"OVIDIUS" UNIVERSITY CONSTANTA
DOCTORAL SCHOOL OF MEDICINE
FIELD OF MEDICINE PHD

PHD THESIS SUMMARY

The study of cellular processes involved in the pathophysiology of Down Syndrome

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CONTENTS (THESIS IN EXTENSO)

PURPOSE OF THESE STUDIES

This study aims to address the diagnosis of Down syndrome (Down syndrome-DS) from a new perspective by updating and deepening into clinical applications through the identification of new elements with applicability in diagnosis, prognosis, optimize and personalize therapies.

Amniotic fluid (amniotic fluid- AF) obtained by amniocentesis could serve as a source of stem cells to regenerate tissues in subjects with DS affected by accelerated aging and degenerative diseases. Cell origin of AF is still very much discussed.

Human AF obtained in the process of amniocentesis was found to contain a variety of cells derived from embryonic tissues and extraembrionare. Proliferative capacity is limited because most cells are differentiated present, few studies have demonstrated the presence of a subset of stem cell properties (amniotic fluid stem cells - AFS). However, AFS differs from both pluripotent embryonic stem cells (embryonic stem cells - ESC) and adult multipotent stem cells, and may represent a new class of stem cells whose plasticity properties are between embryonic and adult stem cells.

Unlike the ESC derived from the blastocyst internal cell mass, the AFS cells do not form tumors after transplantation to mice. In recent years evidences have demonstrated the AFS cells potential to differentiate into multiple lines. As a result, AF could be a safe and readily available source of AFS cells that can be used for therapeutic purposes and that breach any ethical objections, given that amniocentesis is a widely accepted form for prenatal diagnostic tests. Note that a bank of 100,000 AF specimens could potentially serve 99 percent of the US population with a perfect match for the transplant.

AF cells types and properties can vary with gestational age. To make use of this source of stem cells, to be use in tissue regeneration is fundamentally necessary to emphasize their proliferative capacity and ability to differentiate. And to evaluate the possibility of autologous AFS cell transfer collected before birth, to treat adults with DS with degenerative diseases, it is necessary to validate their regeneration capacity.

The specific objectives during my research work were:

1) to determine quantitative, immunophenotypic and morphological changes that AF cells are subjected derived from DS to measure intrauterine growth and proliferative potential of these cells, as compared to those of normal pregnancy.

2) AF cell phenotype characterization in DS for potential use of those analyzes to predict effects on the patient and for designing future approach in tissue engineering to regenerate damaged organs of sick patients.

In order to achieve these objectives I used culturing in vitro and cell biology methods geared towards AF cell characterization.

INTRODUCTION

Down syndrome as identifiable genetic modification prenatal screening tests

DS is the most common genetic disorder in the human species and the most common cause of intellectual disability and mentally retarded (Cuckle HS, 1987).
DS or trisomy 21 is also the most frequent and best known chromosomal disease.

DS is the most common genetic disorder with a frequency of approximately 1/700 newborns. In the world there are about 7 million people diagnosed with this syndrome of which approximately 4,000,000 people are in the US. This rate, however, underestimate the true incidence because it does not include miscarriages and induced abortions (UM Reddy, 2006).

Newborn with trisomy 21 has less length and weighing than gestational age parameters, presents hypotonia, hiperextensibilitate and behavioral reflexes (eg, Moro reflex) reduced.

The head is brachycephalia with fontanels broad and flattened occiput. The face is round, flat and presents a suggestive dysmorphia: epicanthic fold (a repliu in the inner corner of the eye), oblique fissure vents up and out; small nose and nostrils flattened root small and anteversion; small nose and nostrils flattened small and anteversion root; open mouth and lingual protrusion (due to small oral cavity); located below the ears, small and dysplastic. The neck with excess skin on the neck is short; hands are short and broad, with brachydactyly clinodactyly (bowing) finger V and often a single palmar flexion crease (simian crease); inconstant some visceral malformations are present (duodenal atresia, cardiac defects, anal perforation) (M Covic, 2003).

Cytogenetics is essential for diagnosis and mandatory in every case, even if clinical examination is also evident by the analysis result cromozomiale given genetic counseling and calculate the risk of recurrence.
Chromosomal analysis can reveal:

1. Trisomy 21 free and uniform in 92-95% of cases of DS;
2. Trisomy 21 mosaic chromosomal free (type 47/46) in 2-3% of cases;
3. Trisomy 21 by Robertson imbalanced translocation - between chromosome 21 and another chromosome acrocentric;
4. Partial trisomy 21 in 1% of cases (Covic M, 2003).

In the early years many children with DS have various medical problems. The most important ones beeing caused by visceral malformations, particularly cardiac and digestive. Respiratory infections are common (the non-specific immune deficiency) and the risk of leukemia is 15-20 times higher than in the general population. Morbidity is increased: a rate of 10-15% of patients with DS may have one of the following medical conditions: hypothyroidism, impaired vision (strabismus, cataracts, glaucoma), atlanto-axial instability, epilepsy, deafness. After 40 years frequently senile dementia installs and mortality significantly increase through strokes. In developed countries, the survival rate at 50 years is 85% and at 60 years is only 44%, namely half that of the general population (M Covic, 2003).

Amniotic fluid - amniotic stem cell source for regenerative applications in improving Syndrome pathology

AF is a complex and dynamic solution that meets at various gestational age, multiple functions for the developing fetus. In the first trimester of pregnancy, AF consists mainly of maternal plasma passing through the fetal membranes. There is a free diffusion that occurs bidirectional between AF and fetal skin, placenta and umbilical cord. With the maturation of fetal skin and other organs, changes the composition of AF. In the second quarter, AF still has similar levels of fetal and maternal blood electrolytes, only organic matter levels are significantly altered (XL Tong, 2009). AF provides physical protection for the fetus mechanical and thermal insults, and also has immune functions, contains growth factors and cytokines and acts as a nutrient source (MA Underwood, 2005). After the moment of implantation, an extra celomic cavity filled with fluid will become eventually the amniotic space, which will be identified even before the embryo is being recognized. During embryogenesis, amniotic fluid volume grows faster than embryonic dimensions. Water from AF comes from maternal plasma passes through fetal membranes based on osmotic and hydrostatic forces. For the placenta and fetal vessels to develop, water and substances dissolved in plasma mother cross the placenta to the fetus and then AF.

AF contains carbohydrates, proteins and peptides, lipids, lactate, pyruvate, electrolytes, enzymes and hormones. Previous to the production of keratin in fetal skin, amino acids broadcast placental membranes and placenta into the fetal circulation by fetal skin in AF. Later in pregnancy diffusion membranes and placenta still is enhanced by fetal urinary excretion of amino acids (Jauniaux E, 1999).

Amniocentesis was a valuable tool in assessing fetal status since 1970. The most common use of AF is to assess fetal chromosomes. Amniocentesis is usually indicated for women over 35 or who have risk factors for a chromosomal abnormality. For the diagnosis of aneuploidy in first quarter, nuchal translucency ultrasound evaluation, maternal serum markers together with the use of amniocentesis had decreased chorionic villi sampling.

Human AF was evaluated as a source of stem cells with initially encouraging results (Luton D, 2003). The possibility of a non-controversial source of stem cells, could stimulate research in this area.

Stem cells from amniotic fluid (reference, potential in regenerative medicine)

Amniotic stem cells represent the mixture of stem cells that can be obtained from AF (Fauza, D., 2004, Cananzi, Mara, 2009) and amniotic membrane (Kim, m.k, 2014). They can differentiate into different types of tissue, including skin, cartilage, cardiac tissue, nerves, muscle, bone (Antonucci, Ivana, 2009). These cells may have medical applications, especially in organ regeneration (Abdi, Reza, 2008).

It was determined the presence of fetal cells and embryonic germ layers in AF in all and hematopoietic progenitor cells have been identified in AF, since the 12th week of pregnancy. It has been suggested that they originated from the yolk sac (Fauza, D., 2004). As well one study has shown that non-hematopoietic progenitor cells were also present in the AF. This was later confirmed, highlighted cells are mesenchymal stem cells. In addition, it is a proof showing that embryonic stem cells are part of the fluid, though in very small quantities (Fauza, D., 2004).

At the same time, it has been determined that the stem cells from the amniotic membrane also have a multipotent potential. It has been found to differentiate into neuronal and glial cells, such as hepatocytes and precursors (Fauza, D., 2004).

Most of the stem cells present have similar characteristics, suggesting that they may have a common origin (Fauza, D., 2004).

It was also confirmed that AF contains a heterogeneous mixture of multipotent cells after it has been demonstrated that they were able to differentiate into cells from all three germ layers and without forming teratoma. This feature distinguishes them from embryonic stem cells, but shows similarities with adult stem cells (Jacot, JG, 2013). Thus, fetal stem cells from AF are more stable and more plastic than their adult counterparts making them easier to reschedule in order to obtain pluripotent status (De Coppi, P, 2014 Moschidou, D, 2012).

A variety of techniques has been developed for the isolation and cultivation of amniotic stem cells.

One of the most common methods involve obtaining AF isolation through amniocentesis. The cells are then removed from the fluid by the presence of c-Kit. There is a debate about whether c-Kit is a suitable marker to distinguish amniotic stem cells from other types of cells, as cells lacking c-kit presents also the potential for differentiation. Growing conditions can also be adjusted to promote the growth of a particular cell type (Jacot, JG, 2013).

Mesenchymal stem cells (mesenchyme stem cells - MSC) are very abundant in AF and described several techniques for their isolation. They usually involve obtaining AF through amniocentesis and their differentiation from other cells by morphology or presence of certain features (Fauza, D., 2004).

Human leukocyte antigen test was used to confirm that the MSC come from the fetus, not from the mother. It was initially assumed that MSC were removed from the embryo at the end of their life cycle, but because the cells remained viable in AF and were able to proliferate in culture this hypothesis has been invalidated. However, it remains unclear whether the cells come from the fetus, placenta or possibly from blastocyst's inner cell mass (Fauza, D. 2004).

Comparison of MSC derived from AF with those derived from bone marrow showed that the former have a higher growth potential in culture. MSC derived from AF phenotyping revealed a phenotype similar to bone marrow-derived MSC and MSC derived from fetal tissue from the second trimester of fetal development (Fauza, D., 2004). In animals, the MSC appear to have a unique immunological profile, which has been observed following its isolation both in culture and in vitro (Fauza, D., 2004).

Embryonic stem cell-like cells, in contrast to the mesenchymal stem cells are not abundant in AF, they constitute less than 1% of amniocentesis samples. Embryonic-like stem cells were initially identified using common embryonic stem cells markers such as Oct4, CD34, vimentin, alkaline phosphatase, stem cell factor and c-Kit. However, these markers were not necessarily expressed in the same time. In addition, all of the markers may occur alone or in other combinations in other cell types (Fauza, D., 2004).

These pluripotent embryonic stem cells remains to be fully established. Although these cells expressed markers were able to differentiate into muscle cells, fat, bone, nephrotic neuronal and endothelial cells, it has not been necessarily a homogeneous population of undifferentiated cells.

Evidence in favor of their nature of embryonic stem cells is their ability to produce clones (Fauza, D., 2004).

Stem cells from AF or amniotic membrane is a source of multipotent stem cells that could exceed the limits of growth, histocompatibility, tumorigenesis and ethical issues associated with the use of human embryonic cells harvested from the umbilical cord, cord blood, bone marrow and induced pluripotent cells (De Sacco, S, 2010).

Studies have shown that the AFS cells can be used to treat various diseases. One such study showed the usefulness of AFS cells in regenerative mammary gland (BK Young, 2015). Other studies have shown that may be helpful in muscle and cardiac disease (Mauro, A. 2010 şiDelo, D. M., 2011). In addition, AFS cells have shown also that it could have multiple applications in cell therapy and tissue engineering (Cananzi, Mara, 2009 De Sacco, 2010, Young BK, Siyang 2015, X. X, 2009) .These exciting options for cell-based treatment could lead to further legal remedies against human disease (Vieira, M. N., 2010).

Transplant therapy studies with human embryonic stem cells are also prevented by possible immunological rejection and tumorigenicity. As mentioned above, the AFS cells are not tumorigenic after transplantation in mice, in contrast to embryonic stem cells obtained from the cell mass of blastocyte (De Coppi, 2007).

RESULTS AND DISCUSSIONS

Immunophenotyping of amniotic Down syndrome cells and normal cells.

To obtain information on immunophenotyping expression, we optimized cultivation method and evaluation in terms of immunophenotype through flow cytometry.

During flow cytometry analysis, two cell populations were constantly present in AF cells cultures, as identified on FSC/SSC dot (Figure 1A) and contour (Figure 1B) plots. The predominant population exhibited an increased FSC, indicative of large cell size (Figure 1A,B - P2 gate). Second population had variable propensity between samples and decreased FSC (Figure 1A,B – P1 gate), hence small cell volume. We have analyzed the immunophenotype of each of these two cell populations using markers for hematopoietic (CD14, CD34, CD45) and mesenchymal (CD13, CD29, CD90) lineages. Results showed that all samples were negative for hematopoietic markers and positive for CD13 and CD90 mesenchymal markers, regardless of their DS phenotype. CD29 is almost absent on all tested cell lines. CD90 had a variable expression between subjects; cells with high and low CD90 expression levels constituted two peaks with variable ratio (CD90 histograms in Figure 1 and 2). Noteworthy, both cell subpopulations displayed similar expression patterns irrespective of their FSC value (Figure 1 and 2 – C versus D).

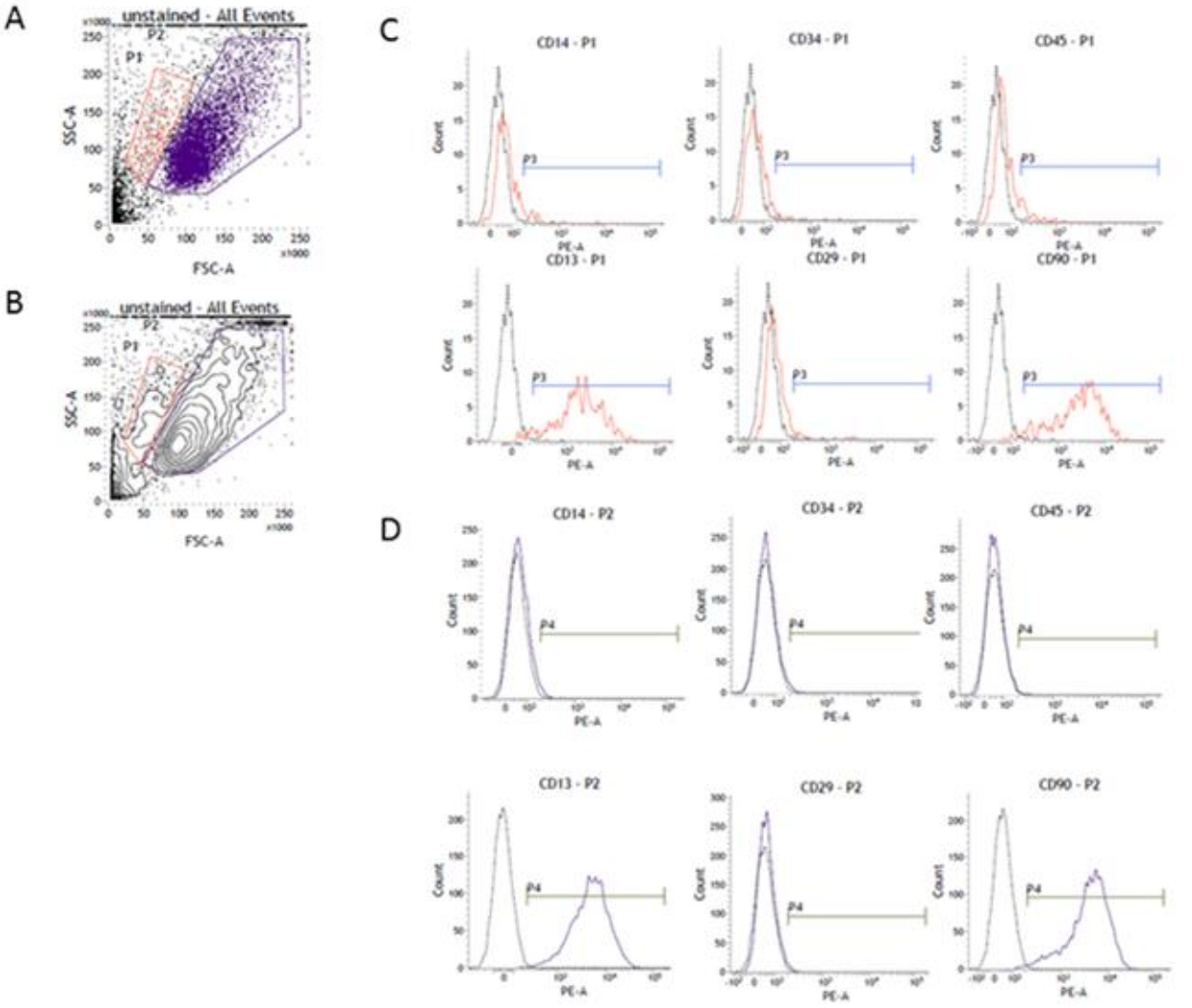


Figure 1: Immunophenotypic normal amniotic fluid cells - line 210.A.FSC / SSC dot plot FSC cell showed a low (P1, red) and an increased FSC (P2, blue); B. FSC / SSC contour plot revealed cell boundary between the two cell populations; C. histogram showed hematopoietic and mesenchymal expression of cellular markers for FSC celulele.cu low; D. histogram showed expression of hematopoietic and mesenchymal markers for cells with increased FSC.

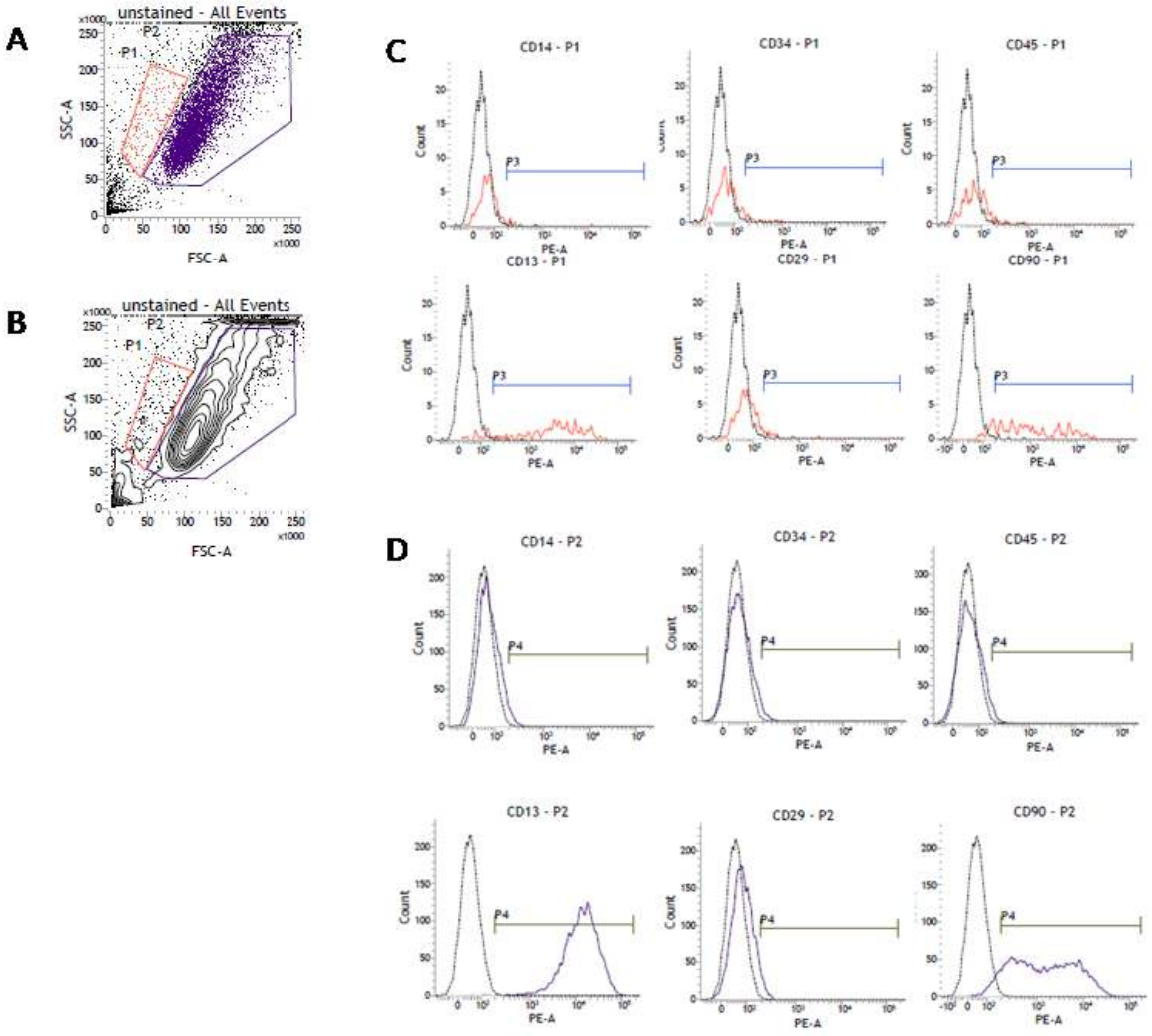


Figure 2: Immunophenotyping cells from amniotic fluid DS - DS line. A. FSC / SSC dot plot of the cells showed a low FSC (P1, red) and FSC high (P2, blue). B. FSC / SSC plot cell outline shows the boundaries between the two cell populations. C. histograms show the expression of hematopoietic and mesenchymal cell markers with low FSC. D. histograms show the expression of hematopoietic and mesenchymal markers on cells with increased FSC.

Image cytometric analysis of amniotic cells from Down syndrome and normal cells.

To obtain information about the parameters of the picture, we have optimized a method of cultivation, marking and evaluation in cytometric terms.

We performed image analysis on normal samples and cell lines derived from pregnancies with Down syndrome. The entire sample area was reconstituted (Figure 3 A) and the cytoskeleton visualized together with the expression of Ki67, a nuclear marker expressed only by cycling cells, in both normal (Figure 3B).and DS cells (Figure 3C).

cells, in both normal (Figure 3B).and DS cells (Figure 3C).

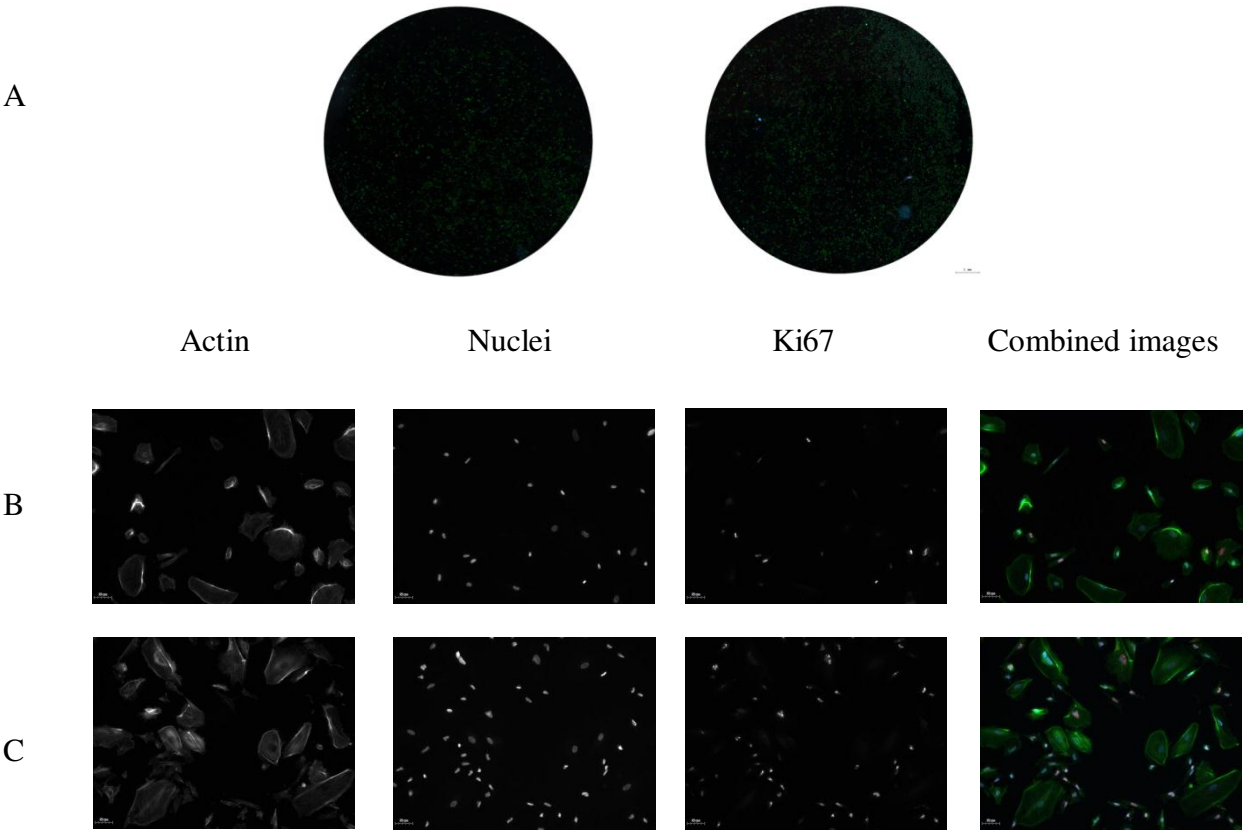


Figure 3: Image analysis cytometry cells and normal amniotic fluid SD.A. Representative images of the normal cover slips (PRA15-0210, left) and SD (right). See the entire sample was obtained by assembling all fields generated by automatic scanning TissueFAXSiPlus. B. scan field of view of normal amniotic cells.C. scan field of view from amniotic cells from DS. Actin filaments were stained with Alexa Fluor 488 conjugated phalloidin (green), nuclei were stained

with Hoechst (blue) and nuclear antigen Ki-67 was labeled with specific antibodies followed by secondary antibodies conjugated to Alexa Fluor 594 (red). Described in each channel and overlapping images merged.

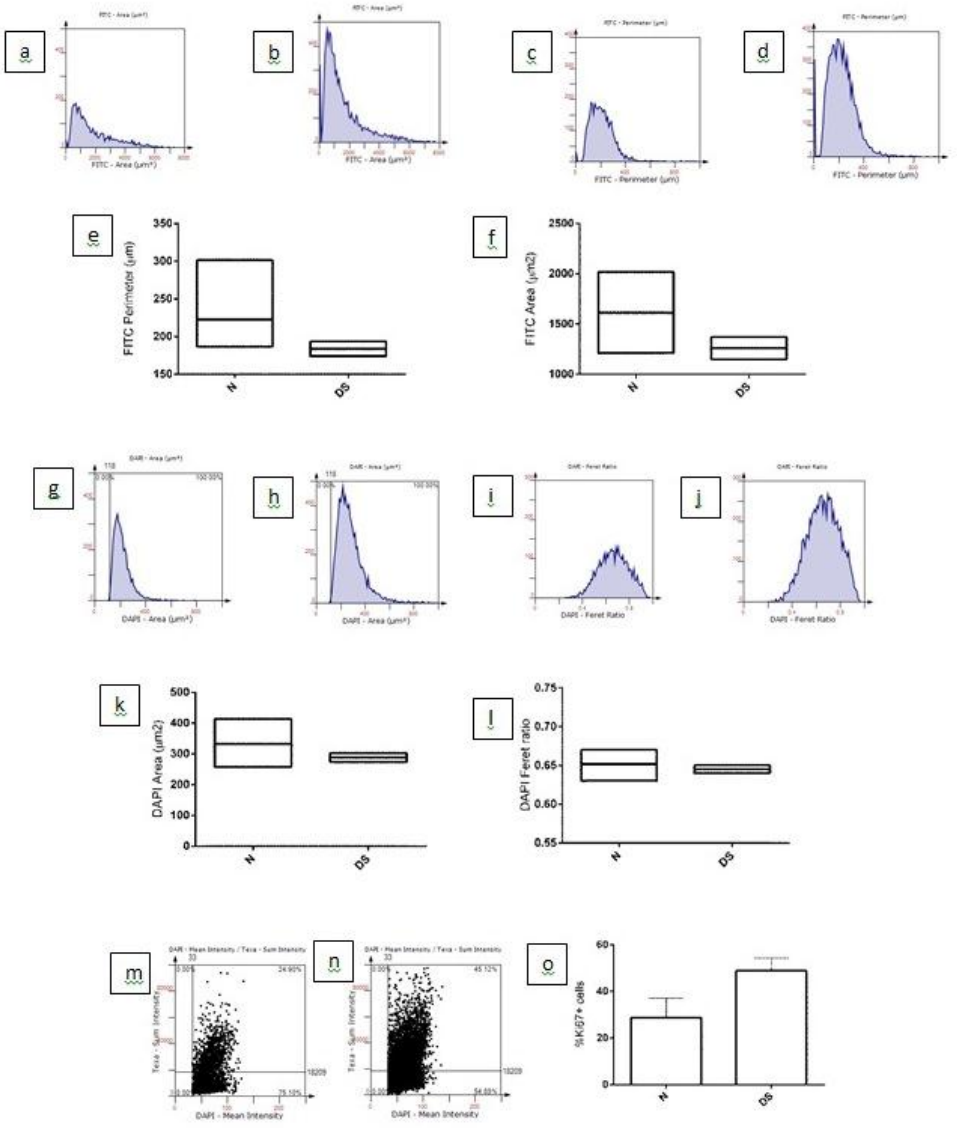


Figure 4: Quantitative analysis of proliferation and cellular parameters. Distributions of cell areas in the representations of normal cell areas (a) and DS (b)are displayed in histograms. The minimum, maximum and average of the areas of the underlying cell are illustrated in the chart (e) in normal (n = 7) and SD (n = 5). Securities distribution cell perimeters of normal amniotic fluid (c) and DS (d) are shown in histograms. Maximum values, average and minimum cell perimeters are illustrated in the graph underlying (f) to normal (n = 7) and DS (n = 5) lines.

Distributions of surface nuclei of normal representations (g) and DS (h) are shown in histograms. The minimum, maximum and average of the underlying core are illustrated in the chart (k) for normal (n = 7) and SD (n = 5). The distribution ratio of nuclei in normal ferret (i) in the case of DS (j) is displayed in histograms. The minimum, maximum and average ratio ferret nuclee are illustrated in the graph underlying (l) normal lines (n = 7) and DS (n = 5). Scattergrama display represents the percentage of Ki67 positive cells in normal AF (m) versus DS (n) They are shown together withaverage percentages and standard deviations (a).

We observed the two characteristic epitheloid and fibroblastoid cell subpopulations and the apparent increase in the propensity of small sized cells in DS samples. To obtain quantitative data, we performed image analyses aimed at measuring cell and nuclei morphometric parameters. Hence, we measured cell areas and perimeters based on actin filaments fluorescent staining, and nuclei areas and ferret ratios (circularity) based on Hoechst staining. Results showed a decrease in cell and nuclei size with no significant modification of nuclear shape (Figura 4 - Al).

Cell cycle analysis of amniotic cells from Down Syndrome and normal cells

In order to assess the fraction of proliferating cells cytometric measurements were performed based on Ki67 nuclear staining. Quantitative analyses scattergrams (Figure 4 – m, n) shown a significant increase of %Ki67+ cells in DS subjects derived AF cells (48.92%, SD = 5.37, n=2) as compared to normal samples (28.68%, SD = 8.44, n=7). With a view to examine in more detail the potential cause of this increase in cell proliferation, we also performed a cell cycle analysis by flow cytometry following propidium iodide staining. Our data showed that in DS derived AF cells the proportion of cells in G0/G1 phases increased (from 61.34% to 84.89%), while the fraction of cells in S and G2/M decreased (from 20.89%+12.02% to 2.69%+1.80%), when compared with normal cells (Figure 5).

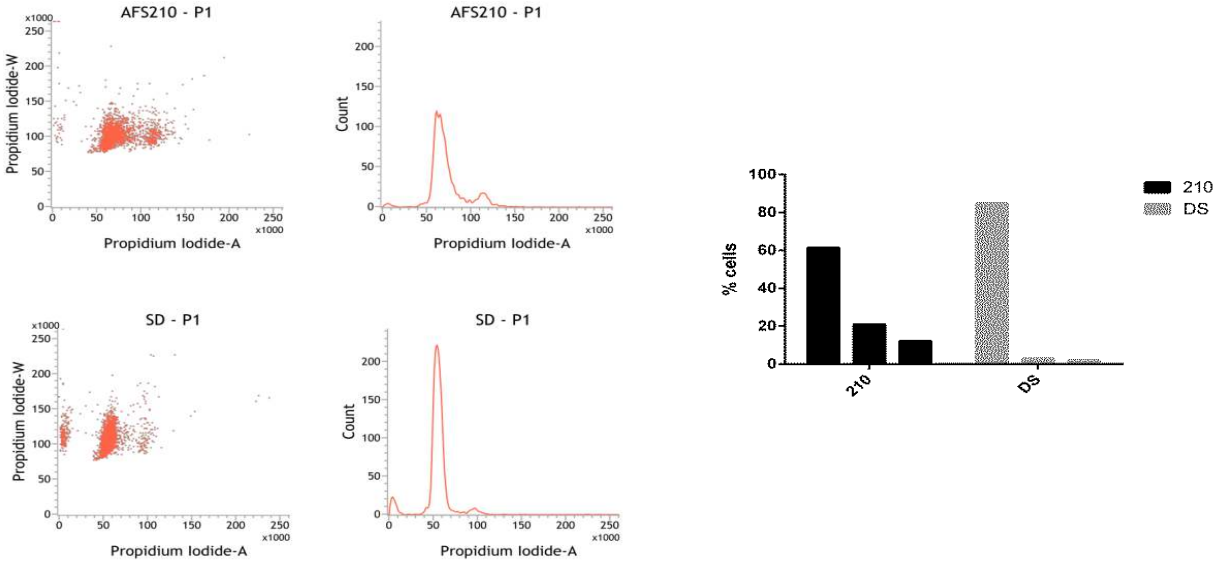


Figure 5: A represents a distribution cytometric analysis of amniotic fluid cells according to the cell cycle in the normal case (upper diagram) and in case of DS (lower diagram). B. Chart showing the proportion of cells in each phase of the cell cycle.

In the second part of the experimental studies we have initiated cell culture samples of amniotic fluid of fetuses with phenotype normal parameters and performed experiments characterizing

them (AFS - amniotic fluid stem cells) in parallel with isolated mesenchymal stem cells from human bone marrow (BM-MSC - bone marrow-derived stem cells mesenchyme) (Figure 6).

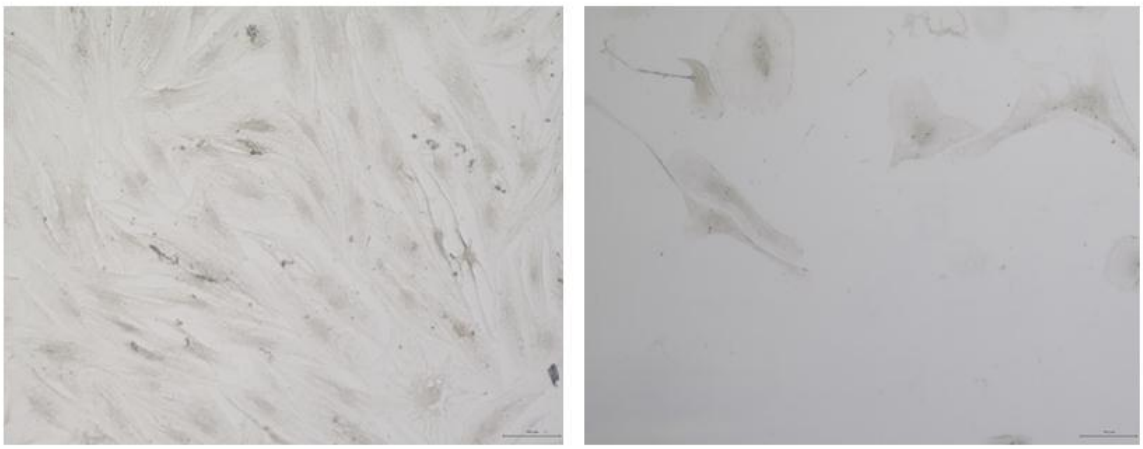


Figure 6: The morphology of cells from amniotic fluid of patients with normal fetuses - eg cell line O.D. (right) as compared to bone marrow mesenchymal stem cells (left).

Analysis of amniotic Down syndrome cell differentiation and normal cells

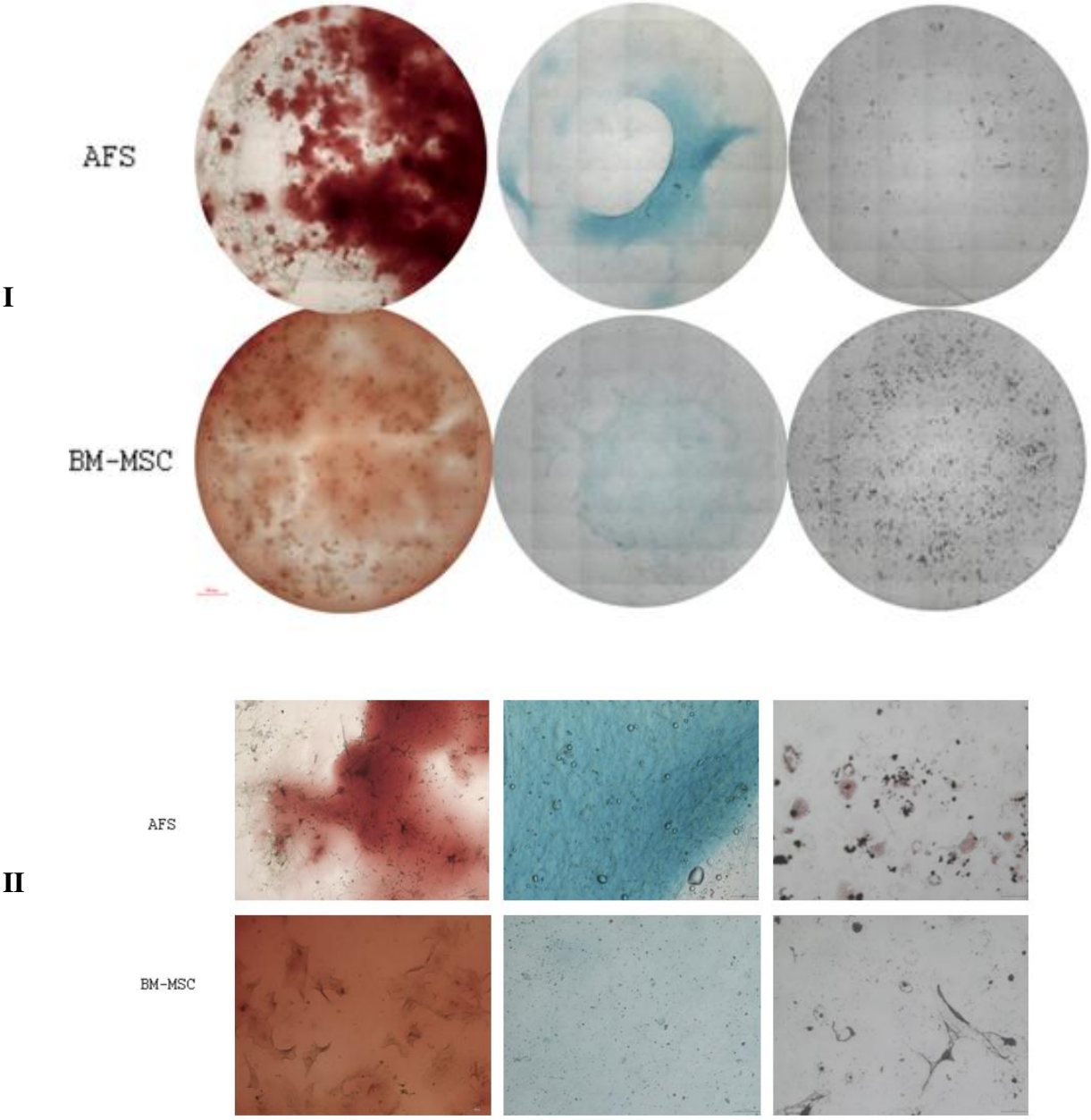


Figure 7: The differentiation of mesenchymal stem cells from amniotic fluid (AFS) and bone marrow (BM-MSC) into osteoblast (left), chondrocyte (middle) and adipocyte (right) cells. In the group of images I the entire well is reconstituted while in group II detailed pictures of cells undergoing differentiation are shown.

As a result of the analysis of the cellular differentiation of the mesenchymal cells in the second biological sources can be seen that the cells of the amniotic differentiate to osteoblasts and chondrocytes, but not in adipocytes under experimental conditions where the cells of the bone marrow subjected to induction of its function to the three phenotypes differentiated (2) .

We currently study different cell lines of normal and DS amniocytes, which will be analyzed in parallel with mesenchymal stem cells from different donors to characterize the molecular differentiation process and potential differences, in case of genetically modified fetal cells.

CFSE staining to analyze cell proliferation

To study the potential to modulate DS proliferation cell, we made the last part of the study, with CFSE labeling experiments and evaluation of its dilution in the presence of normal cells under co-culture. We chosed normal amniotic cells and mesenchymal stem cells to test their effect on cell proliferation DS.

The first experiment consisted of optimizing the concentration of CFSE labeled cells so they can be traced to both microscopy and flow cytometry for a period of 24 hours. We tested two concentrations of 0.5 and 5 μM and have carried out an experiment to evaluate the visibility of microscopic cells on the immediately next day marking (d0) or 24 hours after labeling (d1) (Figure 8). The results showed that both on the amniotic and the mesenchymal cells the optimal tracer concentration is 5 μM.

Using this concentration we performed co-cultures of cells labeled with CFSE and then diluted 1: 1, 1:10 and 1: 100 with unlabeled cells from other lines (AFS MSC) or in the same line as control (Figure 8). As a measure of the proliferative capacity of the cells was quantitated median value CFSE cells that have undergone division during the 24 hours (P3 marker in the histogram shown in Figure 9). The higher the value the proliferative activity is lower and lower values are indicative of an accelerated proliferation.

Data analysis showed that DS 283 cells had a median fluorescence of CFSE 836-1007, while AFS 213 values were 2348-3202. After culturing the cells in the presence of normal cells DS AFS213, median CFSE remain relatively constant (982-1031) .In contrast, 24 hours after culturing in the presence of MSC T DS, they are accelerating their divisions, indicated the median CFSE of 666-831 (Figure 9).

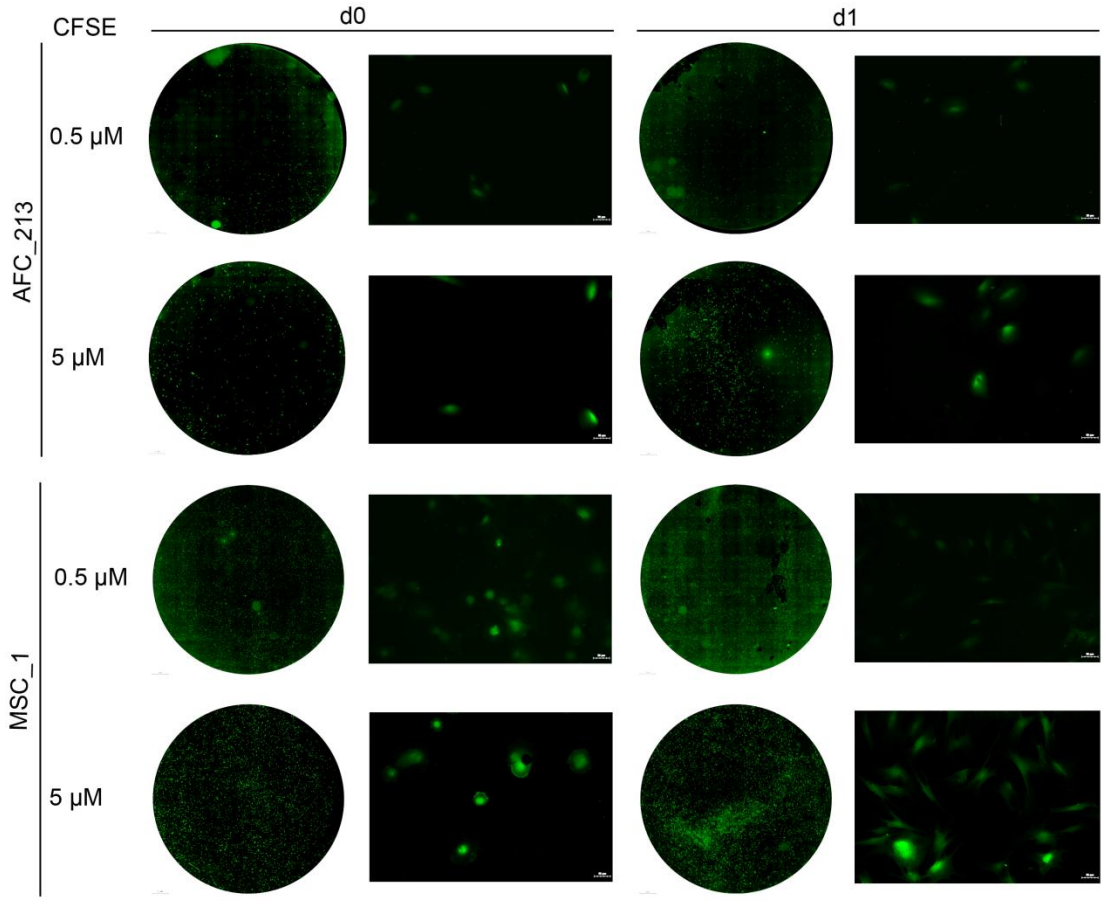
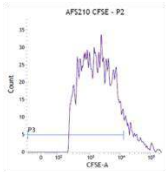
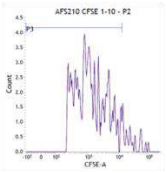
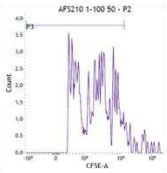
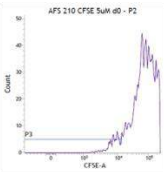
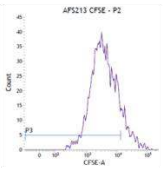
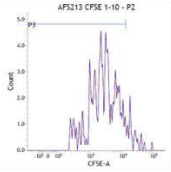
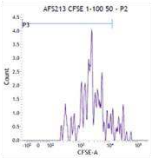
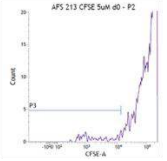


Figure 8: Image analysis of samples labeled with CFSE at a concentration of 0.5 µM, and 5 µM.

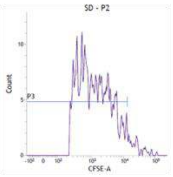
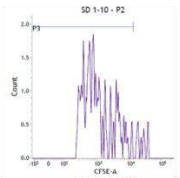
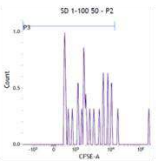
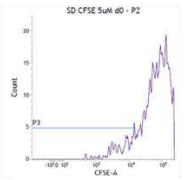
AFS210

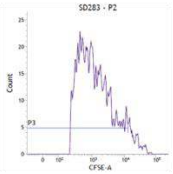
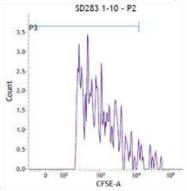
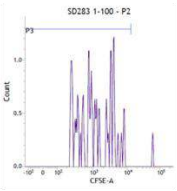
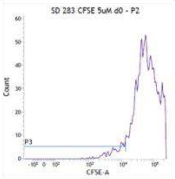
AFS213

SD

SD283

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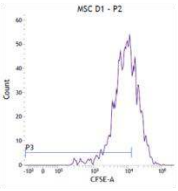
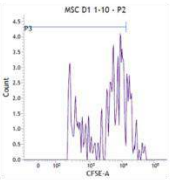
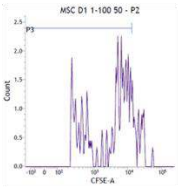
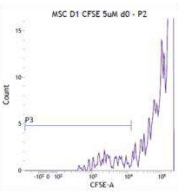

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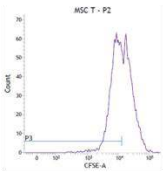
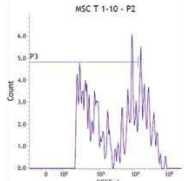
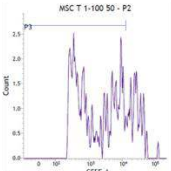
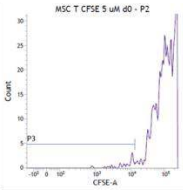

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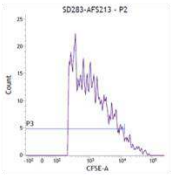
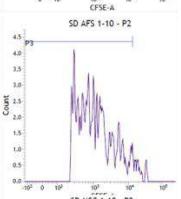
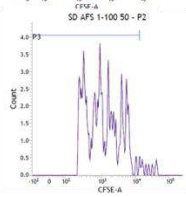

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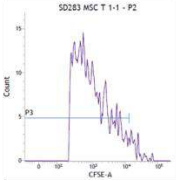
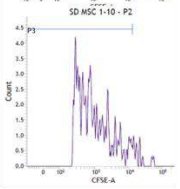
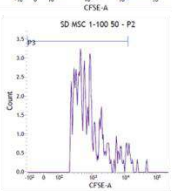

1:1 1:10 1:100

Figure 9 (continuation) : Flow cytometry analysis of the samples of amniotic cells DS, dilution 1: 1, 1:10 and 1: 100 with normal amniotic cells or normal mesenchymal stem cells. Cells were labeled with 5µM CFSE before being diluted with cells in co-culture.

But these studies require further deepening of possible molecular mechanisms by which it can control or modulate the activity of proliferative ds cells pathology to reverse their phenotype and improving physiological consequences of cellular changes specific to this pathology.

CONCLUSIONS (THESIS IN EXTENSO)

During flow cytometric analysis, two cell populations were constantly present in the cell cultures in LA, identified on the dot-plots and contour-plots outline sites and sites, forward scatter (FSC) / side scatter (SSC). FSC shows predominant population increased, which is indicative large cell sizes. FSC shows predominant population increased, which is indicative for large cell sizes. The second population has tended to variability between samples and low CSF, therefore, the volume of the cells was small.

SD cells fall to a greater extent in the cell cycle (indicated by the high percentage of Ki67 + cells), but the majority remain in the G1 phase, slowing down the cell cycle exit. SD co-culturing cells with MSC but not with AFS increase their rate of division. Further research is necessary to identify the specific MSC factors indicating favorable exit from the cell cycle and SD cell phenotype consequences.

The results showed that all samples were negative for hematopoietic markers, positive for mesoderm markers CD 13 and CD 90, irrespective of phenotype. CD 29 is almost absent in all cell lines tested. CD 90 had an expression variable between subjects.

Cytometric analysis of amniotic cell proliferation indicates that the cells suffer from several cell divisions, is more effective than the bone marrow mesenchymal stem cells to proliferate amniotic cells undergo multiple cell divisions, which are more effective than bone marrow mesenchymal stem cells to proliferate.

As a result of the analysis of the cellular differentiation of the mesenchymal cells from two biological sources (amniotic fluid and bone marrow) it is noted that amniotic cells are differentiated to osteoblasts and chondrocytes, but not in adipocytes experimental conditions in which bone marrow cells subjected to induction efficiently reach three differentiated phenotypes.

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Social Fund for Sectoral Operational Programme Human Resources Development.

List of publications:

I. Amniotic Fluid Cells Proliferation in Normal and Down Syndrome Subjects. **Honcea Adina**, Iulian Rujan., Pavel Anca Gabriela, Ion Ileana, Sima Livia Elena.ARS Medica Tomitana - 2016; 1(22): 10.1515/arsm-2016-0001

II. Stem cells from amniotic fluid, an essential tool for regenerative medicine – review. **Honcea Adina**. Fiziologia – Physiology. 2016.26.1(89).

III. Cytometric analysis of cell proliferation and differentiation of amniotic fluid - the scientific poster in the Conference PedDEX, Iasi 2015. **Honcea Adina**, Livia Sima Elena.