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ADRP LOCALIZATION WITH IMMUNOFLUORESCENCE IN BOVINE EMBRYOS

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Background: Adipocyte Differentiation-Related Protein (ADRP) also known as adipophilin is a protein found in bovine embryos which links lipids. Its expression is highly correlated with stored lipids during lipid droplet formation. It could be thus used as a marker of the lipid accumulation and of the embryos' quality.

Methods: The aim of this study was to localize ADRP in bovine embryos using immunofluorescence. Three groups of embryos were produced, 1) *in vivo*, 2) *in vitro* in basic medium (modified synthetic oviduct fluid, mSOF, supplemented with 5% foetal calf serum), and 3) *in vitro* on a bovine oviduct epithelial cell (BOEC) monolayer with basic medium. Embryos produced *in vivo* were recovered from superovulated beef heifers at Day 7.5 post insemination. All blastocysts were fixed with 4% paraformaldehyde, permeabilized with Triton 0.5% and blocked in 2% bovine serum. Then they were incubated with guinea pig anti-ADRP (Progen, #GP-40, 1:1000) overnight and with secondary donkey anti-guinea pig antibody conjugated to fluoroprobe Dylight 649. Lipid droplets were stained with the Nile Red dye and chromatin using Hoechst 33258. The embryos were then individually placed between glass coverslips before submission to laser excitations.

Results: As the commercial anti-ADRP was a polyclonal antibody, it was purified by Western blot and concentrated. The results observed with these 2 anti-ADRP (commercial form and purified form) were similar, thus the commercial form can be considered as "monoclonal". Adipophilin is strongly localized around lipid droplets in embryos produced *in vitro* (figures 1 and 2). The same result was observed in embryos produced *in vivo* (figure 3). ADRP is pointed up by the arrows below.

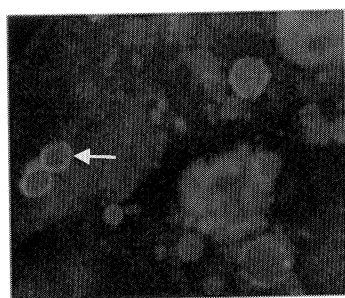


Figure 1: *in vitro* embryos in basic medium, x63

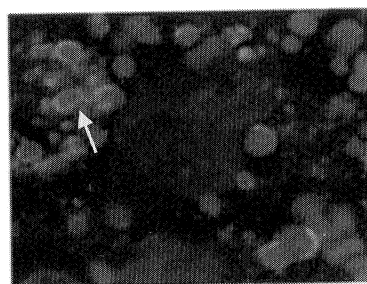


Figure 2: *in vitro* embryos with BOEC monolayer, x63

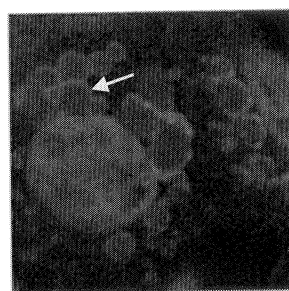


Figure 3: *in vivo* produced embryos, x63

Conclusion: In *in vitro* and *in vivo* bovine embryos, Adipophilin wraps lipid droplets. Its quantification can be a fine marker to evaluate lipid droplets in bovine embryos which are known to be related to embryo cryoresistance.