Introduction:
Since the beginning of in vitro production of embryos, it has been clear that in vitro matured and cultured embryos were less developmentally competent compared to their in vivo counterparts. It has never been clear as to how or why they differ. Therefore, it was our goal from this experiment to use next generation Illumina sequencing to show what differences exist at the transcript level in in vitro matured MII porcine oocytes compared to in vivo matured MII porcine oocytes.

Experimental Design and Methods:
In vivo matured oocytes were collected from three gilts with similar genetic background by surgically flushing the oviduct on day 2 after standing estrus (Day 0). The flush was searched and MII oocytes were collected in pools of 10 and snap frozen in liquid nitrogen (N2). In vitro matured MII oocytes were collected by euthanizing 3 gilts with similar background and recovering ovaries at which point follicles 2-8mm in size were aspirated. Oocytes with multiple cumulus cell layers and uniform cytoplasm were placed in M199 supplemented with 5ug/mL LH and FSH as well as 10ng/mL EGF for 42 hours. At that time, cumulus cells were stripped by vortexing in hyaluronidase. They were then evaluated visually, and MII oocytes were collected in pools of 10 and snap frozen in liquid N2.

For Illumina Sequencing, total RNA was extracted from three pools of 10 oocytes for both treatments with All Prep DNA/RNA Micro Isolation Kit (Qiagen). cDNA was synthesized using oligo (dt) primed reverse transcriptase with SuperScript III (Invitrogen). Second strand cDNA was synthesized using DNA polymerase I and sequenced using Illumina Genome Analyzer II. The resulting 100 bp reads were aligned to a custom built porcine transcription. A normalization factor was generated to take into account variations in total expression and sample loading. A 95% confidence interval was then constructed around the mean number of reads to detect the average number of reads for each transcript that we could be confident was statistically greater than 0.

Transcripts were determined to be different by performing a student’s t-test with a P<0.1, minimum of 7 reads in at least one of the treatments (found by the 95% confidence interval) and ≥2 fold difference.

Real-time PCR was done to validate the sequencing using the comparative $C_t$ method on IQ Real-time PCR system with Bio-Rad SYBR green mix. Expression levels are calculated relative to the reference sample and the housekeeping gene, YWHAG. The $\Delta\Delta C_t$ values were checked for normality and then either analyzed or log transformed and then analyzed using PROC GLM in Statistical Analysis System (SAS; SAS Institute, Cary NC).

The misrepresented transcripts were further characterized using the functional annotation tool DAVID (http://david.abcc.ncifcrf.gov/tools.jsp). DAVID was used to determine which pathways and biological processes are enriched in the differentially regulated transcripts.

Misrepresented pathways characterized by DAVID
Overrepresented in IVM Oocytes
1. Pathways to cancer
2. Spliceosome
3. Cell Cycle
4. Ubiquitin Mediated Proteolysis

Underrepresented in IVM oocytes
1. Cytoskeleton Regulation
2. T cell receptor Signaling Pathway
3. Ubiquitin Mediated Proteolysis
4. Cell Cycle

Conclusion:
This data shows that there is an abundance of misrepresented transcripts and several altered pathways in in vitro matured oocytes. This data set provides itself to be a tool that may give clues to improve the in vitro maturation process, making in vitro matured oocytes more like their in vivo counterparts and thus have improved subsequent developmental competence.