Advanced protocols for tissue disaggregation and preparation of cell suspensions

M. Montanari[1,2], S. Burattini[1,2], W. Balduini[1], S. Carloni[1], F. Luchetti[1,2], P. Ambrogini[1],
MG. Nasoni[1,2], C. Ortolani[1,2], S. Papa[1,2] and B. Canonico[1,2]

[1] Department of Biomolecular Sciences, [2] Center of Microscopy and Flow Cytometry, University of Urbino Carlo Bo

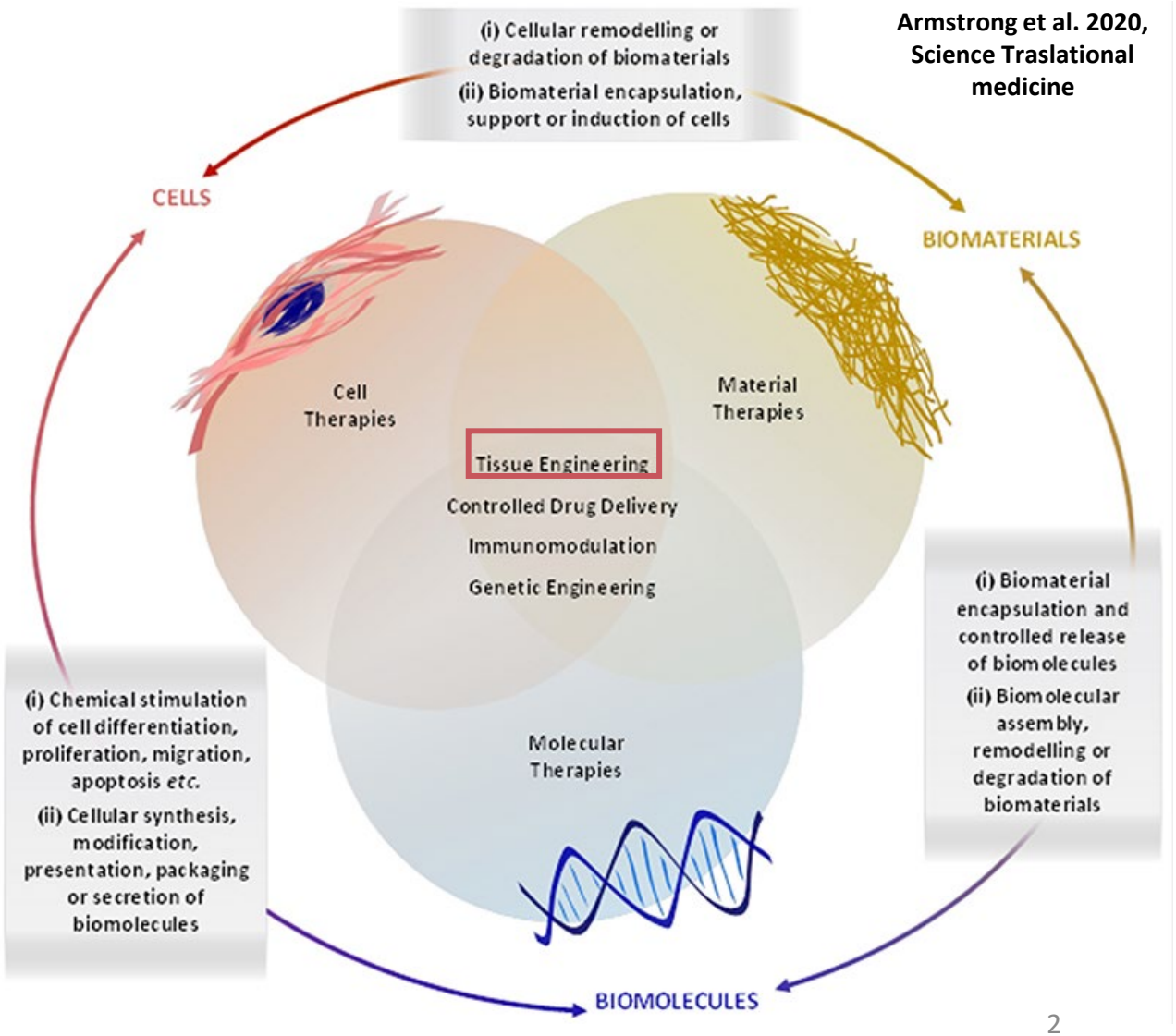
1506
UNIVERSITÀ DEGLI STUDI DI URBINO CARLO BO

DISB
DIPARTIMENTO DI SCIENZE BIOMOLECOLARI

Introduction

The aging population demographic has led to the rise of **regenerative medicine**, which typically employs combinations of **cells**, **biomaterials** and **biomolecules** in order **to regenerate or replace damaged or diseased tissue**.

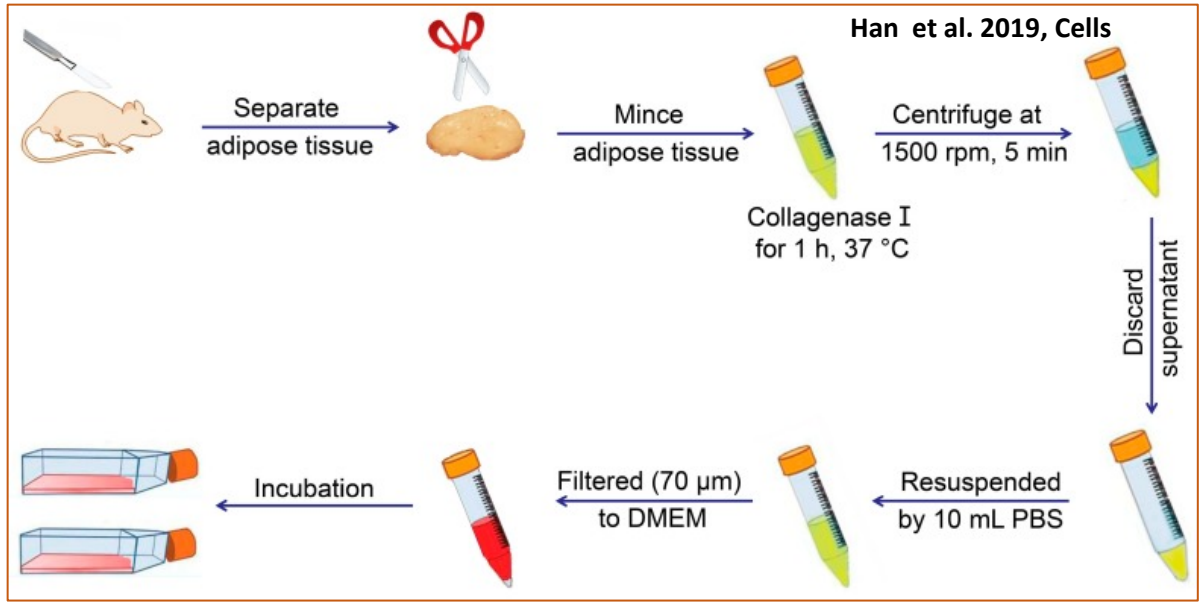
Significant promise for applications in tissue engineering and regenerative medicine (cell turnover, cell replacement or repair, rejuvenation and Immunomodulation) are Mesenchymal stem cells (**MSCs**) . (Kobolak et al. 2016)



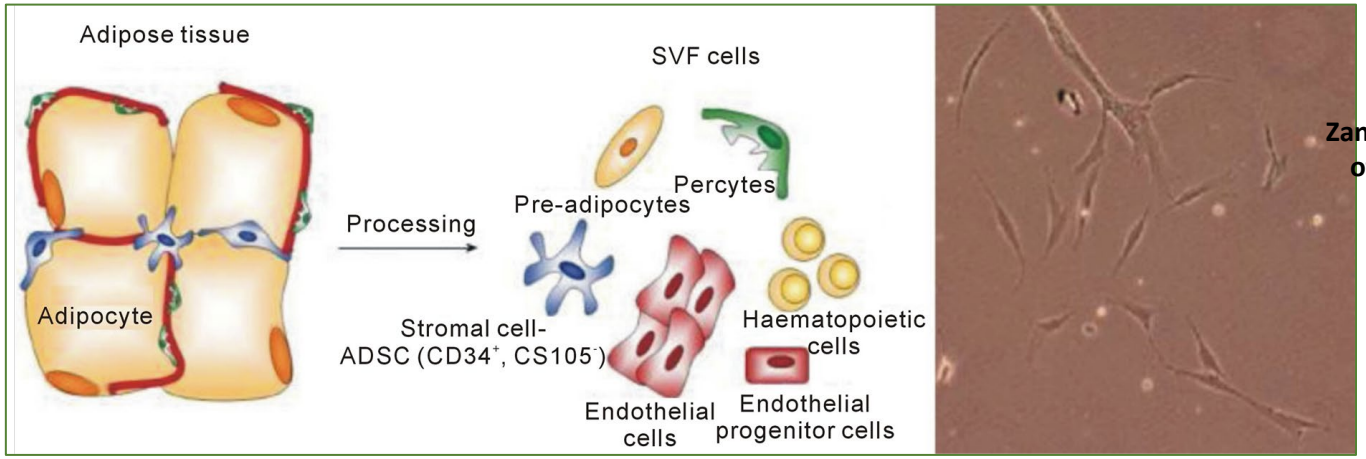
Armstrong et al. 2020, Science Traslational medicine

Introduction

The procedure for MSC (and various cell) extraction on *mouse adipose tissue* usually use digestive enzymes. (Han et al. 2019)



Other procedures (Zanzottera, F. et al. 2014) adopt mechanical disaggregation to obtain cellular suspensions rich in adipose derived MSCs and growth factors from *human adipose tissue*, useful for wound healing and engraftment.



Zanzottera, F. et al. 2014, Journal of Cosmetics, Dermatological Sciences and Applications

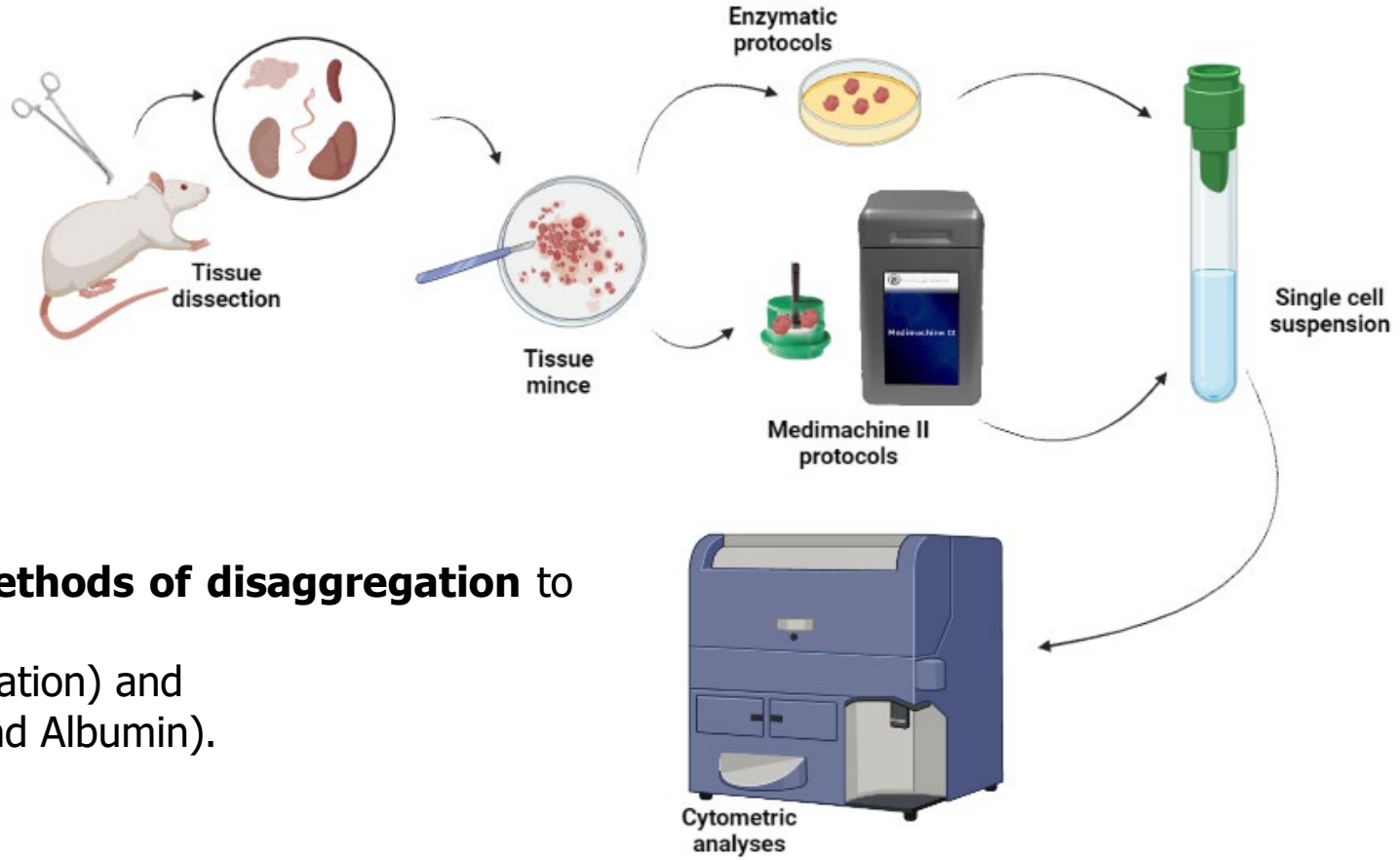
Introduction

The first step to obtain a **cellular suspension from tissues** is the disaggregation procedure.

The main goal of a cell suspension method is:

- provide **A rich and representative sample** of the different cellular subpopulations,
- get the greatest **number of viable cells** and
- **avoid cell clumps**.

Current protocols for the preparation of cell suspensions from solid tissues are usually **time-consuming**, **highly operator-dependent**, and may **selectively damage certain cell types**.



In this study, we compared **two frequently used methods of disaggregation** to obtain a single cell suspension from different tissues:

- Medimachine II (enzyme-free mechanical disaggregation) and
- low concentrations of the enzyme Trypsin (EDTA, and Albumin).

Methods

Here we compared **two different approaches** a **mechanical tissue disaggregation** method (by Medimachine II) and **enzymatic procedure**, for mice (C57BL/6), rat (albino Sprague-Dawley) tissues.

Flow cytometric, confocal and ultrastrucural (TEM) analyses were applied to **spleen**, **testicular**, **kidney** and **nervous** tissues (from newborn mice).

Samples were treated by **trypsin/EDTA** (0.125% with BSA 5% and DNAse 0.005%) for 10 min at 37°C or **processed by Medimachine II** (a mechanical system working independently to operator's ability), adding 1ml PBS into Medicons chambers.

Protocols were **optimized on each specific tissue** and the cell suspension **filtered** using particular Filcons **on the basis of the size of the main cell type**.

ENZYMATIC DISGREGATION

AUTOMATED MECHANICAL DISGREGATION (Medimachine II)

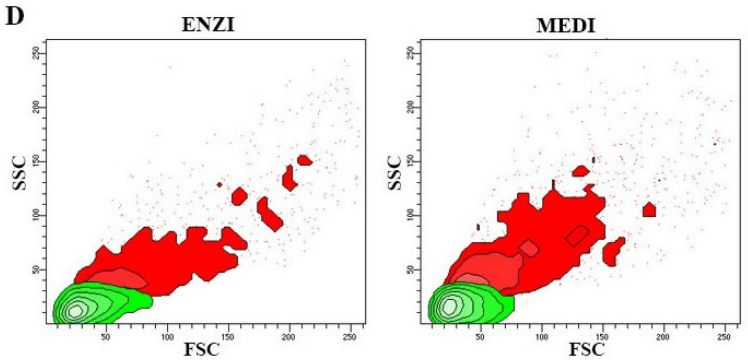
1506
UNIVERSITÀ DEGLI STUDI DI URBINO CARLO BO
DISB DIPARTIMENTO DI SCIENZE BIOMOLECOLARI

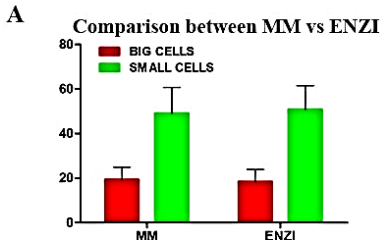
Results

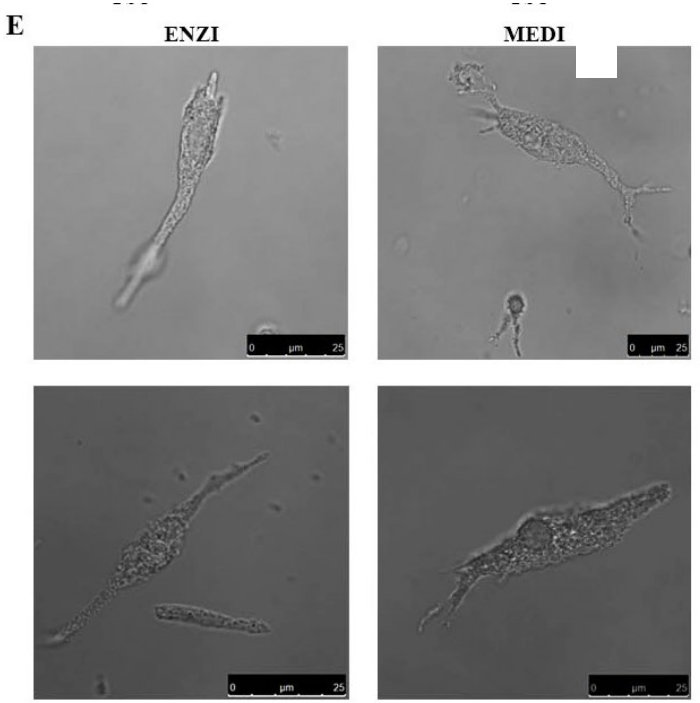
We used different cellular markers analyzed by flow cytometry and confocal microscopy.

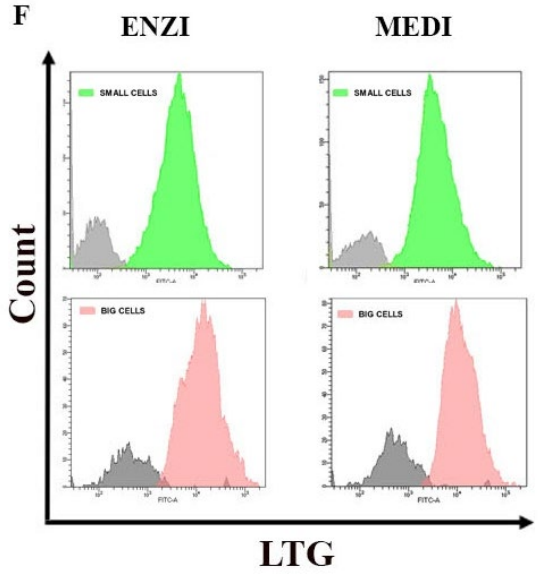
Nervous tissues Results: Organotypic hippocampal slices

Flow Cytometry and Confocal microscopy enable us to distinguish two subpopulations, in both methodics. Therefore, Small and Big cells aren't dependent from the type of disaggregation we used.
For **viability** evaluations we used PI positivity, that highlights that the two approaches induce a **similar number of cell death**. Another investigated parameter was the **lysosomal compartment** by LysoTracker Green (LTG) fluorescence, that follows the same trend in both procedures.



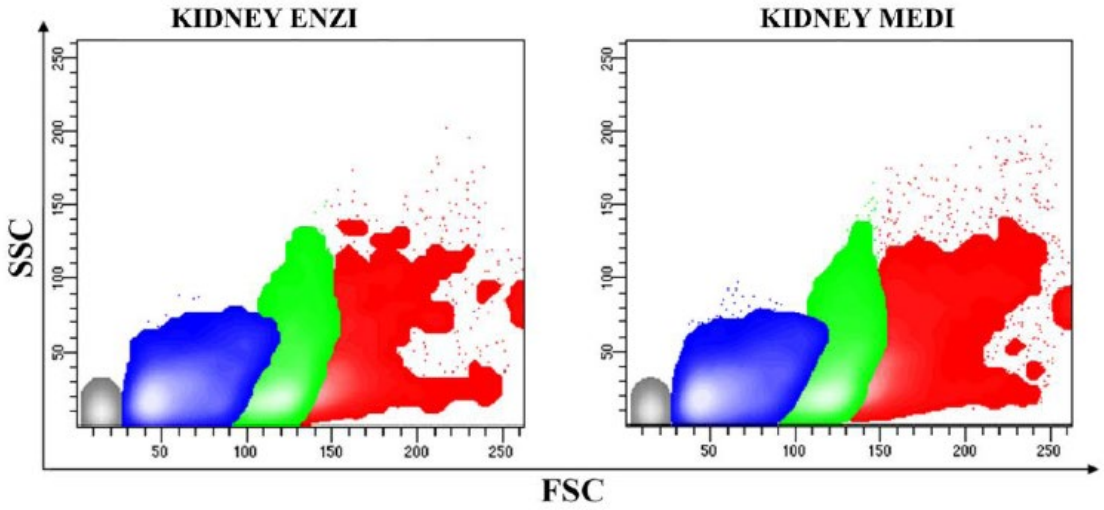


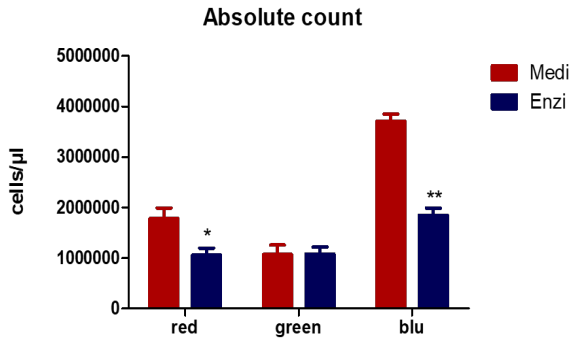




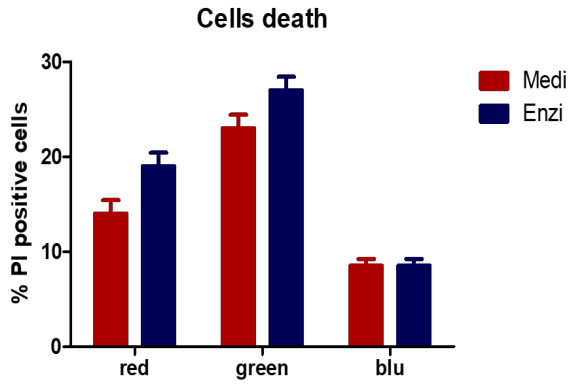
Kidney Results

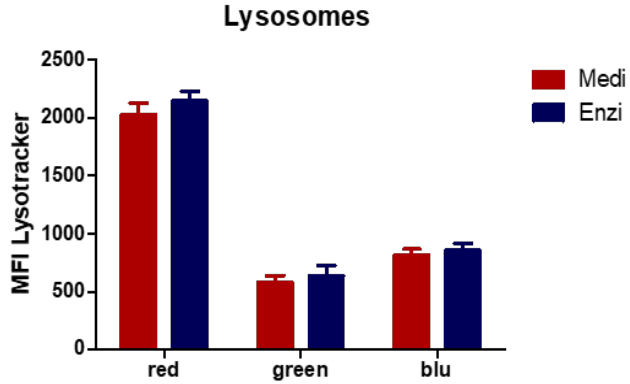
Cell populations were selected with a strategy that allows us to exclude most of the debris.
The Medimachine II procedure allows to obtain a **greater cell yield** with a percentage of viability that is similar, even **slightly higher than the enzymatic** procedure. The percentage of **PI-positive cells** obtained by the two methods of disintegration is **quite similar**.





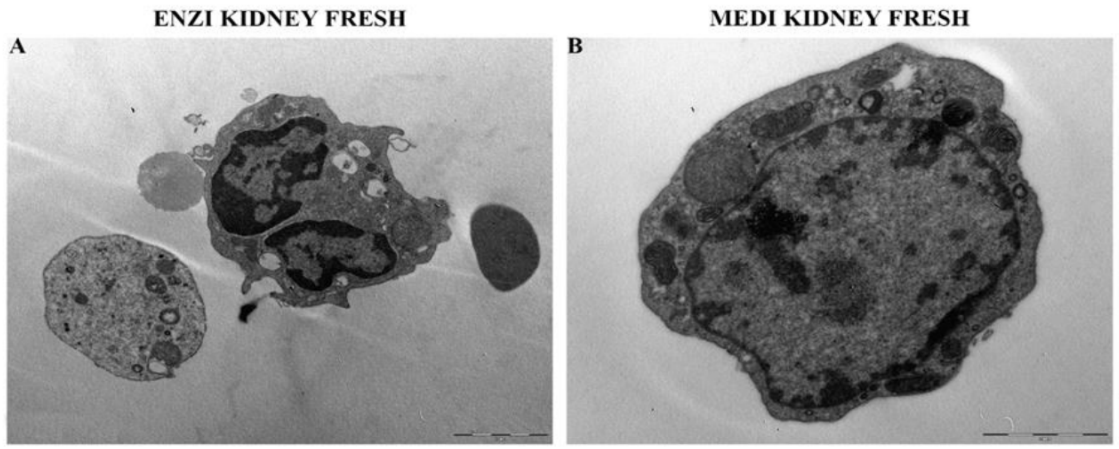
In the **red population**, with enzymatic dissociation we have 18% PI positivity, whereas with Medimachine II we have only 13%. In the **Green population**, with enzymatic we have 26% of PI positive cells, nevertheless with Medimachine II we get 22%; In the **Blue population**, with both enzymatic and Medimachine, we found 8% PI positivity.
The intensity of LTG is therefore overlapping in the two procedures.



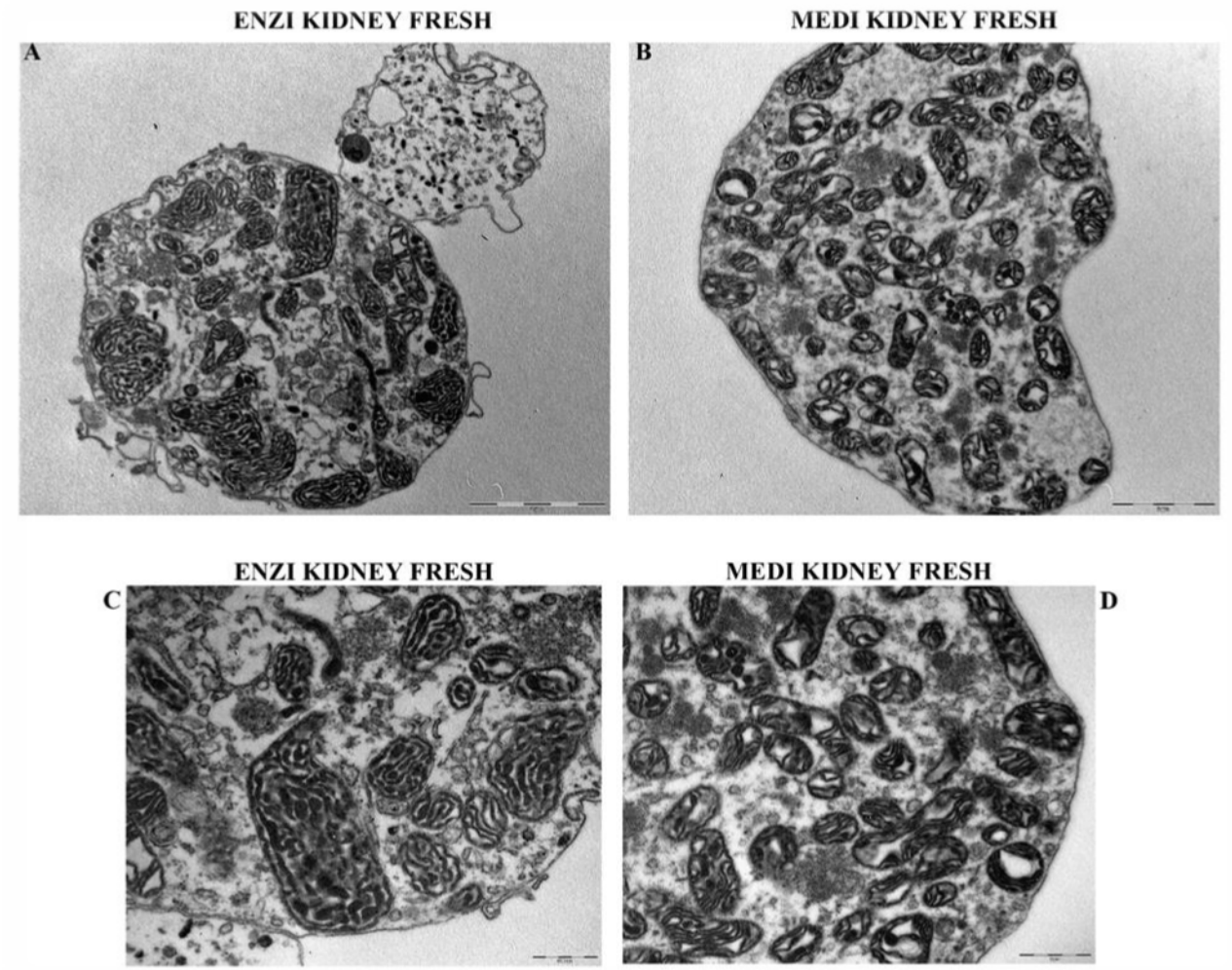


1506
UNIVERSITÀ DEGLI STUDI DI URBINO CARLO BO
DISB
DIPARTIMENTO DI SCIENZE BIOMOLECOLARI

Kidney Results



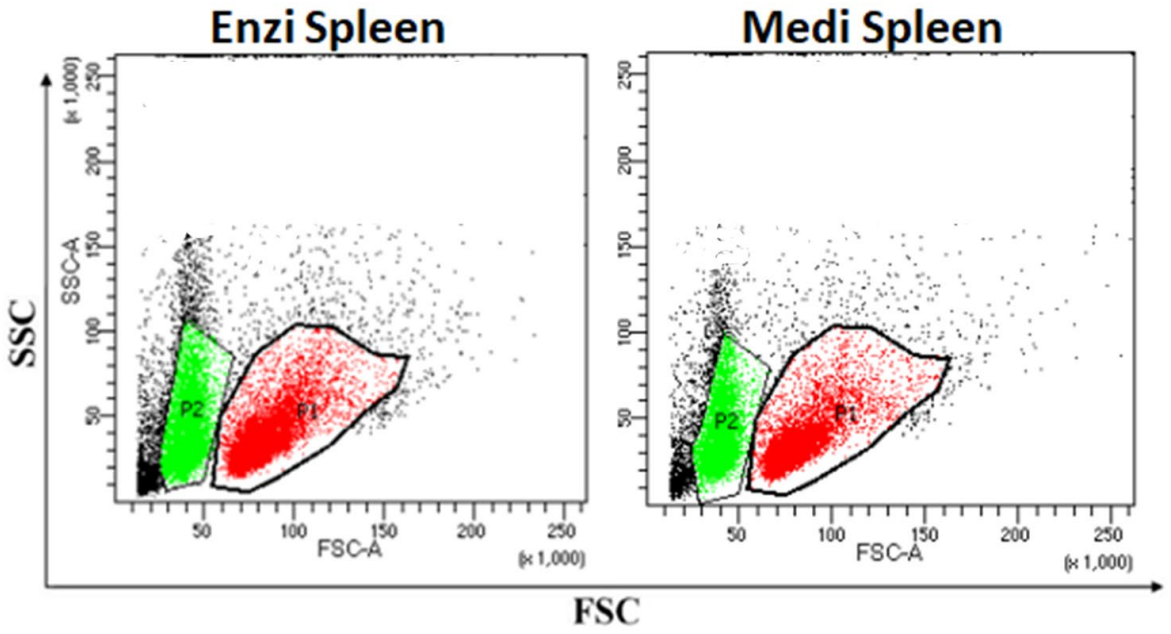
Transmission electron microscope (TEM) analysis of renal cell suspension allowed to highlight the cell ultrastructural and the mitochondrial content.
It shows the **richness in mitochondria** and **good conditions of preservation of cell** and **mitochondrial membranes**.

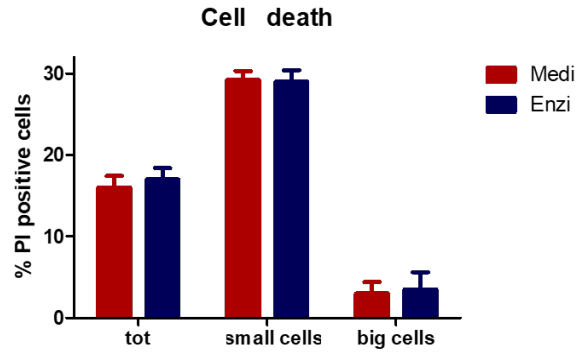


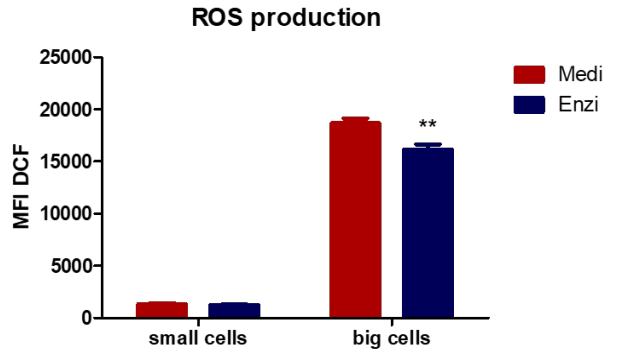
Spleen Results

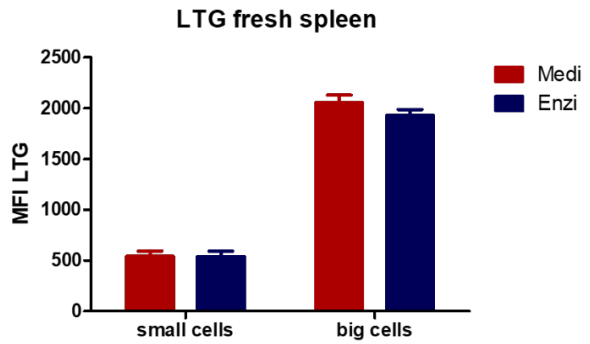
The trend for **cell death** looks very similar in both protocols, furthermore **ROS (**H_2O_2 content by DCF staining) shows comparable results in small cells.

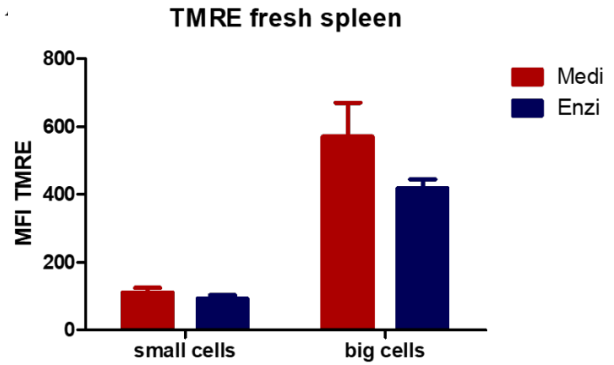
The lysosomal compartment and the mitochondrial membrane potential have been studied by **LTG** and **TMRE** staining, the statistical analysis shows a slightly higher mean fluorescence intensity (MFI) for the Medimachine II procedure.



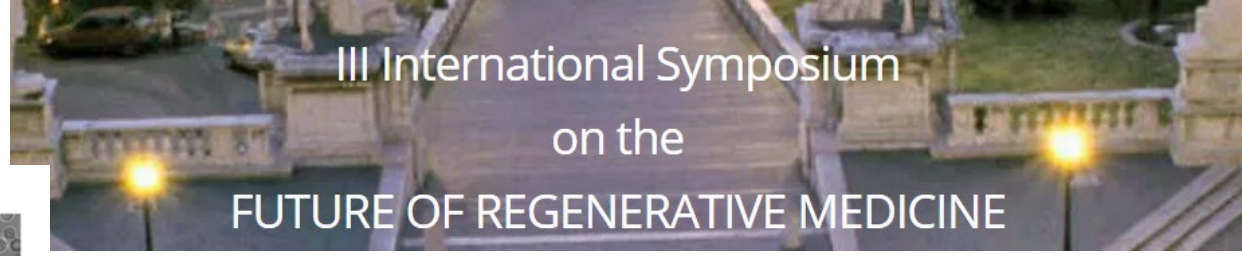




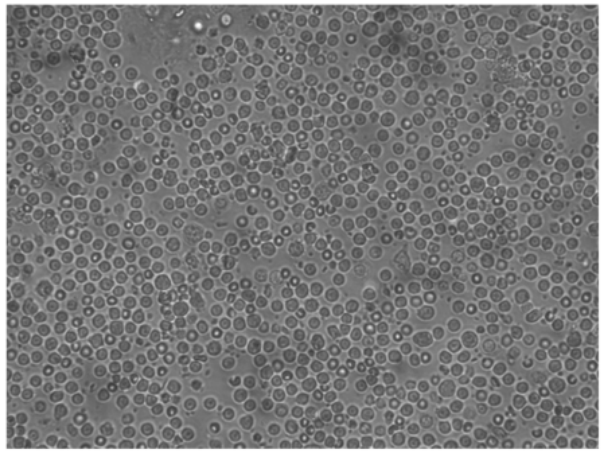




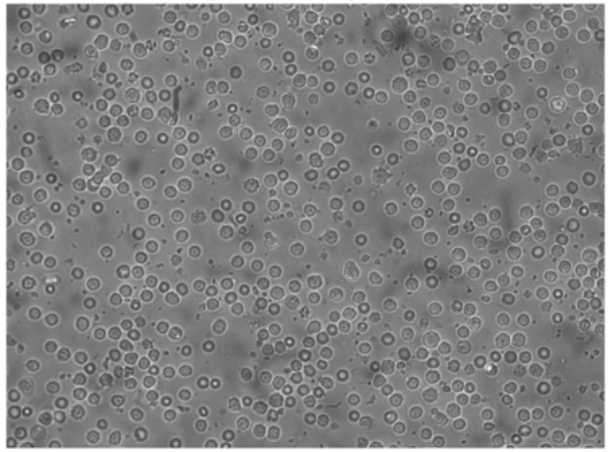
Spleen Results



ENZI SPLEEN FRESH

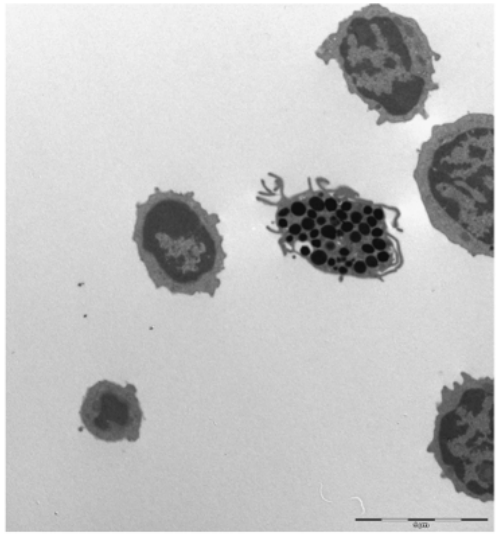


MEDI SPLEEN FRESH

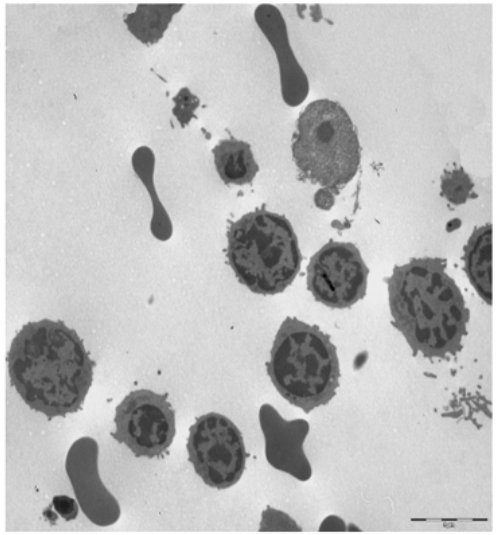


TEM made it possible to obtain a **comparison** for **ultrastructure** between the two methods of tissue's dissociation.
In Medimachine-obtained suspension it is possible to appreciate well-preserved **splenocytes** and **erythrocytes** with characteristic morphology.

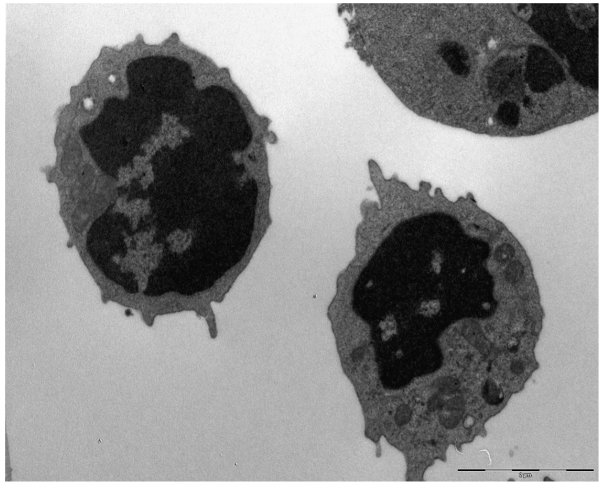
ENZI SPLEEN FRESH



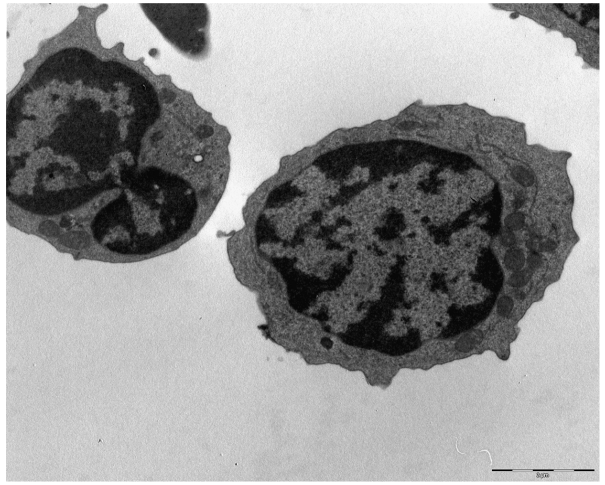
MEDI SPLEEN FRESH



ENZI SPLEEN FRESH



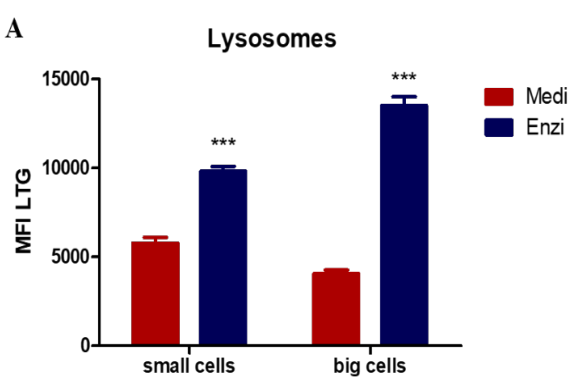
MEDI SPLEEN FRESH

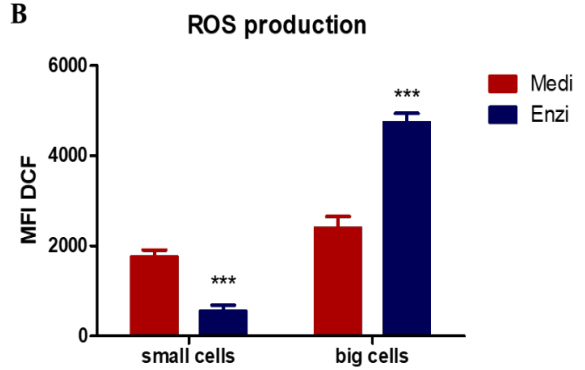


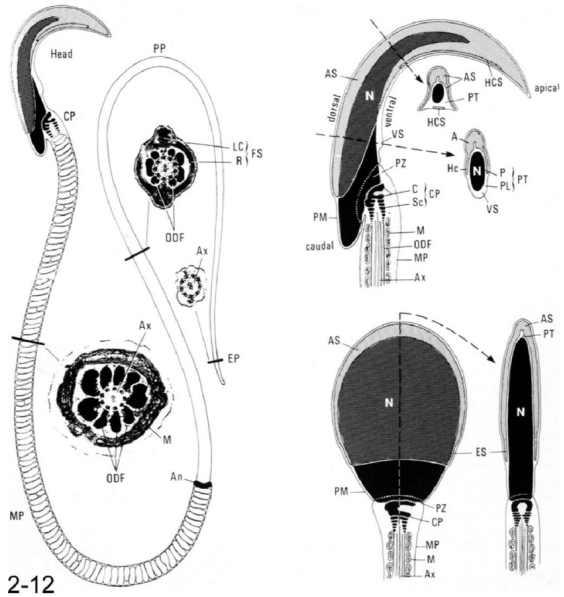
Testicular Results

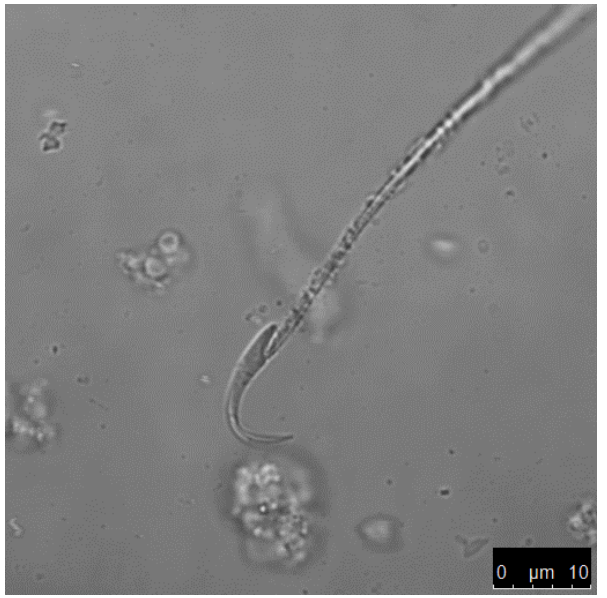
Big cells show a higher level of **LTG fluorescence** than small cells, demonstrating in these a higher content in lysosomes. Small cells, probably mature spermatozoa, show significantly lower IF levels than big ones; this coloration is greatest in cells obtained by Medimachine II disintegration.

The **ROS analyses** show that small cells have a lower amount of ROS than found in big cells, but with an important difference: it is evident that the disintegration technique using Medimachine II, in the case of small cells, allows to obtain higher values than those detected by enzymatic technique. On the contrary big show higher ROS values with enzymatic technique.







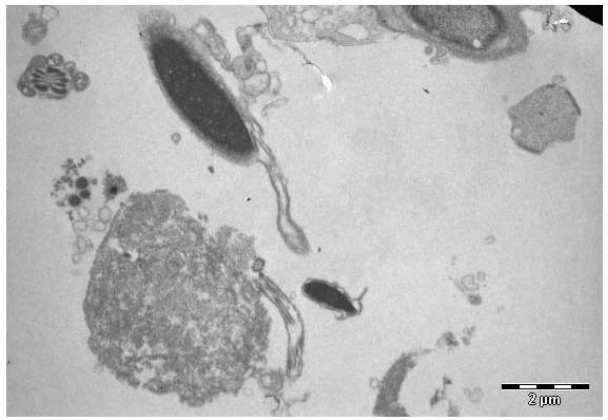


Testicular Results

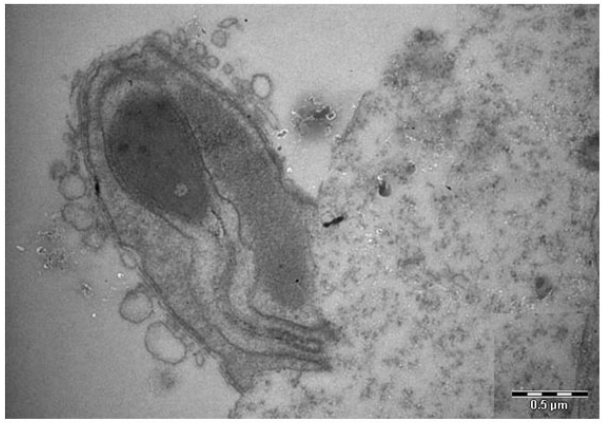
Confocal microscope and TEM analysis of sperm obtained from disintegration with both methods shows the integrity of the **spermatozoa** that keep the head **hook-shaped** especially evident with Medimachine II and the scourges preserved in both methods can be seen.

A peculiarity is the green corona resulting from LTG that specific marker of both the lysosomal and acrosomal content, while the red-orange halo due to TMRE that marking mitochondrial content, in fact fluorescence is obtained in the initial part, that is, in the area of the neck of the scourge where the mitochondria are concentrated.

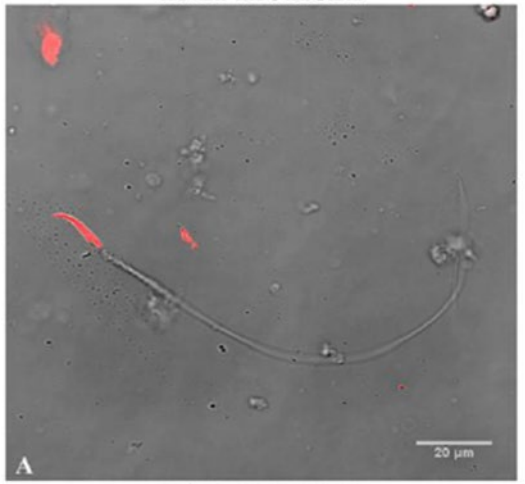
ENZI TESTICLE



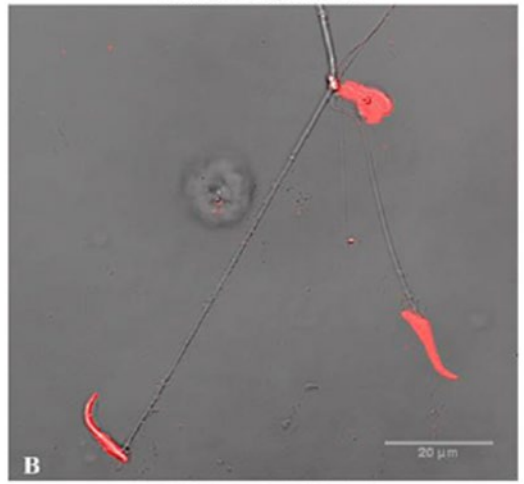
MEDI TESTICLE



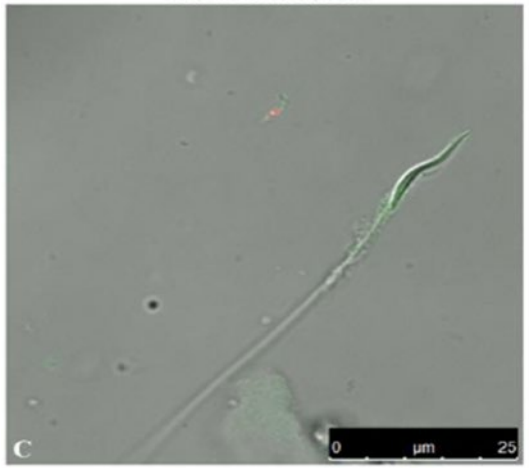
ENZI TESTICLE



MEDI TESTICLE

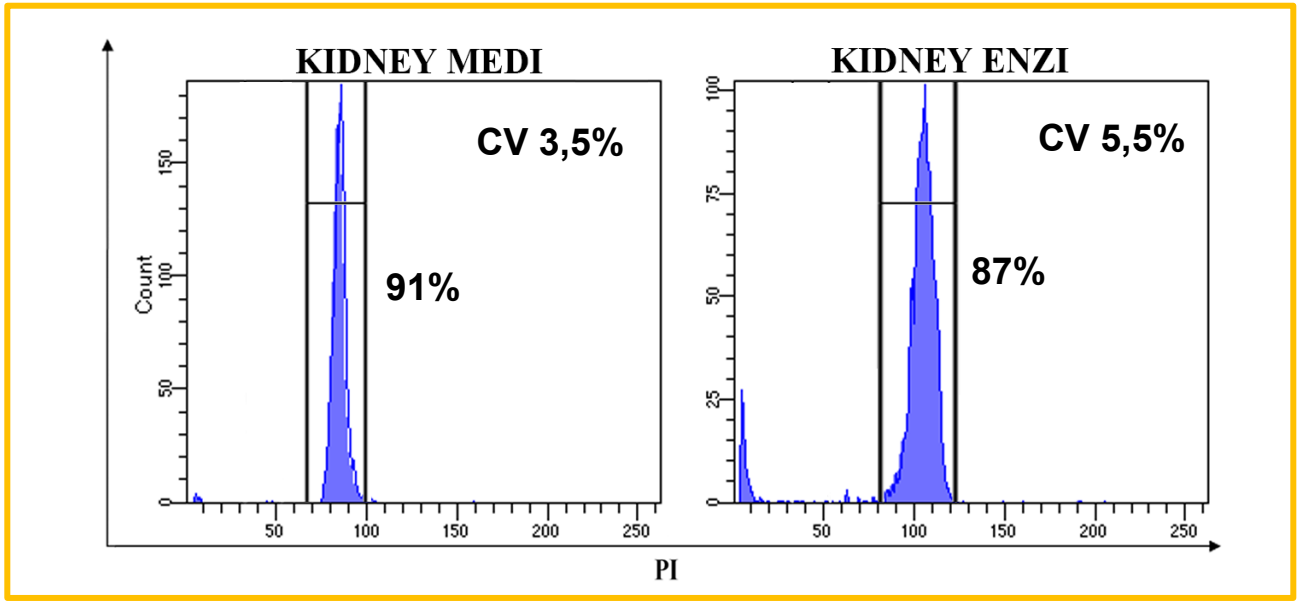


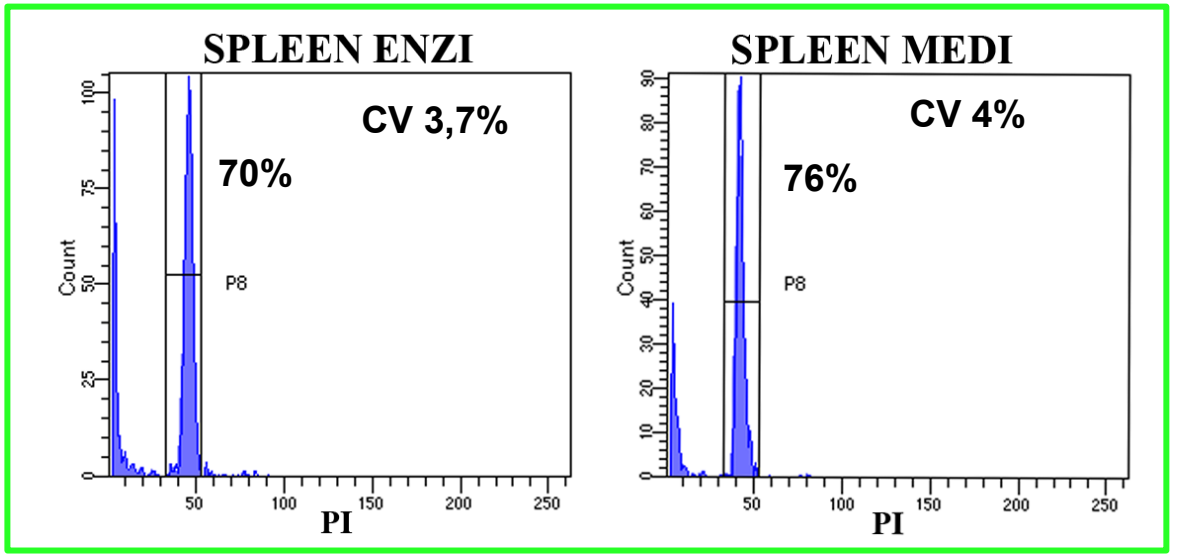
ENZI TESTICLE

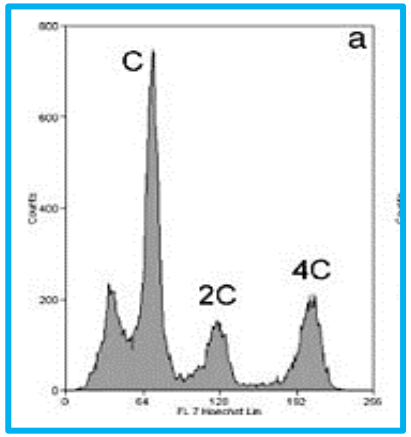


MEDI TESTICLE

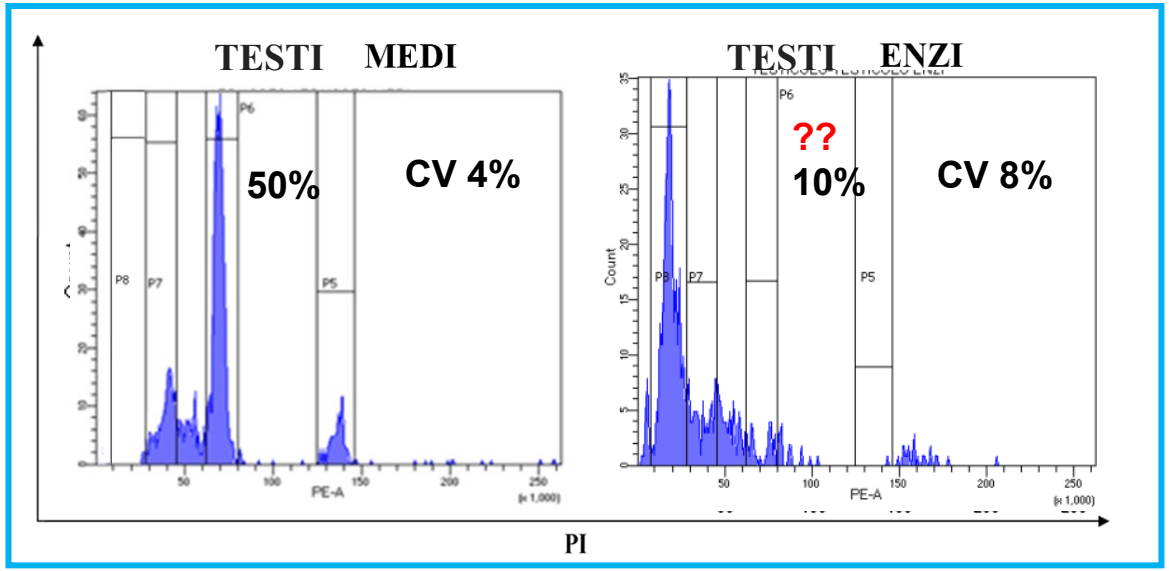
Cell cycle Results







DNA content analysis of RAT testicular cell suspensions from (Rodríguez-Casuriaga et al. 2009)



1506
UNIVERSITÀ DEGLI STUDI DI URBINO CARLO BO

DISB
DIPARTIMENTO DI SCIENZE BIOMOLECOLARI

CONCLUSION (1):

Enzymatic protocols

Medimachine II protocols

Combines the advantages of a **high reproducibility** and allowed the **rapid obtainment** of enriched cell suspension.

1. **similar** *physical characteristics* **and** *intracellular organelles* **and** *cellular parameters*.
2. **comparable rates of apoptotic/necrotic cells.**

Conclusion (2)

Medimachine II and Medicons represents a simple, fast, and standardized method for tissue processing, useful not only in the research field, but also in the clinical settings (i.e. bioptic, autoptic sample management or regenerative surgery, by the sterile Rigeneracons and the MediGraft Kit). In conclusion, the automated protocol allows to minimize bias arising from the operator's ability.

1506
UNIVERSITÀ DEGLI STUDI DI URBINO CARLO BO
DISB DIPARTIMENTO DI SCIENZE BIOMOLECOLARI

Acknowledgment

We want to thank CTSV to provide us the newly developed MediMachine II, Medicons and Filcons and to have supplied Medicons by their early days of marketing (the late 1990s).

THANK YOU FOR YOUR ATTENTION

1506
UNIVERSITÀ DEGLI STUDI DI URBINO CARLO BO

DISB
DIPARTIMENTO DI SCIENZE BIOMOLECOLARI