

## **Isolation and characterization of progenitor cells derived from bone marrow and yolk sac of canine fetus.**

**Wenceslau Cristiane Valverde, MS**, Departamento de Cirurgia da Faculdade de Medicina Veterinária da Universidade de São Paulo, Av. Prof. Dr. Orlando Marques de Paiva, 87 CEP 05508 270 - Cidade Universitária São Paulo/SP – Brasil. Tel/Fax: +55 11 30917690, wenceslauvet@yahoo.com.br

**Wenceslau Cristiane V.<sup>1</sup> MS, Ambrosio Carlos E.<sup>1</sup> PhD, Martins Daniele S.<sup>1</sup> PhD Lizier Nelson F.<sup>2</sup> MS, Rubtsova, Nadezda V.<sup>3</sup> PhD, Kerkis Irina<sup>2</sup> PhD, Miglino Maria A.<sup>1</sup> PhD.**

<sup>1</sup> Departamento de Cirurgia da Faculdade de Medicina Veterinária da Universidade de São Paulo São Paulo, <sup>2</sup> Laboratório de Genética, Instituto Butantan, SP, Brasil; Institute of Cytology and Genetics of the Siberian Branch of the Russia of Academy of Science, Novosibirsk, Russia.

**Introduction** The fetus is a source of non-embryonic stem cells (SC), which has already resulted in numerous instances of actual clinical benefit to patients, such as Parkinson's disease, autoimmune diseases and others. They are easy to obtain and to expand undifferentiated, while presenting high differentiation abilities. The dog models mimic important aspects of human anatomy, physiology and pathology producing safety pre-clinical results after xenotransplantation of stem cells. On the other hand, the pet is a growing market of stem cells application. In our present study we aimed at isolation and characterization of yolk sac (YS) and bone marrow (BM) progenitor cells from canine fetus.

**Methods** All experimental procedures were approved by the Ethical Committee of the School of Veterinary Medicine and Animal Science of São Paulo University (Nº931/2006). Canine fetuses at 30 and 55 days gestation were obtained through an ovarian hysterectomy and under general anesthesia. The explants of YS tissues and BM cells flashed from femur bone were cultured in  $\alpha$ -MEM + 15% fetal bovine serum. Morphology of the cells was evaluated under inverted and by transmission electron microscopy (TEM). Antibodies: goat anti-Oct3/4; mouse anti-vimentin; mouse anti-VE-cadherin; rabbit anti-nestin; mouse anti-cytokeratin. Expression of anti-CD44 antibody was analyzed by flow cytometry. Osteogenic, adipogenic, neuronal differentiation assays as well as karyotype analysis were performed according routing protocols.

**Results** Two days after cultivation first fibroblast-like colonies appeared in BM cells culture as well as outgrowth of fibroblastic cells around YS explants was observed. TEM analysis demonstrates that the cells from both sources showed two cell populations: with a high nuclear-to-cytoplasmic ratio and fibroblast-like morphology. Both, YS and BM fibroblast-like cells react positively and uniformly with vimentin antibody and majority of cells were positive to nestin antibody. In addition cells isolated from BM present positive immunostaining with CD44 (~ 96,6 %) and cytokeratin. Several fibroblast-like cells isolated from YS were also positive for Oct 3/4 and VEGF antibodies. Both cell populations showed successful osteogenic and chondrogenic differentiation. In addition, BM derived fibroblast-like cells was able to produce neuron-like cells. The analysis of karyotype performed at the passages 6 and 7 revealed normal diploid chromosomes number (2n=78).

**Conclusions:** Our data suggest that undifferentiated cells can be isolated from BM and YS and maintained in culture during successive passages, presenting normal karyotype. TEM analysis indicates that BM and YS undifferentiated cells are composed by two different cell populations: i. cells present characteristic similar to very small embryonic-like (VSEL) stem and ii. cells present characteristic of bone marrow mesenchymal stem cells (MSC). Both described previously for human BM – MSC. Unexpectedly, antigen expression profile BM progenitor cells and differentiation capacity suggest their ectodermal commitment.