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Study of the biochemical changes in the cytoplasm of *in vitro* matured bovine oocytes in media enriched with Vascular Endothelial Growth Factor-D by Raman microscopy

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In recent years, numerous studies have focused on the optimization of *in vitro* embryo production (IVP) of animals of productive interest, including bovines. *In vitro* maturation (IVM) of oocytes is the first stage of the IVP and can determine the performance of the process and the quality of the future embryo. Therefore, the optimization of the IVM media would make it possible to increase the number of competent oocytes. The aim of this work is to evaluate the impact of vascular endothelial growth factor-D (VEGF-D) on the cytoplasm of bovine oocytes during the IVM, by Raman microscopy.

The spectra were taken from the cytoplasm of oocytes that were subjected to the IVM process for 18 and 22 h in the absence (control: 18H and 22H) and in the presence of the factor (18H-F and 22H-F). For a more precise analysis of cytoplasmic changes during IVM, the zona pellucida was removed from the oocytes by enzymatic digestion. The average spectrum of the 18H-F group showed a marked increase in the intensities of the characteristic protein bands with respect to the 18H spectrum. When the spectra that completed the maturation time (22h) were related to each other, only subtle changes in intensity were observed in some bands assigned to amino acids. A principal component analysis (PCA), multivariate statistical calculation, was implemented for the signals that showed the greatest spectral difference. The scatter graph obtained by the PCA revealed that the oocytes of groups 18H-F and 22H-F are grouped in the sector of the graph representing the presence of intense characteristic protein bands. We previously demonstrated that, throughout IVM, there is a gradual accumulation of proteins in the cytoplasm of bovine oocytes, being greater at the end of the process (22h). All these data confirm that the supplementation of the maturation media with VEGF-D increases the protein content in the cytoplasm of bovine oocytes and, therefore, could have a favourable impact on the performance of IVP.

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