

***In vitro* development of bovine embryos: morphological analysis of β -catenin and actin**

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SUMMARY

During embryonic preimplantation development, cell adhesion plays an important role in the differentiation of trophectoderm and morphogenesis of blastocyst. Cell adhesion begins at compaction and requires a package of molecules consisting of α -catenin, β -catenin, uvomorulin as well as other proteins linking catenins to cadherins and actin. These molecules are synthesised well in advance of compaction, and at this time they redistribute from all the membranes to those associated with intercellular contact.

Compaction in bovine embryos begins at 16-32 cell stage, one cell cycle after the activation of embryonic genome: this is a crucial phase since an high percentage of in vitro generated embryos arrest their growth around this stage.

Aim of the present study was to verify whether the developmental block is associated with an altered localisation of cytoskeletal components taking place around compaction.

Bovine embryos generated in vitro were fixed on Day 5 post insemination, when compaction events begin, and processed for the immunolocalisation of β -catenin, filamentous (F-actin) and non-filamentous actin (G-actin). Embryos were at different developmental stages, due to individual differences of developmental kinetic. Embryos with >16 blastomeres were considered to be of highest quality because their percentage (38,2%) corresponded ($r=0,96$) to the rate of embryos that develop to the blastocyst stage on Day 7 post insemination (38.8 %) in the control group.

While F-actin and G-actin morphology did not differ between embryos, β -catenin distribution was different depending on the stage of development. β -catenin was localized at cytoplasmic and cortical level in all the blastomeres in $93.4\pm 3.8\%$ of embryos with >16 cells. The proportion of embryos whose blastomeres presented this pattern was inversely related to the number of blastomeres, ranging from $56.6\pm 7.6\%$ in 8-16-cell embryos to only $12.4\pm 3.9\%$ in 0-4-cell ones.

Our results indicate that the developmental block observed in in vitro generated embryos is accompanied by an altered localisation of cytoskeletal components.