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Comparative proteomic analysis of bovine embryos developed in vivo or in vitro up to the blastocyst stage

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Despite many improvements in *in vitro* systems and embryo culture media, *in vitro* derived embryos still display morphologic and metabolic differences making them less viable and cryoresistant compared to their *in vivo* counterparts. To bring knowledge to this issue, we used a quantitative proteomic approach to compare early bovine embryos developed *in vivo* or *in vitro*.

Eleven Holstein females were synchronized for estrus, treated for ovarian superovulation and inseminated twice with frozen-thawed semen. Between days 1.7 and 7.5 after the first artificial insemination, *in vivo* embryos were recovered after slaughter by flushing of the oviducts and uterus. In addition, embryos were produced *in vitro* using slaughterhouse bovine ovaries, the same male semen and a culture medium with no serum or protein supplementation. All embryos were washed three times and stored at −80°C before analysis. Proteins from pools of grade-1 embryos at the 4-6 cells, 8-12 cells, morula, compact morula and blastocyst stages (4 embryos/pool; 3-4 pools/stage, total of 38 pools) were analyzed by nanoliquid chromatography coupled to tandem mass spectrometry (nanoLC-MS/MS). Proteins were identified using the UniProt *Bos taurus* database and quantified by label-free spectral counting. Proteins quantified with minimum 2 normalized weighted spectra (NWS) in at least one condition were analyzed using principal component analysis (PCA) and ANOVA. The hierarchical clustering of differentially abundant proteins (DAPs; ANOVA p-value < 0.05) were done using Spearman correlations and the gplots package of RStudio. Functional analysis of DAPs was carried out using the Metascape on-line tool.

A total of 3,028 proteins were identified in embryos, of which 227 were specific to *in vivo* embryos and 49 to *in vitro* embryos. The PCA of the 2,186 proteins quantified with more than 2 NWS showed a clear separation of embryo pools according to their stage of development and origin (*in vivo* vs. *in vitro*). Oviductin, also known as oviduct-specific glycoprotein 1 (OVGP1), and clusterin were among the most overabundant proteins in *in vivo* compared to *in vitro* embryos at all stages. Three clusters of 999 DAPs (ANOVA's p-value <0.05) according to the origin were evidenced: 463 DAPs with higher abundance *in vivo* than *in vitro* across development (cluster 1); 314 DAPs with less abundance *in vivo* than *in vitro* before the morula stage (cluster 2); and 222 DAPs with less abundance *in vivo* than *in vitro* after the morula stage (cluster 3). Proteins in cluster 1 were mainly involved in carbohydrate metabolic pathways, cellular detoxification and cadherin binding. Proteins in cluster 2 were mainly involved in protein synthesis. Proteins in cluster 3 were mainly involved in mitochondria-dependent activity and cytoskeleton organization.

These data provide a first exhaustive proteomic comparison between *in vivo* and *in vitro* embryos in cattle and bring new insights into the molecular contribution of the maternal environment (ovarian follicle, oviduct and uterus) to the preimplantation embryo. Moreover, the DAPs identified constitute valuable markers of embryo quality for the assessment of new *in vitro* systems, closer to *in vivo* conditions.

Keywords: Embryo, proteomics, cattle.