ALTERED PROTEIN COMPOSITION OF PORCINE FOLLICULAR FLUID DUE TO A HIGH-FIBRE DIET AND THE POTENTIAL FOR OPTIMISATION OF IN VITRO CULTURE MEDIA

Altered protein composition of porcine follicular fluid due to a high fibre diet and the potential for optimisation of *in vitro* culture media.

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This study reports a proteomic analyses on porcine follicular fluid (FF) obtained from a previous nutritional trial, where oocytes from gilts fed a high fibre (HF) diet for the first 19 days of their third oestrous cycle produced blastocysts with more cells following *in vitro* maturation (IVM) and IVF compared with oocytes from control-fed (CON) pigs. Oocytes were matured in TCM-199 supplemented with LH and FSH at 0.5 mg mL1 and 10% of the animals' own pooled FF. Following IVF, resultant embryos were cultured in NCSU-23 medium for 6 to 7 days. We hypothesize that FF protein composition is altered by the HF diet and that this confers the reproductive benefits previously observed. The FF had previously been stored at 808C after the IVF trials and was thawed for the current study, which compared the protein composition of pooled Day 19 FF from 12 CON pigs and 12 HF pigs. These gilts were a subset of the pigs described above with the largest FF volumes. The protein composition of pooled FF from 6 CON pigs whose oocytes produced blastocysts was compared with FF from 6 CON pigs whose oocytes did not produce blastocysts. The same analysis was carried out with the 6 HF pigs that produced blastocysts and the 6 HF pigs that did not produce blastocysts. Equal numbers of samples from animals were selected for experimental balance. The proteomic study was carried out in duplicate. Abundant proteins were depleted from FF by Proteominer enrichment. Samples were labelled by isotopic di-methylation, where in each analysis, one sample was labelled with a heavy methyl group, the other with a light methyl group. Proteins were detected by liquid chromatography tandem mass spectrometry. Protein identifications were filtered using a 1% false discovery threshold and a requirement for two or more peptides detected for each protein. Differentially expressed proteins (DEPs) were identified as having heavy/light ratios greater than 1.2 or less than 0.8, which are recognised cut-off points for differential expression in proteomics. Over 140 DEPs were detected between CON and HF samples, indicating a nutritional influence on FF protein composition. Over one-third (37%) of these DEPs were also differentially expressed in the blastocyst versus no blastocyst analyses, suggesting that the altered FF protein composition may affect IVF outcome. DEPs were submitted into Ingenuity Pathway Analysis to highlight associated canonical pathways and upstream regulators. Top ranking canonical pathways detected included coagulation system, acute phase response, and LXR/RXR activation pathways. Potential upstream regulators detected by IPA included transforming growth factor beta, tumour protein P53, and beta-oestradiol. These pathways and upstream regulators could serve as potential avenues for elucidating the mechanism(s) by which the HF diet results in the reproductive benefits and could lead to the refinement of IVM and IVF culture conditions.

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