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## **Development of a O/W nanoemulsion as a delivering vehicle of lipids of interest for application on bovine embryonic growth.**

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The objective of the present study was to develop an O/W nanoemulsion (NE), with drops below 100 nm of diameter, emulsified by lecithin and encapsulating cholesterol and L-alpha phosphatidylcholine. The purpose of this NE is the application during the early stages of bovine embryonic growth, seeking to increase its viability. Firstly, the effects of lecithin concentration, homogenization pressure with a microfluidizer, and O/W ratio on the emulsion stability index (ESI) and mean droplet size were investigated. NE was prepared by the emulsification-evaporation technique. For that, the organic phase was mixed under stirring for 10 min, and then mixed with aqueous phase using an Ultra-Turrax® homogenizer. Subsequently, this coarse emulsion was homogenized with a microfluidizer to reduce its droplets size. And then, the NE was placed in a rotaevaporator to eliminate residual chloroform. The obtained NE was homogeneous, white and opaque with a milky appearance. A central rotational composite design was used to optimize the NE fabrication parameters, which were as follows; pressure (100 MPa), O/W fraction (20/80) and percentage of lecithin (1%). The three optimized formulations (F1, F2 and F3) used in the embryo application showed an ESI between 0.046 and 0.086, which reflects a high stability with a very low incidence of destabilization phenomena such as creaminess or phase separation. The average droplet diameter analyzed by laser diffraction was approximately 70-80 nm, being able to transit across the embryonic zona pellucida with pores of average 90 nm of diameter. Atomic force microscopy images clearly confirm morphology of spherical droplets with an average diameter size of lower than 100 nm. The NE behave as Newtonian fluid with a viscosity of  $\sim 2.1 \times 10^{-3}$  Pa.s. Embryonic developmental analysis was not affected by the NE and the blastocyst rates were similar between control and NE groups, and the cleavage rate was higher in the embryos treated with the F2 formulation when compared to the control group. As conclusions, the optimized manufacturing parameters allowed the production of a very stable NE, able to be used as a deliver vehicle for lipids with potential application to increase and improve bovine embryonic development.