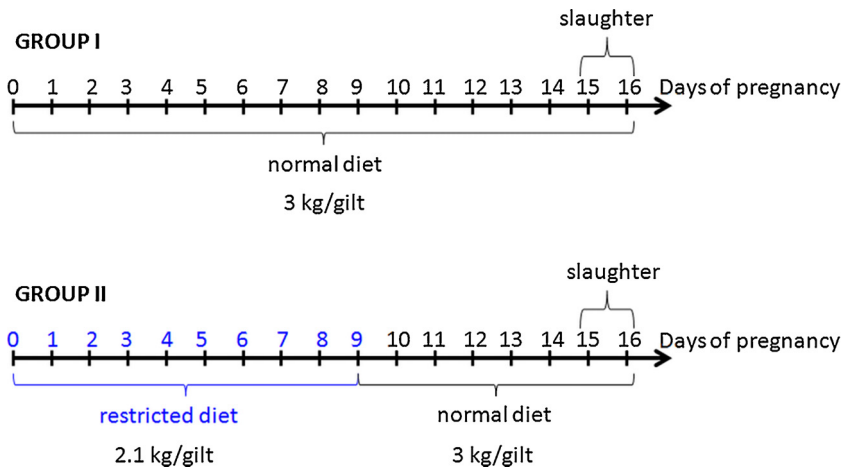

Fig. 1. Experimental design for the manner of feeding. Group I – normal-diet-fed gilts. The daily amount of feed was 3 kg/gilt until days 15–16 of pregnancy. Group II – restricted-diet-fed gilts during peri-conceptional period, whereas gilts were fed for 9 days amounts of 2.1 kg/gilt. After day 9 of pregnancy, gilts of group II were fed 3 kg/gilt until days 15–16 of pregnancy.