In many species including humans, rats, mice and monkeys embryonic stem cells (ESCs) can be derived from the inner cell mass of pre-implantation embryos, so-called blastocysts. These cell lines can self-renew and grow indefinitely *in vitro*. However, despite many efforts, *bona fide* pluripotent stem cell lines could not yet be derived from cattle. While some progress has been made, reported bovine ESC lines do not meet the stringent criteria for pluripotency, i.e. the capacity to form germ line chimeras. Recently, embryonic stem cell lines were derived from porcine embryos using novel, so-called "expanded culture conditions". These cells, named Expanded Potential Stem Cells (EPSCs), possess the special characteristics of being able to differentiate not only into different cell types of the body but also into extraembryonic tissue.

The aim of the proposed research is to establish bovine Expanded Potential Stem Cells (bEPSCs) from *in vitro* produced embryos. These cells should remain undifferentiated indefinitely under appropriate *in vitro* culture conditions and at the same time possess the ability to differentiate into any cell type of the body *in vitro* and *in vivo,* including their contribution to the germ line and eventually the production of functional gametes.

In the proposed studies, we plan to use a novel Expanded Potential Stem Cell Medium (EPSCM) to establish bovine embryonic stem cell lines. Embryonic stem cells will be isolated from 8-to-16-cell-stage embryos and from blastocysts **(Objective 1)** and characterized by morphology, *in vitro* growth, karyotype stability, pluripotency gene expression, accessibility for genetic manipulation and differentiation potential *in vitro* **(Objective 2).** We anticipate that by using EPSC culture conditions we will be able to establish bovine Expanded Potential Stem Cell (bEPSC) lines. These stem cells would be similar to pre-implantation blastomeres, with the ability to generate germline chimeras after re-introduction into bovine pre-implantation embryos **(Objective 3)**.

The proposed work will provide unique bovine stem cell lines with expanded potential that can be great candidates to introduce multiple genetic modifications and enable the generation of transgenic animal models. These cell lines with expanded potential to differentiate into all embryonic and extraembryonic cell types could be of great interest for reproductive biotechnology as well as for innovative *in vitro* breeding methods.