The Volume of Mesenchymal Stem Cells in the Peripheral Blood in Horses is Associated with Physical Stress

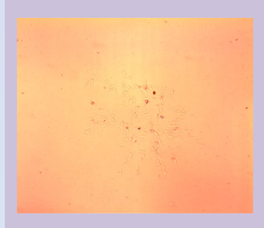
M. Trunda, **miroslav.trunda@genetickabanka.cz**,
K. Pavelcova, P. Hodacova, R. Klubal
Czech Gene Bank, Prague, Czech Republic

Introduction:

Peripheral blood is one of many sources of mesenchymal stem cells (MSCs). It is the easiest way to obtain them. However, the number of MSCs in peripheral blood is very low. Our objective was to determine whether physical exercise increases the amount of precursors MSCs in the peripheral blood of horses.

Materials and methods

In our study we used five warmblood sports horses. Peripheral blood was collected from the *vena jugularis*. Peripheral blood was collected from five horses in three phases. The first phase was at rest (horses were not used in work for three days, peripheral blood was collected in the morning in the stable). The second phase was two hours after the work and the third phase was 20 hours after the work. The work was one hour trot and gallop under rider. Collection of peripheral blood was 10 ml in syringe with heparine. The peripheral blood was transported to the laboratory during one hour. In the laboratory, the peripheral blood was separated. The mononuclear cells (MNCs) were separated from the peripheral blood immediatelly after the collection with help of Histopaq. Afterwards the mononuclear cells were cultured in A-MEM 89%, FBS 10%, PenStrep 1% (37°C, 5% CO2). Number, viability, and CD markers (CD44, CD90, CD105) of MSCs were determined using Bürker´s chamber and flow cytometry (FACS). Change of cultivation medium was performed after 3-4 days . During the three weeks of cultivation were controlled morphology of mesenchymal stem cells (MSCs).



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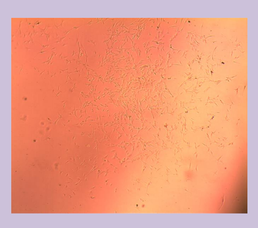
Day 5



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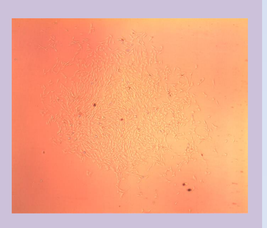

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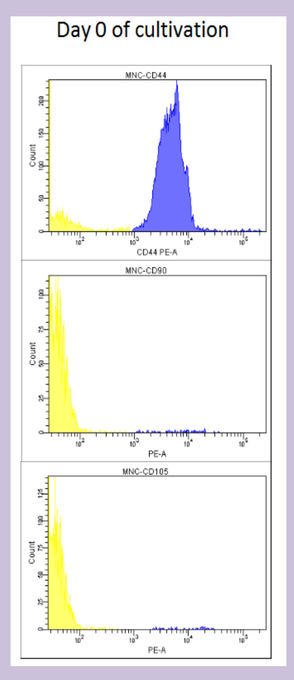
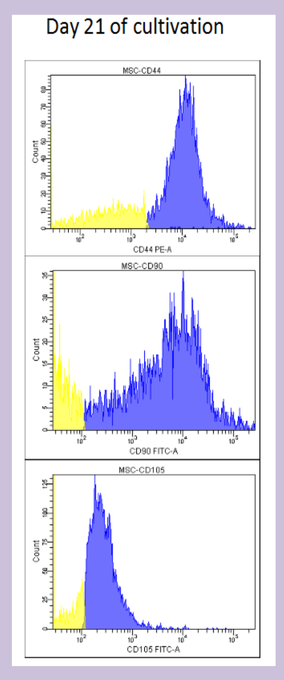
After three weeks of cultivation were trypsinized MSCs subsequently counted in a Bürker´s chamber, and viability analysis of the expression of CD markers (CD44 , CD90 , CD105) was performed on the FACS.





Number, CD markers an viability of MSCs in different phases

	Name of Horses	Number of MSCs	CD44/%	CD90/%	CD105/%	Viability/%
The first phase	Z	0.8×10^6	97.9	49.2	0.0	98.9
	S	0	88.6	4.5	3.3	95.8
	K	0	86.8	4.4	2.2	94.1
	M	0	90.6	7.0	2.5	95.5
	J	0	92.2	4.7	5.1	96.3

	Name of Horses	Number of MSCs	CD44/%	CD90/%	CD105/%	Viability/%
The second phase	Z	0	95.4	8.0	4.8	90.4
	S	0	96.9	9.7	4.1	90.9
	K	1.2×10^6	98.0	36.4	1.5	92.6
	M	0.7×10^6	82.3	88.1	0.0	77.7
	J	0	86.0	7.8	5.6	51.5

	Name of Horses	Number of MSCs	CD44/%	CD90/%	CD105/%	Viability/%
	Z	0	61.1	10.7	4.0	39.1
The third phase	S	1.4×10^6	95.6	66.7	1.5	78.7
	K	1.6×10^6	93.0	69.6	0.1	85.5
	M	1.9×10^6	94.5	36.0	2.9	72.1
	J	0.8×10^6	96.8	67.5	0.0	84.0

Results :

<u>The first phase (at rest):</u>

MSCs were isolated *in vitro* only in one horse. The horse was about 10 days after the diagnosis of a hoof abscess. Fibroblasts were isolated from two horses. In the remaining two horses did not adhere any MSCs.

<u>The second phase (2h after work):</u>

MSCs were isolated *in vitro* in the two horses in the previous group were negative for MSCs cultivation. Fibroblasts were isolated from one horse. Horse, which was positive in the first phase, did not adhere any MSCs.

<u>The third phase (20h after work):</u>

MSCs were isolated *in vitro* in the four horses. Horse, which was positive in the first phase, did not adhere any MSCs.

Conclusions/ Discussion:

From our work, it is obvious that physical stress has an effect on the migration of MSCs from the place of origin (niche) into the peripheral blood. It is a result of physiological tissue regeneration. For the horse (Z) that had a hoof abscess, we isolated enough of MSCs in the peripheral blood even without physical excercise. After healing of hoof abscess, no MSCs in phase 2 or 3 were isolated.

In our study is evident that physical stress and inflammation are factors for migration MSCs to peripheral blood.