Global proteomic analysis of tertiary follicles from sheep

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Introduction

Folliculogenesis is the process of formation, activation, growth and maturation of ovarian follicles, the functional unit of the ovary composed of an oocyte surrounded by somatic cells (Figueiredo et al., 2008). The transition from preantral to antral follicles is marked by the appearance of a cavity in the granulosa cells and/or beginning antrum formation, controlled by autocrine/paracrine mechanisms and other metabolic processes (Erickson, 1983). The present study was conducted to identify the major proteome signature tertiary follicles from ewes.

Methodology

Ovaries from five adult, cross-breed ewes were collected and under sterile conditions, the ovarian cortex was sliced and antral follicles were dissected individually. Fifty tertiary follicles were pooled and resuspended in lysis buffer for protein extraction. Then, proteins were digested with trypsin, desalted and lyophilized. Next, tryptic peptides were loaded into LC-MS/MS, followed by analysis of raw data using PatternLab's software, with *Ovis aries* protein database downloaded from UniProt. Identified proteins were analyzed for gene ontology terms using FunRich software, protein clusters and metabolic pathways using DAVID software.

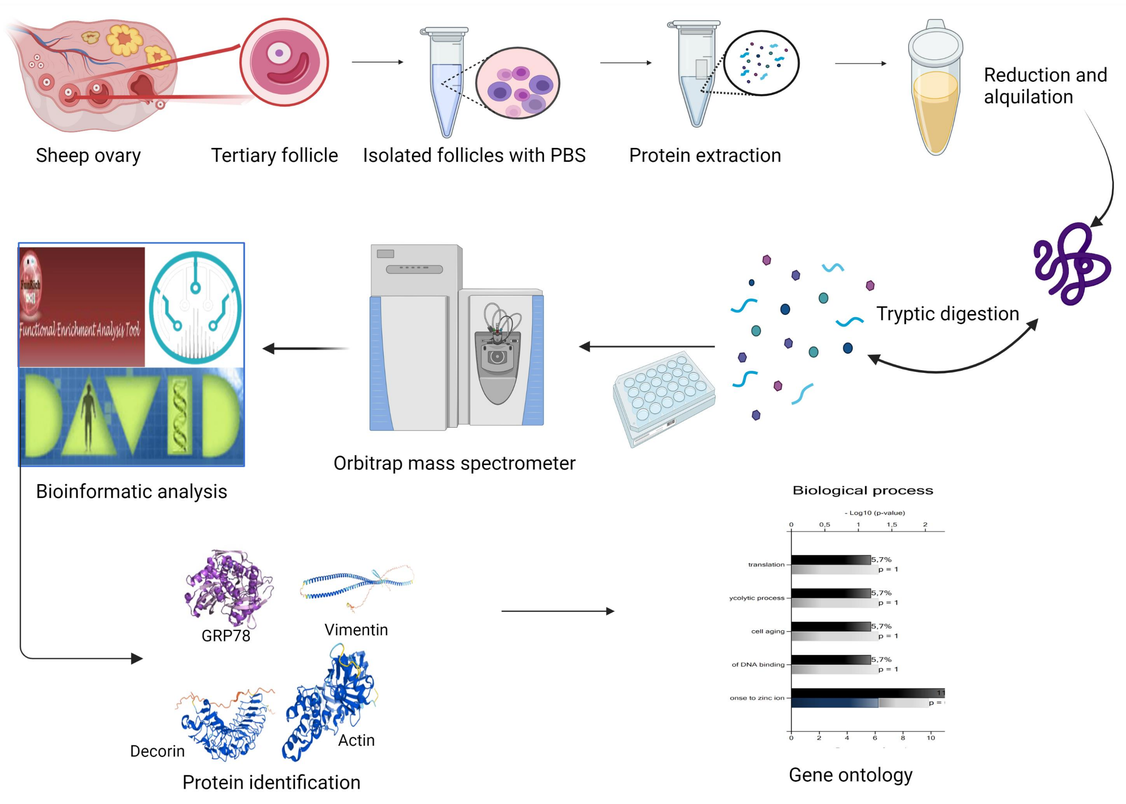

Figure 1. Proteomic strategy applied for protein identification and bioinformatic analysis of proteins identified in early tertiary follicles.

Results and Discussion

There were 1.536 proteins detected in the tertiary ovine follicles, based on the current methods. Based on enrichment score and spectrum count, vimentin and actin cytoplasmic were the most abundant proteins in the ovine tertiary follicles, followed by 78 kDa glucose-regulated protein, albumin, disulfide isomerase, HATPase_c domain-containing protein, heat shock protein alpha, among others.

Important biological processes linked to follicle proteins were cellular response to zinc ion (11,8%), translation, glycolytic process and cell aging (5,7%). The most expressive cellular components were defined as cytoplasm (36,2%), nucleus (27,7%), cytosol (23,4%), extracellular region (12,8%) and cytoskeleton (6,4%). Molecular functions were mainly reported as metal ion binding (24,4%), zinc ion binding (12,2%), oxygen binding (9,8%) and ATP binding (9,8 %).

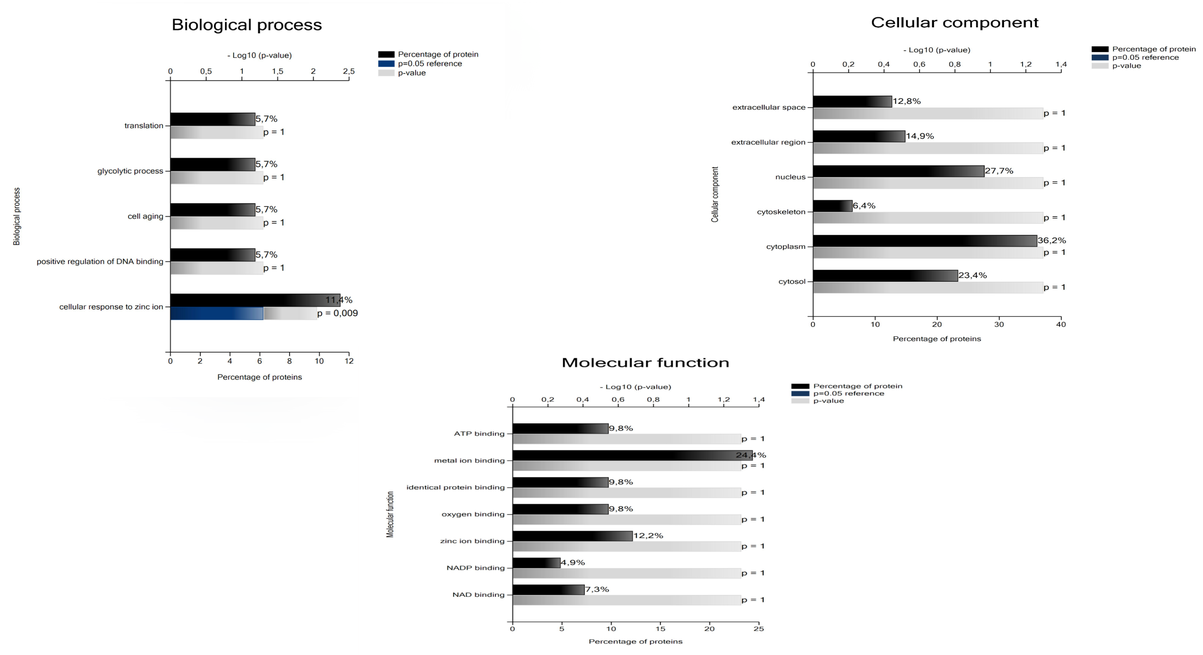

Figure 2. Gene ontology diagram showing the functional distribution of the most abundant detected proteins in ovine early follicles (biological processes, molecular function and cellular components).

Conclusion

The current study is the first comprehensive description of the proteome of tertiary follicles in the ovine species. We also describe a detailed method for extraction and characterization of follicle proteins using HPLC-coupled mass spectrometry and bioinformatic tools. The present results should contribute to the identification, in future studies, of genes and functional proteins important for follicular development and oocyte maturation.

Bibliographic references

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Acknowledgment

CAPES

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