Increased male sex ratio and significantly altered gene expression coincide with increased pace of development in bovine embryos produced and cultured in vitro

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Studies from several mammalian species as mouse, pig, bovine or human indicated a higher male-to-female sex ratio among the fastest developing embryos. These observations suggest differences in the pace of embryonic development after in vitro fertilization (IVF) and culture. However, the outcome of similar studies is still inconclusive in several species. Here we studied the sex ratio in our bovine in vitro production (IVP) system. In order to elucidate possible molecular reasons for differential developmental speed, we focused on the latest in vitro stage, the hatched blastocyst, and analyzed gene expression by RNAseq and subsequent bioinformatics analysis of affected pathways. Sexing of individual blastocysts was performed by a Y-chromosome-specific PCR test.

We observed that also in our IVP system, in vitro produced and cultured bovine embryos showed a remarkably different pace of development. Most blastocysts reached the hatching stage eight days (day 8) after fertilization. However, hatching of a considerable number of blastocysts was observed one day earlier (day 7) or one day later (day 9). Sexing revealed that among embryos with the fastest pace of development, reaching the stage of hatching seven days after fertilization, there was an excess number of males (>60%). This excess was still present but showed a decreasing tendency among blastocysts hatching eight or even nine days after fertilization.

Transcriptome analysis by RNAseq revealed 256 differentially expressed genes (DEGs) between fast and normally developing embryos, reaching the stage of hatching seven or eight days after fertilization. Among these genes, 79 showed higher and 177 lower abundance in fast developing embryos. Ingenuity pathway analysis revealed Oxidative Phosphorylation, Estrogen Receptor Signaling, Neutrophil Extracellular Trap Signaling Pathway as the most up-regulated and Mitochondrial Dysfunction, Sirtuin Signaling Pathway, Glycolysis I, Endoplasmic Reticulum Stress Pathway as the most down-regulated canonical pathways in fast compared to normally developing embryos.

From these data, we conclude that IVP bovine embryos show quite different paces of development, with males being more frequently in the most advanced group. An increased developmental pace was clearly associated with a differential gene expression pattern. However, it is unclear, if this is the consequence of the unbalanced sex ratio in early hatching embryos or due to the increased developmental pace.

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