Journal of Equine Veterinary Science 34 (2014) 188

Contents lists available at ScienceDirect

Journal of Equine Veterinary Science

journal homepage: www.j-evs.com

# "Cumulomics": Mapping the equine cumulus cells' proteome

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The cumulus accompanies the oocyte in intimate contact and represents a promising source to study metabolism during *in-vitro* maturation (IVM) non-invasively for the respective oocyte. Mapping of the proteome and it's alteration during the maturation process can provide novel insights in metabolism during IVM with the potential to adjust media and protocols for this *in-vitro* procedure, which furthermore may help to resolve limitations of *in-vitro* fertilization (IVF) in horses. Using proteomics a wide range of proteins can be detected in minimal sample amounts, providing the opportunity to overcome the necessity to use large pools of cumulus complexes (CCs) and study the cumulus cells' proteome even in species where access to ovaries is limited. For this reasons the aim of this study was to define the proteome of the equine cumulus complex in small pools of six CCs for compact (n = 3 pools) and expanded cumulus (n = 3 pools). Cumulus oocyte complexes (COCs) were collected from excised ovaries by follicular scraping and separated into compact and expanded according to morphology. Two of the three expanded pools were retrieved after *in-vitro* maturation. COCs were washed four times in PBS to remove follicular fluid and culture media prior to any downstream analysis. CCs were separated from their oocytes using a stripper pipette, quick-frozen and stored in liquid nitrogen until further analysis. Proteomic workflow on cumulus cells was conducted by a "hotpot" method based on high-power ultrasound technology for cell lysis and tryptic digestion. Samples were analyzed by nano-HPLC MS/MS technology and data analyzed using an equine specific protein database. In total 374 different proteins (FDR <1%) were detected in the six samples containing pooled compact or expanded cumulus cells (Figure 1). The unique protein detected in all three samples containing compact cumulus and in none of the samples containing expanded cumulus was Thrombospondin-1. In bovine cumulus cells the Thrombospondin-1 gene (THBS1) is overexpressed 6h after the LH surge in preovulatory follicles compared to two hours prior LH surge This is not necessarily a discrepancy as developmental competent equine oocytes are accompanied by an expanded and bovine oocytes by a compact CC.Data of this study assign the "Cumulomics" approach to be capable to reveal changes in cumulus cell metabolism during maturation using a minimal sample amount and revealed one protein, which was only in the compact CC consistent above detection limit.

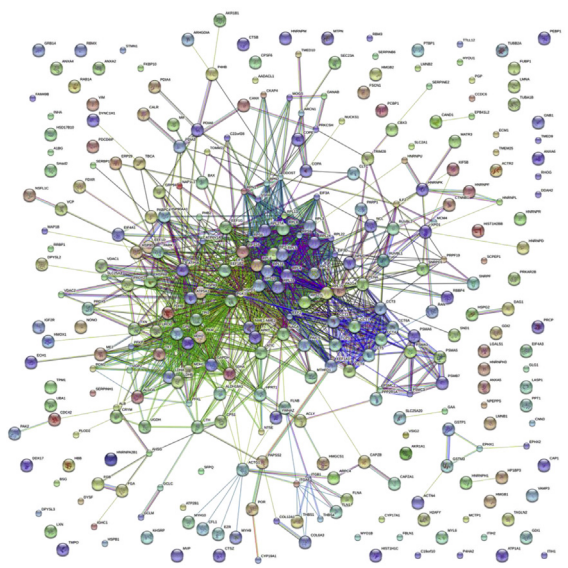

**Fig. 1.** STRING simulation of protein-interactions for proteins detected in compact and expanded cumulus complexes [ Jensen LJ,et.al, Nucleic Acids Res 2009;37:D412–6].

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