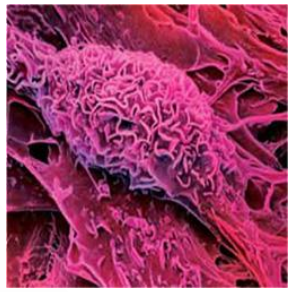
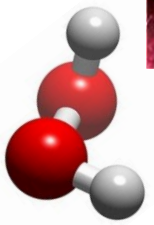
FEDERAL UNIVERSITY OF SANTA MARIA
HEALTHY SCIENCE CENTRE
POSTGRADUATE PROGRAM OF PHARMACOLOGY

HUMAN ADIPOSE-DERIVED STEM CELLS OBTAINED FROM LIPOASPIRATES ARE HIGHLY CYTOGENOTOXIC SUSCEPTIBLE TO HYDROGEN PEROXIDE

Alencar Kolinski Machado, Francine Carla Cadoná, Verônica Farina Azzolin, Gustavo Scola, Marta Maria Medeiros Frescura Duarte, José Raul Pinto Saldanha, Luana Lenz, Thais Doeler Algarve, Ana Cristina Andreazza, Ivana Beatrice Mânica da Cruz.

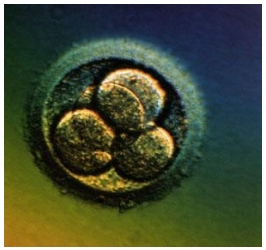




Chicago, march 24th, 2015.

INTRODUCTION

STEM CELLS

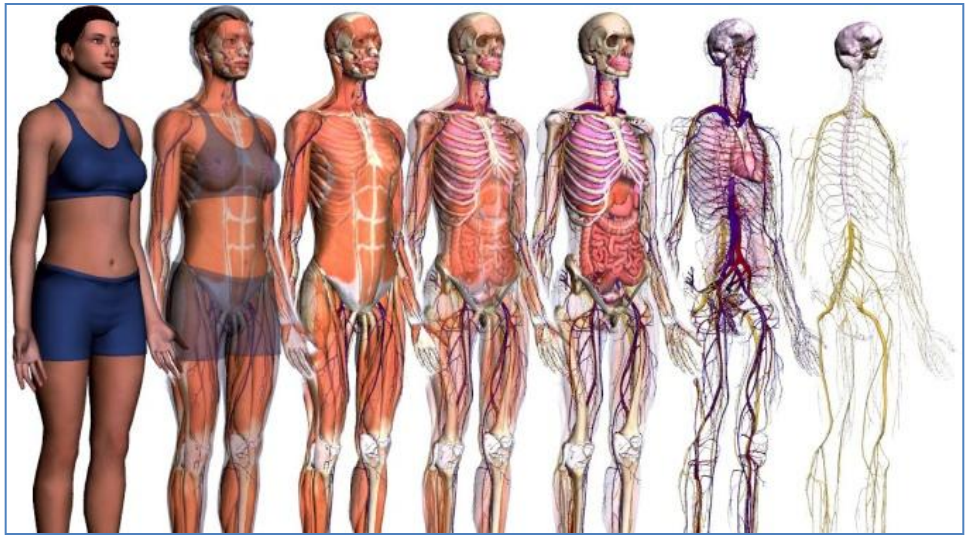




EMBRYONIC STEM CELLS

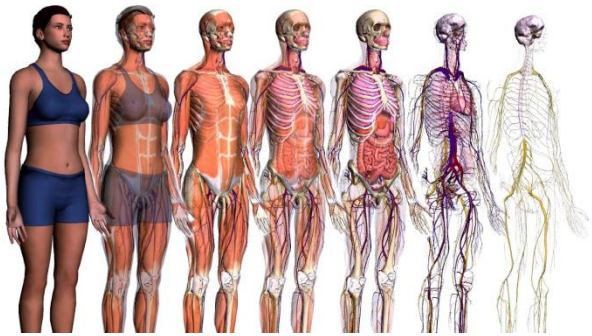
Proliferation

Differentiation



INTRODUCTION

STEM CELLS



Apoptosis

Cellular Renovation

Proliferation | Differentiation

❖ Maintaining the homeostasis

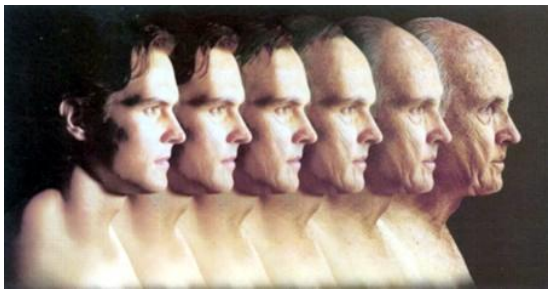
Cellular renovation and tissue repair

STEM CELLS



INTRODUCTION

STEM CELLS

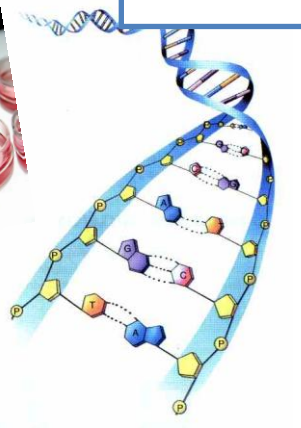


Chronic Degenerative Diseases

Therapeutic Strategies!!!

Stem Cells (SCs) on Medicine Alternative

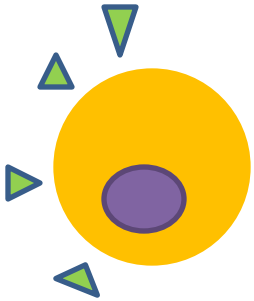




Advance in the SCs researches

INTRODUCTION

STEM CELLS



Detection of specific receptors

Recognition of SCs in the adult body

⬇

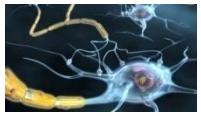
Clinic studies and experimental researches

Facility to obtain

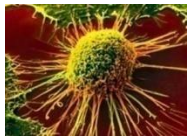


Positive therapeutic response

Mesenchymal Stem Cells







INTRODUCTION

STEM CELLS

In vivo

Adult SCs

Cell renovation

There isn't senescence

Differentiation



Cell aging

In vitro

Adult SCs

Premature senescence

Proliferation and differentiation

Inability to use for regenerative therapy

INTRODUCTION

STEM CELLS

- Stem cells use: high number of cells

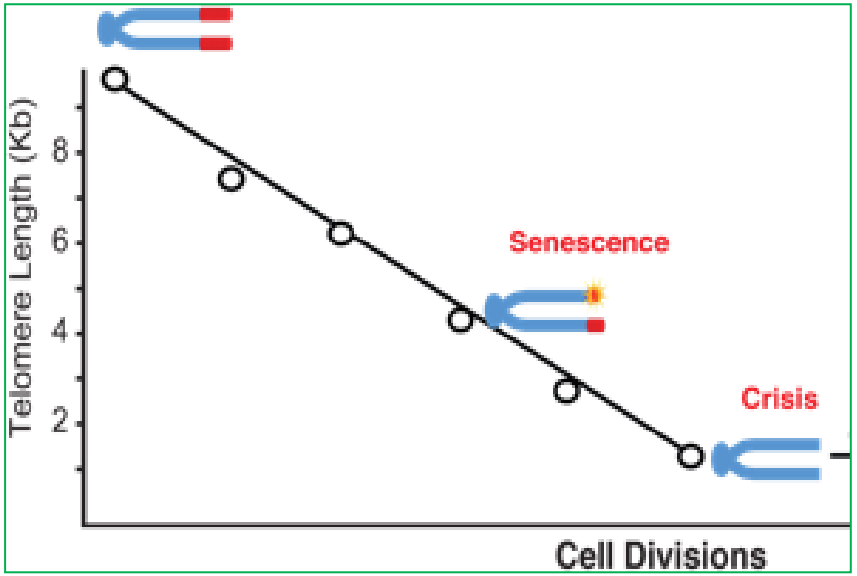
⬇

- Long time in cell culture conditions

⬇

- Cellular senescence

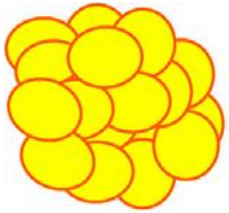
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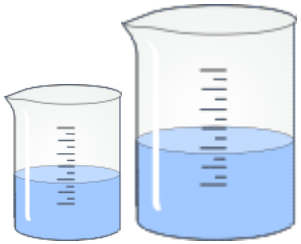
PROBLEM???

METHODS

ASCs ISOLATION AND CULTURE



Lipoaspirates



PBS Washes



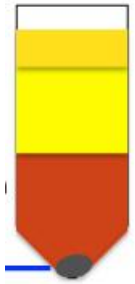
Enzymatic Digestion









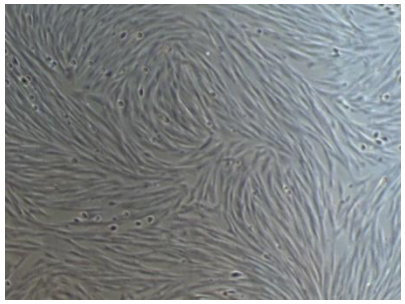




CO_2 Incubator (5%), 37ºC

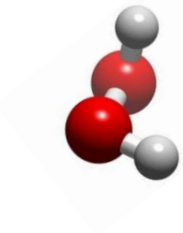
METHODS

H_2O_2 EXPOSITION



ASCs at 4th passagem





1-1000 µM H_2O_2

2 h



ACUTE EXPOSITION

CYTOGENOTOXIC EFFECT



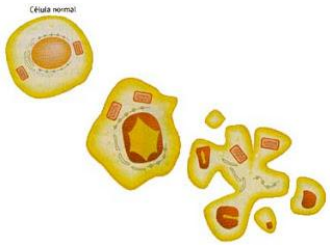
EXPERIMENTAL EVALUATIONS

(ZHANG et al., 2012)

METHODS

Free dsDNA quantification

Cytotoxicity Evaluation



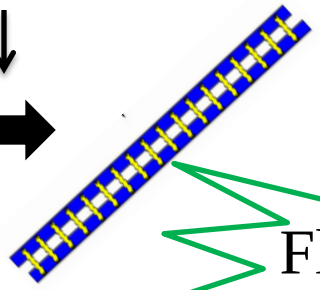
Cell death and membrane rupture



Free dsDNA on medium



PicoGreen



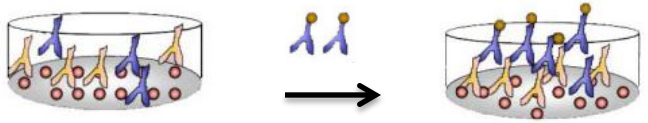
Fluorescence

480nm – excitation
520nm - emission

Caspases 1, 3 e 8 determination

Quantikine Imunoassay Caspase Human®





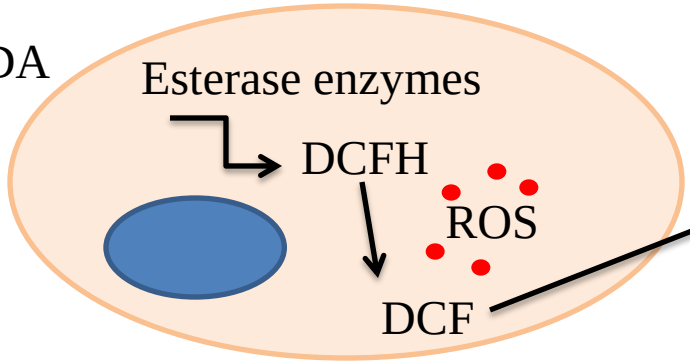
450 nm

METHODS

Dichlorofluorescein assay (DCF)

ROS quantification

DCFH-DA



Fluorescence

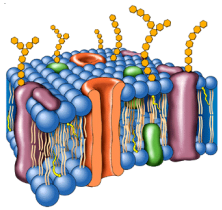
488nm – excitation
525nm - emission

(AHN, 1996)

Oxidative Biomarkers

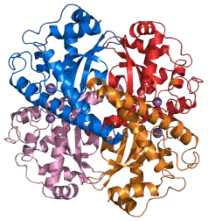
TBARS

Lipid peroxidation



(OHKAWA et al., 1979)

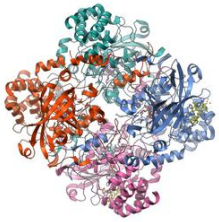
SOD activity



Epinephrine

(BOVERIS e CADENAS, 1997)

CAT activity



H_2O_2

(AEBI, 1984)

METHODS

DNA comet alkaline assay

Genotoxicity evaluation

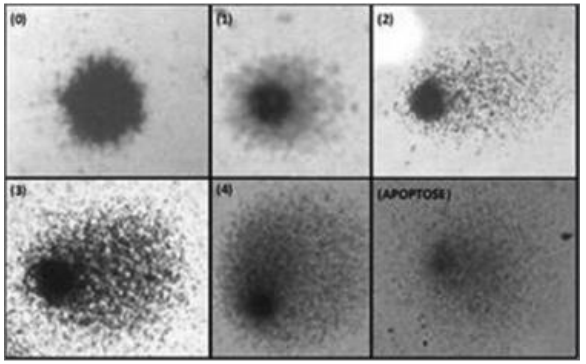


Slide confection

- Cell lysis
- Electrophoresis
- Staining with silver nitrate



Microscopic analysis



Fronza e colaboradores (2010)

(SINGH et al., 1979; NADIN et al., 2001)



Cell morphology evaluation



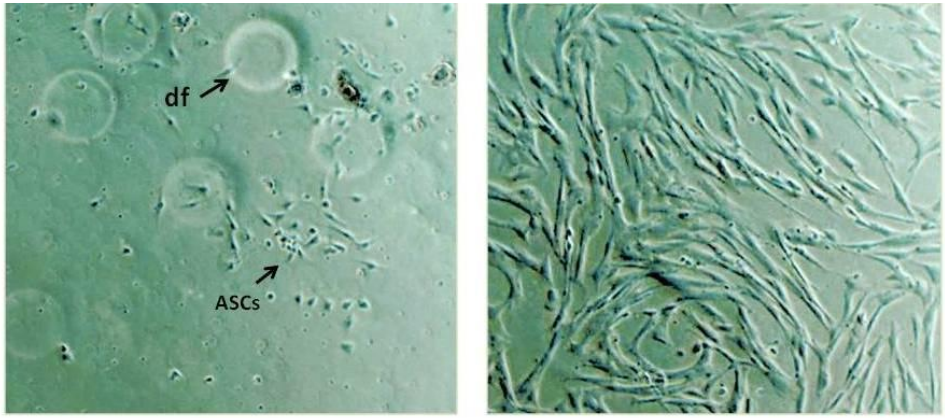
Slide confection

- Fixation
- Giemsa staining



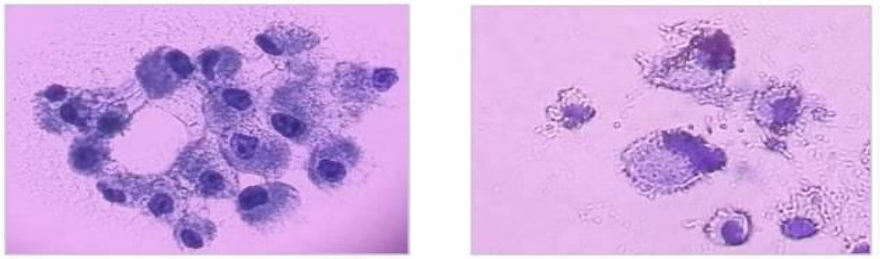
Microscopic analysis

RESULTS



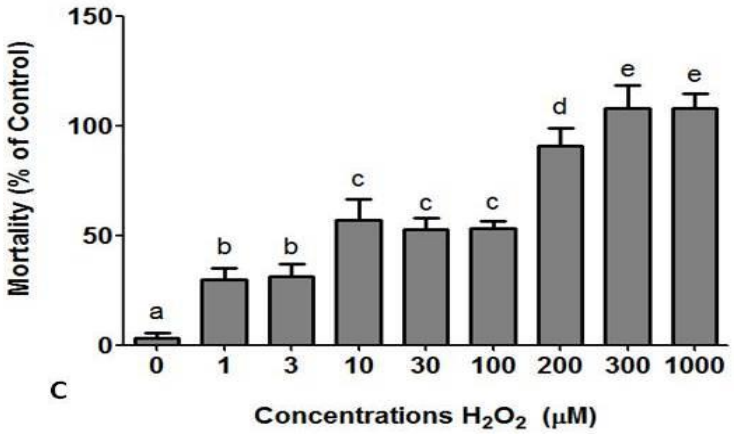
A B

ASCs isolation



A B

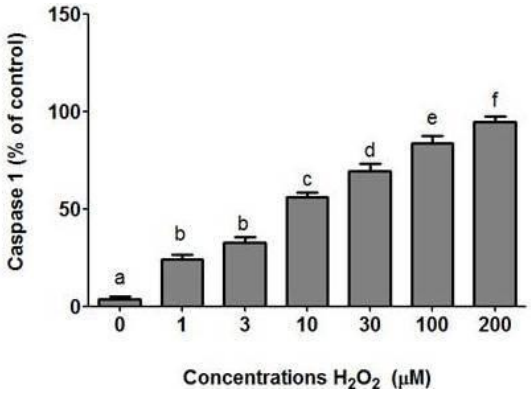
Cytotoxic effect and cell morphology alterations



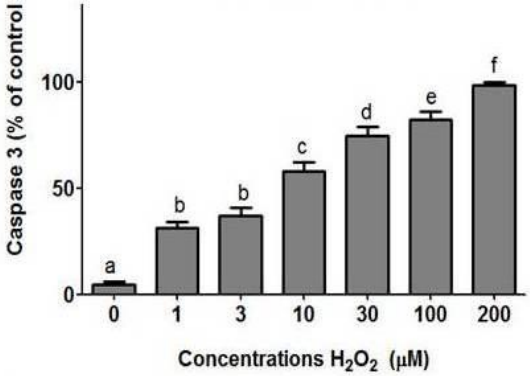
C

RESULTS

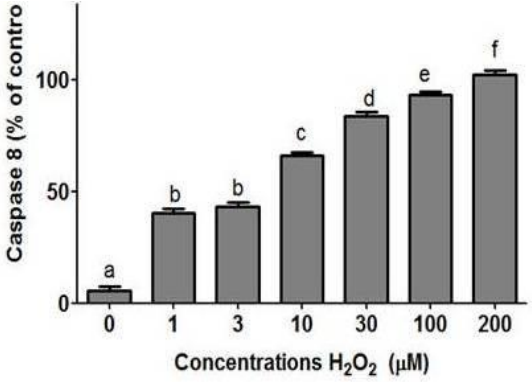
Caspases 1, 3 e 8 induction



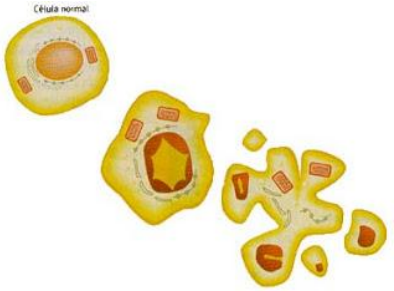
A



B



C



RESULTS

Oxidative metabolism parameters

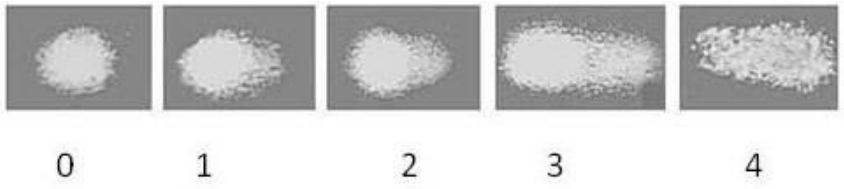
Table 1: Oxidative metabolism parameters of ASCs exposed to different concentrations of H_2O_2.

	H2O2 Concentration (µM)						
	0	1	3	10	30	100	200
ROS	100.3 ± 7.5[a]	106.0 ± 9.7[b]	108.8 ± 11.9[b]	166.8 ± 23.5[c]	165.1 ± 22.0[c]	185.8 ± 31.3[d]	368.3 ± 11.1[e]
TBARS	100.4 ± 5.3[a]	305.4 ± 18.0[b]	656.3 ± 40.7[c]	776.3 ± 40.7[d]	838.2 ± 28.6[e]	915.0 ± 90.6[f]	1823.0 ± 35.2[g]
CAT	100.1 ± 3.3[a]	152.3 ± 11.5[b]	151.5 ± 10.5[b]	154.1 ± 8.8[b]	159.3 ± 10.8[b]	156.3 ± 7.6[b]	156.3 ± 7.6[b]
SOD	100.0 ± 1.5[a]	211,7 ± 25,8[b]	89.9 ± 0.8[c]	22.1 ± 3.5[d]	24.9 ± 2.8[d]	30.5 ± 6.3[d]	34.12± 4.6[d]

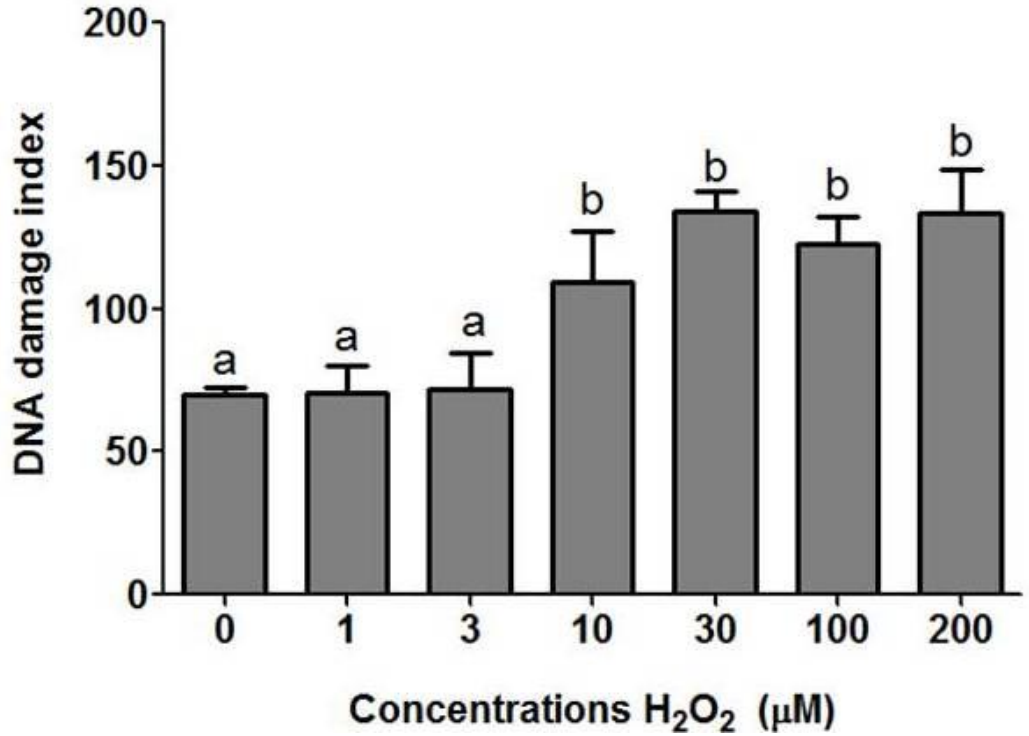


RESULTS

Genotoxic effect







DISCUSSION

Oxidative Metabolism: proliferation, differentiation and senescence of SCs

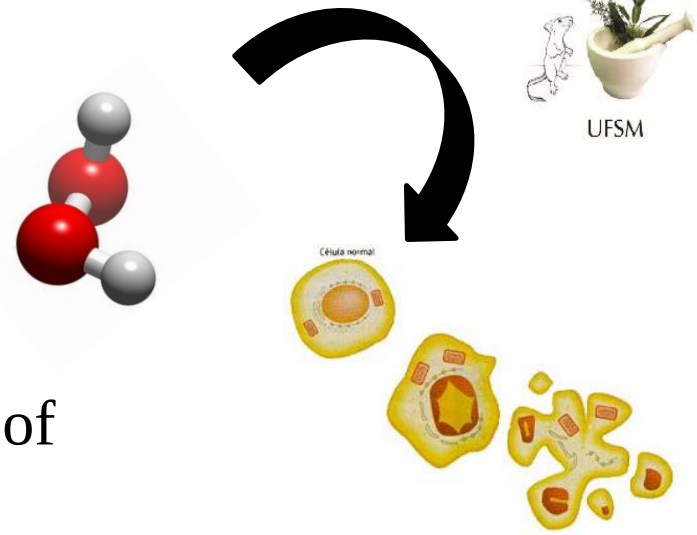
Murry et al (1986): were the first to study the effect of H_2O_2 in adult stem cells;

Zhang et al (2012): *in vitro* effect of H_2O_2 in the cell expansion adult stem cells;

Choe et al (2012): differentiation to osteoblasts from SCs using H_2O_2.

~~Genotoxic effects~~

DISCUSSION



Our results...

❖ASCs high sensibility to the increase of ROS;

❖ 100% of death from 200 µM treatment;

❖ Apoptotic process activation: caspase 1, 3 and 8;

As waited ➡

❖ Increase of ROS ratio and lipid peroxidation, agreeing with Tuteja et al (2009).

DISCUSSION

Our results...

❖Significant increase in the CAT activity;

Enzymatic function

(GAETANI et al., 1996)

❖SOD modulation not dose dependent manner, agreeing with Gottfredsen et al (2013);

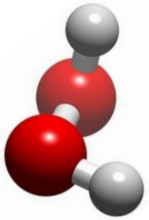
Embryonic stem cells + H_2O_2 = SOD inhibition

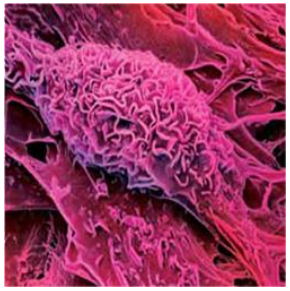
❖ Significant DNA damages, with a decrease of damages 0;

❖ Cytomorphologics alterations.

DISCUSSION

In this way...





Despite the H_2O_2 is able to increase the adult SCs proliferation, but the acute exposition, specialy from 3µM, of this kind of treatment is also able to conduct to citotoxic effects, oxidative stress and mainly genomic damages.

Acknowledgements

CNPq

FAPERGS

CAPES





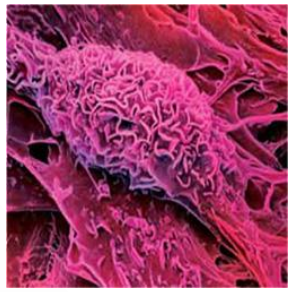


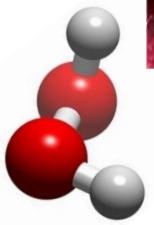


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Chicago, march 24th, 2015.