**Summary of the doctoral thesis. Joanna Romanek**

# HIGH HYDROSTATIC PRESSURE IMPACT ON CRYOPRESERVATED PORCINE MESENCHYMAL STEM CELLS QUALITY

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The aim of the present study was to examine the influence of five varied HHP values on porcine mesenchymal stem cells (MSC) on survival rate, proliferation rate, cells multipotency (transcript expression of *C–myc, Rex1 and Sox2*) and apoptosis level (expression of phosphatidylserine–PS, *survivin* at RNA level and Bax at protein level). Based on the obtained results, two HHP values were selected for further evaluation.

The MSC were isolated from the porcine bone marrow and cultured *in vitro*. Before cryopreservation and storage in liquid nitrogen, MSC were subjected to HHP 20MPa, 30MPa, 40MPa, 50MPa and 60MPa for 1 h at 24°C. Directly after thawing and/or after 8 days of *in vitro* culture cells were subjected to trypan blue staining, cells counting, real–time PCR, western–blotting and fluorescence microscopy. Only Bax protein expression was estimated immediately after HHP treatment to estimate exclusive impact of HHP on cells.

MSC subjected to HHP 40MPa and 60MPa were selected for further experiments. After cryopreservation and thawing, cells were analyzed for caspase 8 activity (by fluorescence microscopy), protein expression of survivin, CAD (by whole mount immunofluorescence) and protein expression of Bax, $Bclx_L$ and $Bclx_S$ (by western–blotting). The statistical differences in survival rate, proliferation rate, transcripts and proteins levels were assessed by Tukey's post–hoc One–way ANOVA test.

To test indirectly influence of HHP on functional activity of MSC, bovine *in vitro* maturated oocytes were *in vitro* fertilizated and obtained embryos were cultured in 4 different systems: 1.SOF medium; 2.SOF medium in coculture with MSC; 3. SOF medium in coculture with MSC subjected to 40MPa HHP and 4. SOF medium in coculture with MSC subjected to 60MPa HHP. The quality of developed blastocysts was analized by TUNEL method. Differences in blastocysts rates) were assessed using chi–square test ($\chi^2$). The TUNEL results regarding all blastocysts' nuclei, apoptotic nuclei, and DCI were calculated by Tukey's post–hoc One–way ANOVA test. In all tests differences with a probability value of 0.05 or less were considered significant.

No significant difference was noted in the Bax protein expression in MSC subjected only to HHP in any of six analyzed groups.

The high significant difference (P<0.02) was observed in proliferation rate between MSC subjected to 40MPa HHP and control group. The high significant difference (P<0.001) was noted between MSC viability immediately after thawing in cells subjected to 60MPa HHP and control and significant difference (P<0.05) was observed between MSC subjected to both 40MPa and 50MPa HHP and control. No significant difference was noted in the survival rate after 8 days of *in vitro* culture, PS exposure, level of *C–myc*, *Rex1*, *Sox2* and *survivin* genes expression in all analyzed groups and control. Also no significant difference was observed in the caspase 8 activity and CAD, survivin, Bax, $Bclx_L$, $Bclx_S$ proteins expression between MSC subjected to HHP (40MPa, 60MPa) and control. The significant differences were observed in the level of *Rex1* gene expression between MSC subjected to 60MPa and 30MPa (P<0.002) and between 60MPa and 20MPa (P<0.05). Also the significant difference was noted in *survivin* gene expression between cells subjected to 60MPa and 30MPa (P<0.05). The highest number of obtained blastocysts was observed when embryos were cultured in the control group in relation to both embryos cultured in presence of MSC after 60MPa HHP and embryos cocultured with nontreated MSC (*P*<0.005). Additionally, the significant difference (*P*<0.05) was noted between control group and embryos cultured in presence of MSC after 40MPa HHP.

In conclusion: MSC subjected to 40MPa HHP showed increased survival and proliferation rates. HHP of 50MPa and 60MPa has influence on MSC survival rate only. HHP doesn't have impact on MSC multilineage potential and does not induce apoptosis in these cells. Obtained results of blastocysts *in vitro* culture in coculture with MSC (HHP treated and non treated) imply that co culture with MSC have negative impact on the blastocysts' developmental rates. Although the apoptotic index of blastocysts cultured with MSC subjected to HHP was lower comparing to both remaining groups, these results are not reliable due to limited number of analysed embryos.