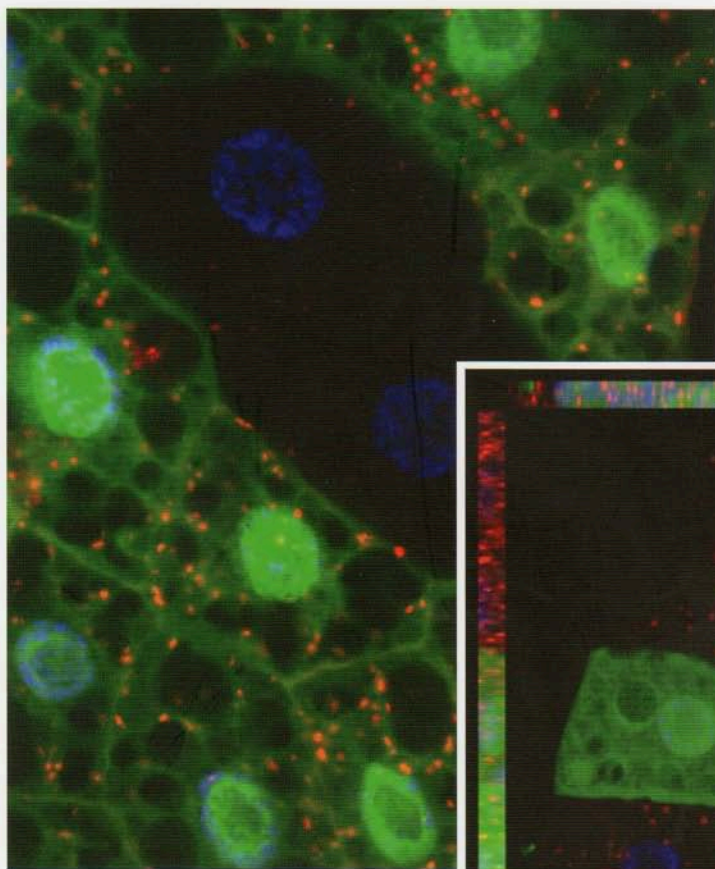


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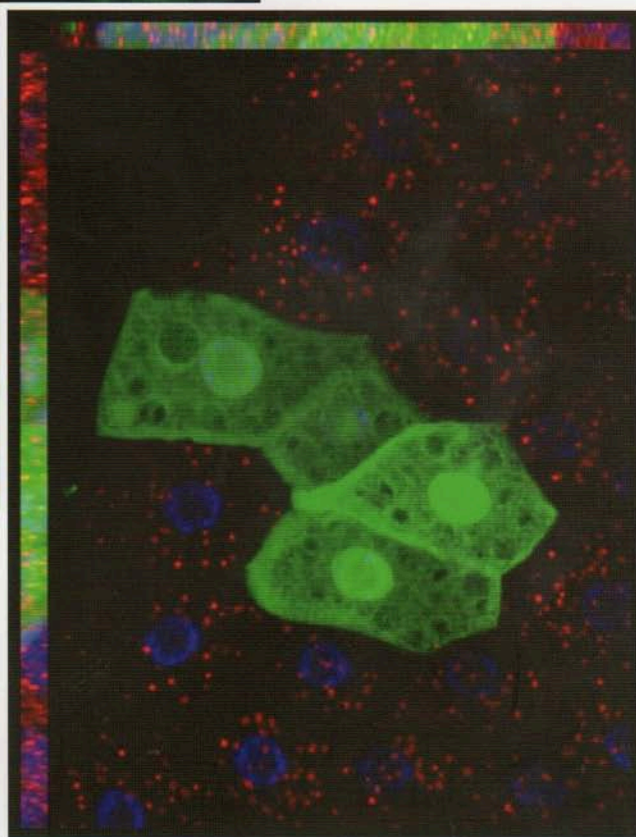
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THE EFFECT OF EARLY DIFFERENTIATION PATHWAY INHIBITORS ON CELL FATE DECISION IN PREIMPLANTATION RABBIT EMBRYOS

Babett Bontovics¹, Jaroslav Slamecka^{2,3}, Pouneh Maraghechi¹, László Hiripi¹, Alexander V. Makarevich², Peter Chrenek^{2,3}, Kinga Németh¹, Zsuzsanna Bősze¹, Elen Gócza¹

¹ *Agricultural Biotechnology Center, Gödöllő*

² *Animal Production Research Centre, Nitra, Slovak Republic*

³ *Faculty of Biotechnology and Food Science, Slovak University of Agriculture, Slovak Republic*

Genes that direct pluripotency in the mouse are well described, however, their expression pattern during the rabbit embryonic development is not yet known. Culture medium supplemented with different inhibitors (PD032590 (PD), CHIR99021 (CH), Y27632 (Y) and A8301 (A)) enabled embryonic stem cell (ESC) lines to be derived efficiently from mouse and rat embryos.

The aim of our work was to analyse the expression pattern of embryonic stem cell-specific markers in rabbit embryos and attached blastocysts following cultivation in different combinations of the inhibitors of GSK3 (CH), MEK (PD) and TGFβ-R (A), involved in differentiation; and a ROCK inhibitor (Y). We cultured rabbit embryos from two or from eight-cell-stage till 4.5day or 6 days,



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than compared the development of rabbit embryos in different conditions. We supplemented the normal embryo cell culture medium (RDH) with 1i (RDH+PD), 2i (RDH+PD+CH), 2i+ (RDH+PD+Y), 3i (RDH+PD+CH+Y) and 4i (RDH+CH+Y+A). The expression level of epiblast-specific Oct4 and hypoblast-specific Gata4 and Gata6 were examined in blastocysts and attached embryoblasts. At 4.5 day, the expression level of Oct4 was significantly higher in blastocysts, cultured in 2i culture medium, but there was no significant difference in GATA4, GATA6 expression on mRNA and protein level, compared to embryos cultured in the other conditions. On day 6, we observed reduced expression levels of epiblast- and hypoblast-specific factors. We noticed that rabbit embryos cultured in 3i and 4i were retarded in their development.

We confirmed that the culture medium supplemented with 2i is the most appropriate condition for rabbit embryo development in terms of preserving the viability of embryos and increasing the proportion of epiblast cells in the blastocysts. We hope that we can maintain the pluripotency of the epiblast-derived rabbit embryonic stem cells in 2i medium supplemented with LIF or other factors during long-term cultivation.

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