



Centre de recherche en
biologie de la reproduction



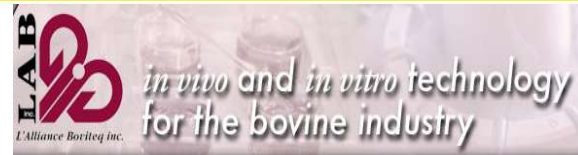
“Comparative genomic and phenotypic analyses
between Holstein and Jersey embryos to address the
known breed differences namely in cryotolerance”

EmbryoGENE 2011 AGM

Luis Baldoceca

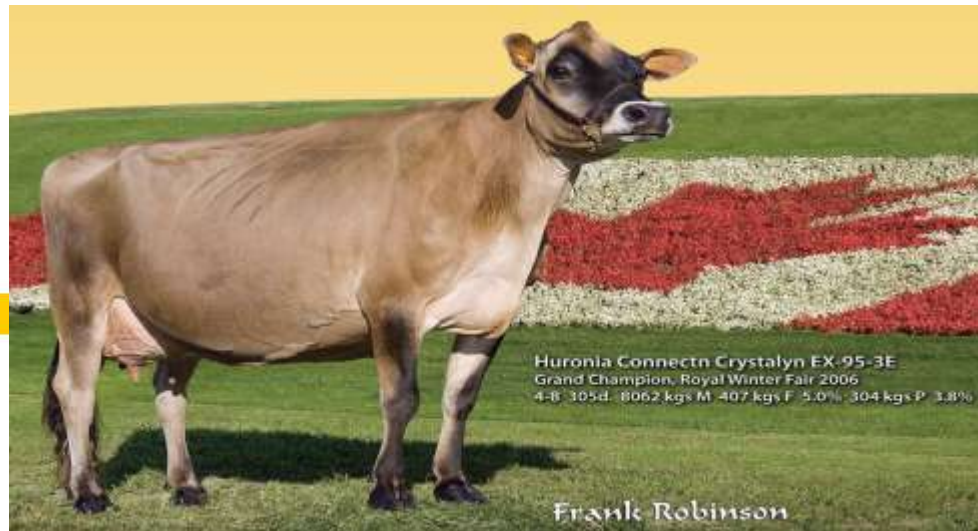


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Introduction

- There is increasing interest in cryopreservation of bovine embryos, specially previously problematic embryos.
- The Jersey cattle is one of the breeds with the highest fat milk index.
- The main defining characteristic of the Jersey cattle breed is their genetic predisposition to produce more butterfat than other bovine breeds, suggesting that their cellular biochemical makeup might have include more lipid content (Beaulieu et al., 1995).



Introduction

- Although it is not well documented in a scientific literature, Jersey breed embryos (JE) were demonstrated to have lower pregnancy rates following cryopreservation compared with Holstein breed embryos (HO) (Steel and Hasler, 2004).

Table 1. Pregnancy rates resulting from fresh and frozen embryo transfers in Holstein and Jersey breeds.

Embryos	Holstein Embryos		Jersey embryos	
	N. Transfers	% Pregnant	N. Transfers	% Pregnant
Fresh	3376	65.3	1161	62.8
Frozen-Gly	836	57.7 ^a	593	46.2 ^b
Frozen-EG	1111	53.8 ^a	193	39.9 ^b

^{a,b} Values in rows without superscripts differ significantly ($P < 0,001$), chi-square analysis.

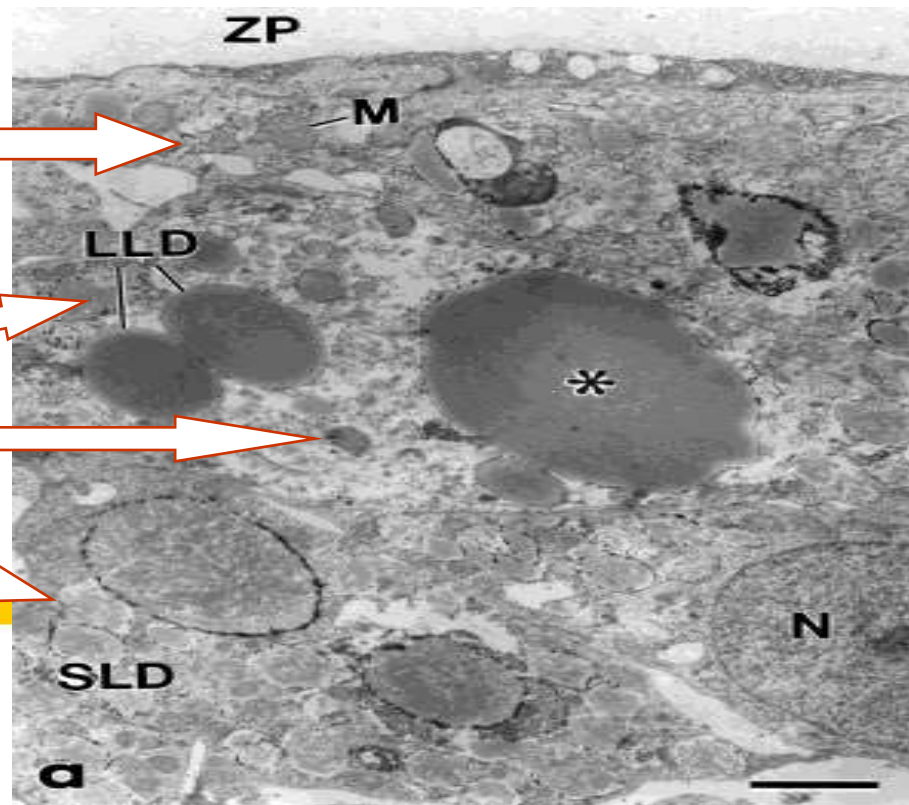
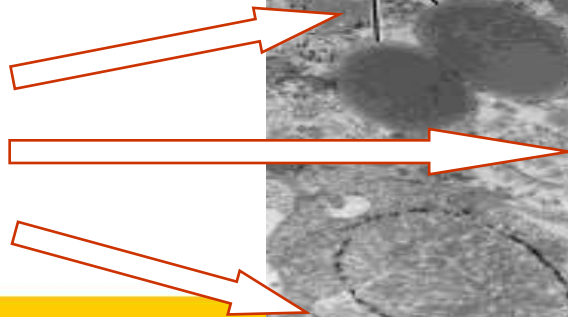
Introduction

- The lower success in frozen JE is associated with the amount of intracellular lipids in the embryo, that result in cell damage during cryopreservation.
- The intracellular lipid amount in the embryo seems to be related with depletion of stored products by impaired mitochondrial function (Abe et al., 2002).

Mitochondria

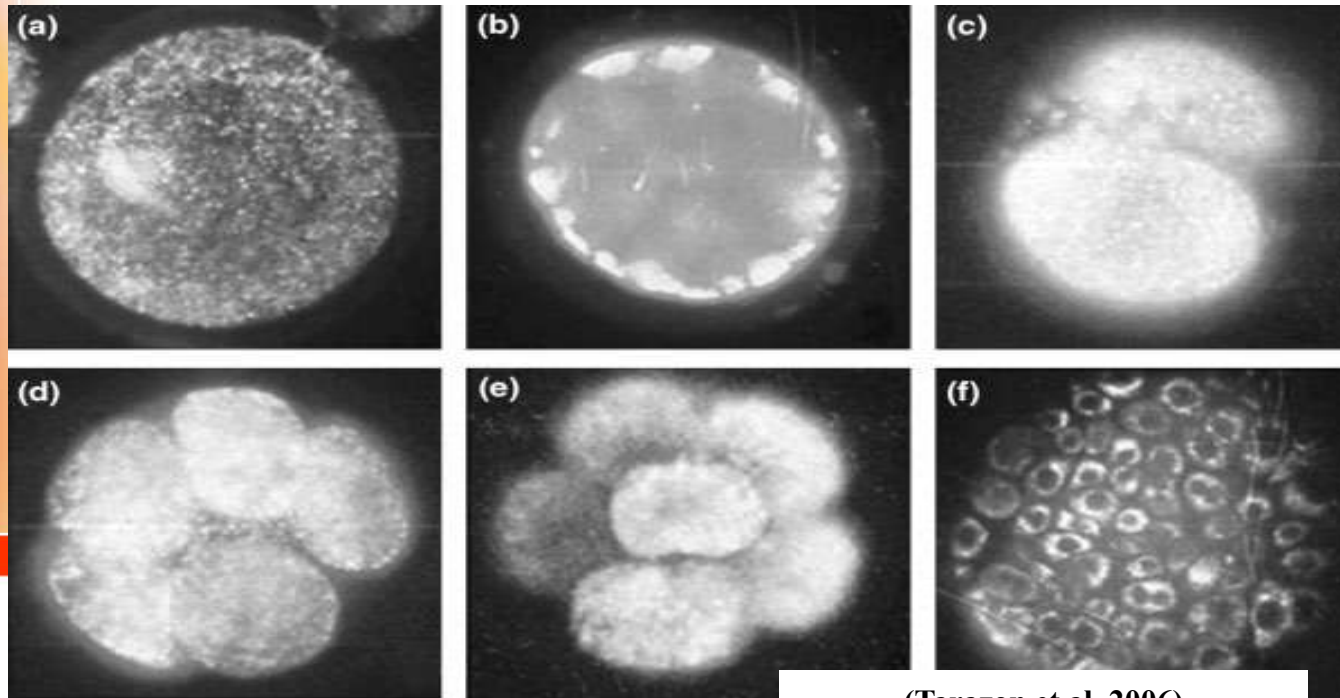


Lipid droplet



Mitochondria

- Mitochondria are vital determinants of normal pre-implantation embryonic development (Dumollard et al., 2007).
- Mitochondria are not static organelles, as their position in cell are localized to regions where the ATP supply must be high.



(Tarazon et al. 2006)

Lipid Profile

- Van Soom et al. (2003) observed that the color of bovine embryos depends on the breed.
- In comparison with Nelore breed embryos, HO were darker, and this was correlated with lower mitochondria number. (Visintin et al., 2001).

Table 2. Morphologic differences among Nelore and Holstein embryos.^a

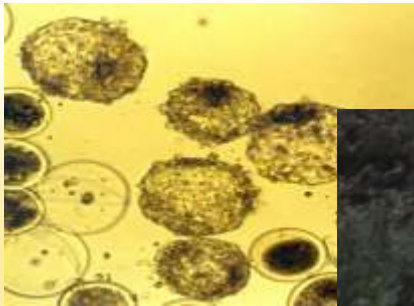
Characteristics	Nelore	Holstein
Mitochondria	++	+
Lipid droplets	++	+++
Endosomic cistern vesicles	+++	++

^aThe values had been estimated on the basis of morphologic analysis: + (few), ++ (intermediate values), and +++ (many).

- Do JE and HO are different lipid profile in embryo ???

Hypothesis

- The JE embryos have low rate of success after cryopreservation in comparison to HO due to a different lipid metabolism that relies on variations of gene expression.



Objective

- Characterisation of the phenotype and gene expression profile of JE embryos to identify candidate target genes that could shed light on the causes of the phenotypic breed difference between HO and JE.



Material and Methods

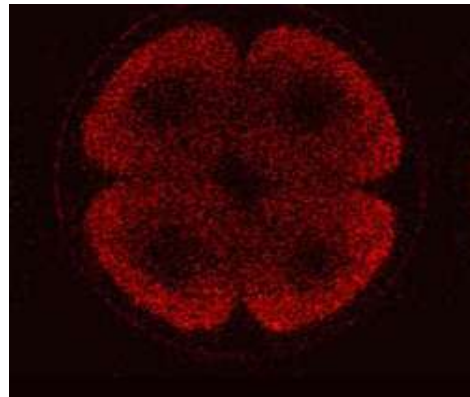
- HO IVF-morulae and blastocysts
- Phenotypic evaluation(Morphology)
 - Mitochondria
 - Distribution
 - Mitochondrial activity
 - Lipids
 - Cytoplasmic droplets size
 - Droplet number
 - Lipid Profile

Dyes and Confocal Microscopy

- Mitotracker Red (Invitrogen™) is commonly used to provide a means to assess mitochondrial survival or to assess the load of functional mitochondria.

Oxidation State : oxidized

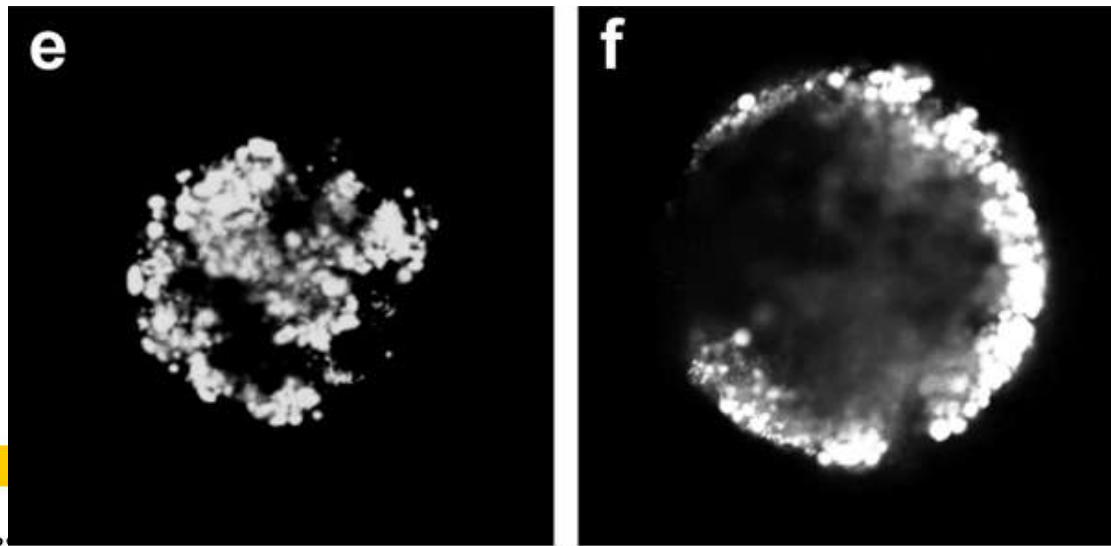
- Calcein AM (Invitrogen™) is uniformly distributed in all cells. Will be used as a mean to normalize fluorescence across different planes/depths.



Urchin embryo (Mitotracker Red) Agnello et al., 2008

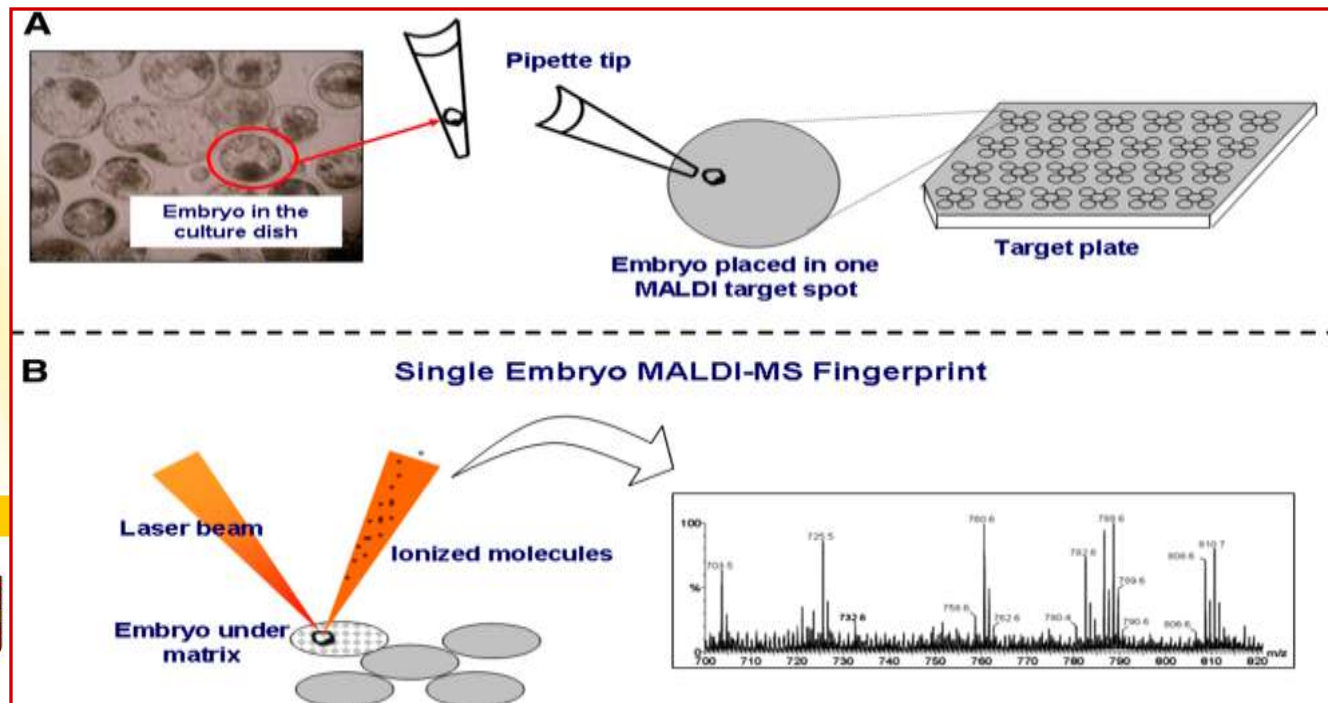
Dyes and Confocal Microscopy

- Nile Red is applied to detect intracellular lipid droplets and to quantify the total lipid content in mammalian embryo (Romek et al., 2010).
- Objectives:
 - Size of lipid Droplet
 - Quantification of lipid Drop

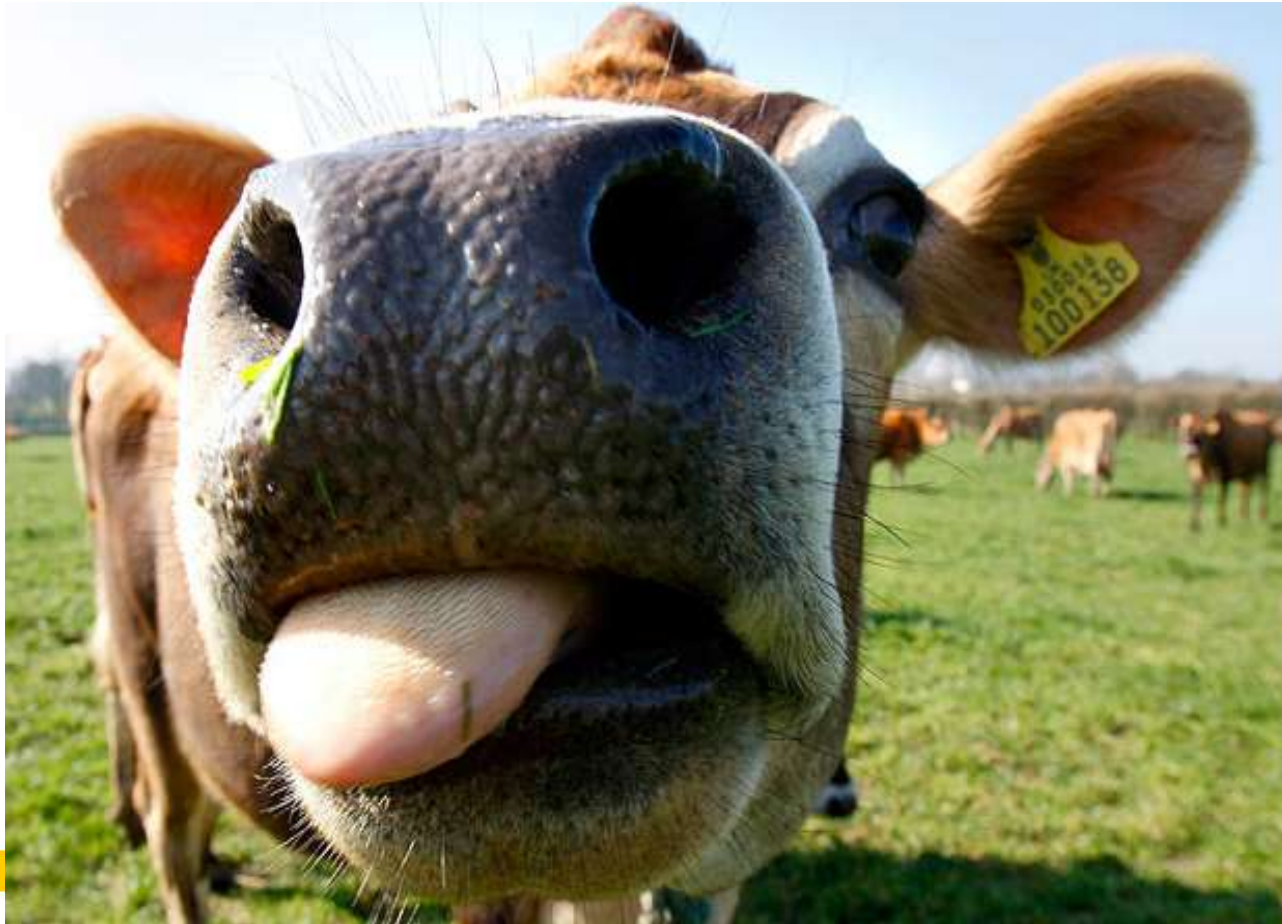


Mass spectrometry

- Mass spectrometry (MS) provides fast and simple acquisition of mass spectra of lipid profiles in embryos.
- Matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS) of single and intact embryos that can report direct analysis of lipid fingerprinting (Ferreira et al., 2010).

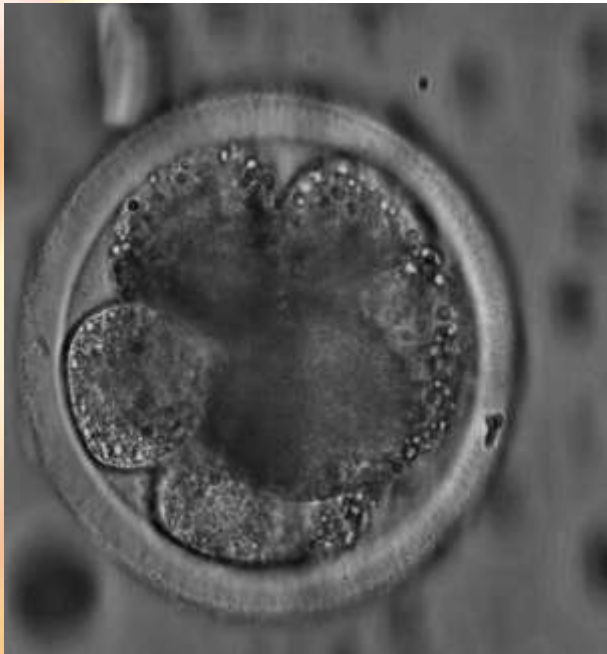


Preliminary Results

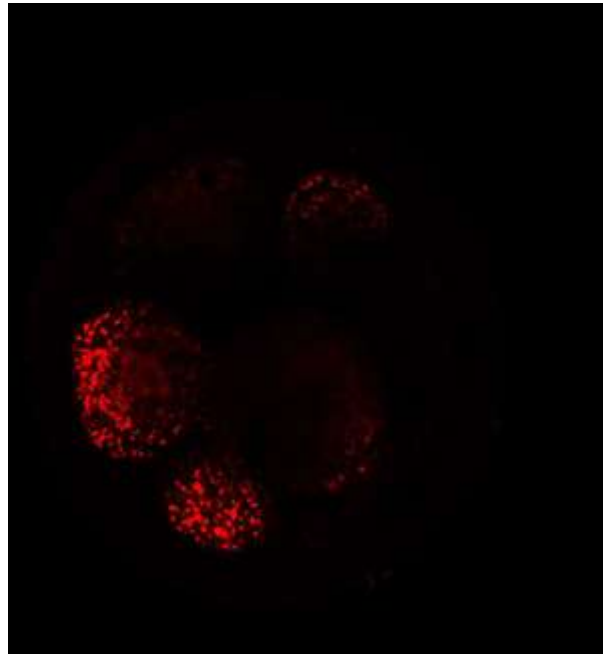


Preliminary Results

Distribution of functional mitochondria



Bright field



Mitotracker Red

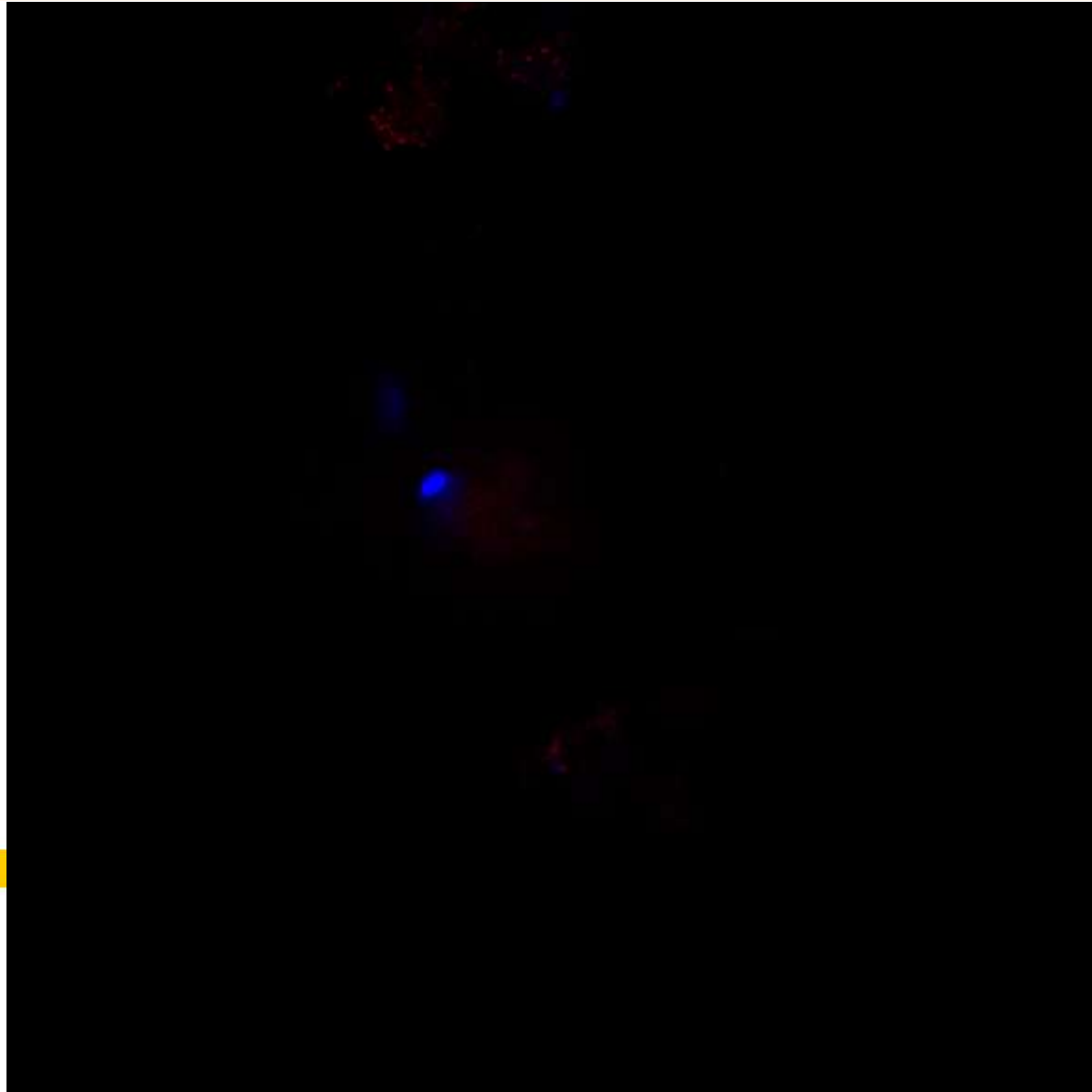


Merge

Preliminary Results

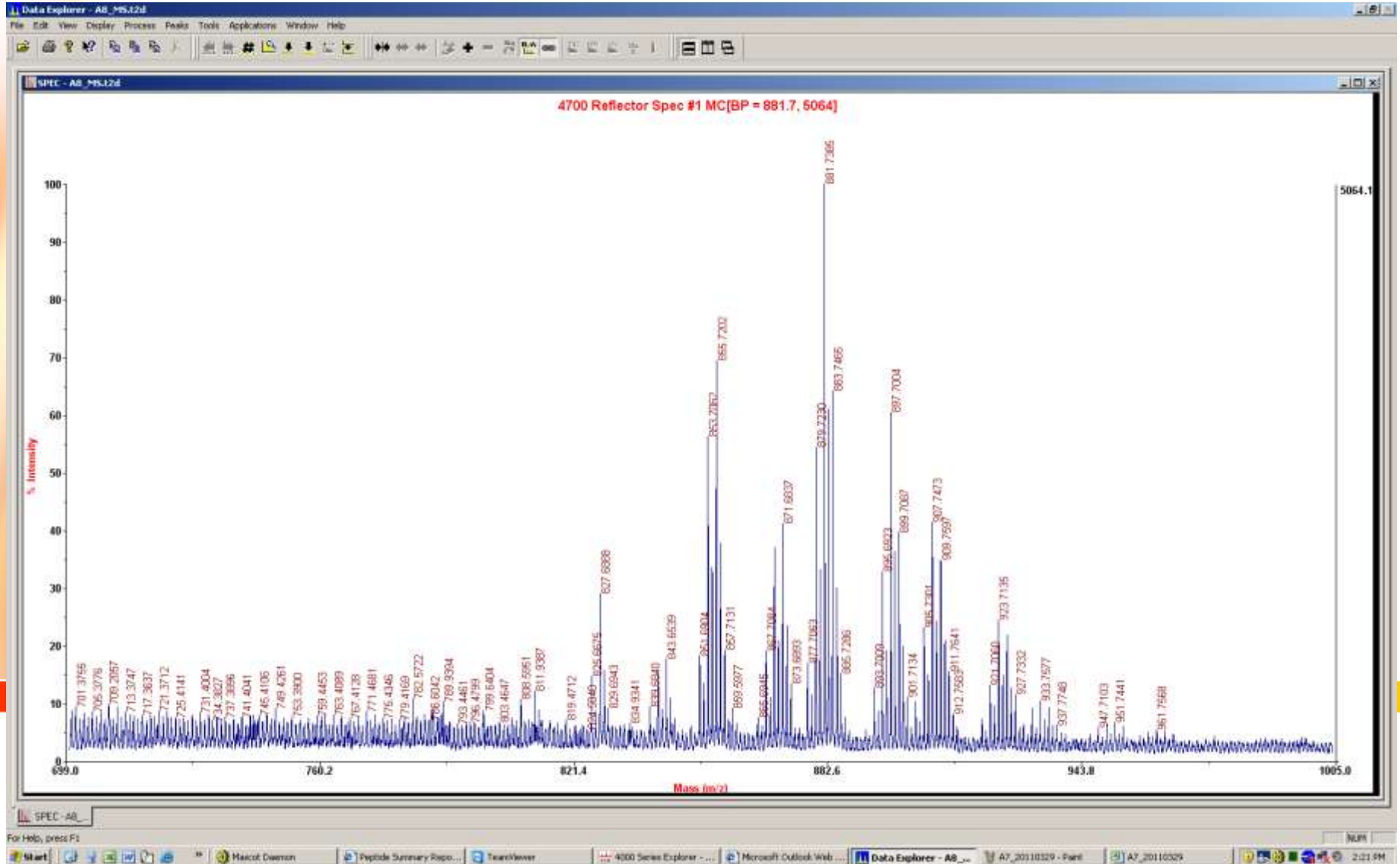
Nile Red: Lipid Droplets

We are recording
the amount and
the size of lipid
droplets per
embryo.



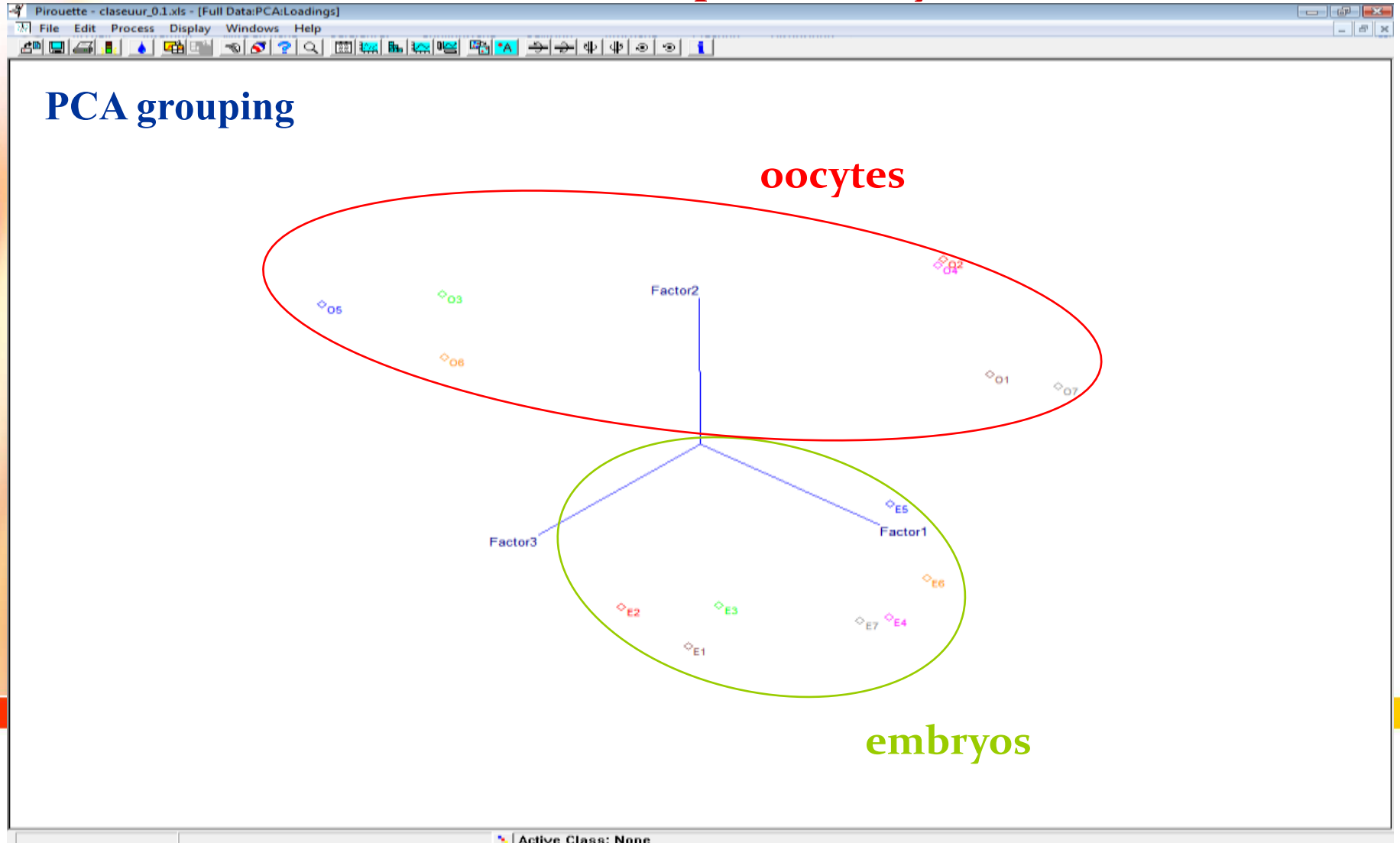
Preliminary Results

Mass spectrometry



Preliminary Results

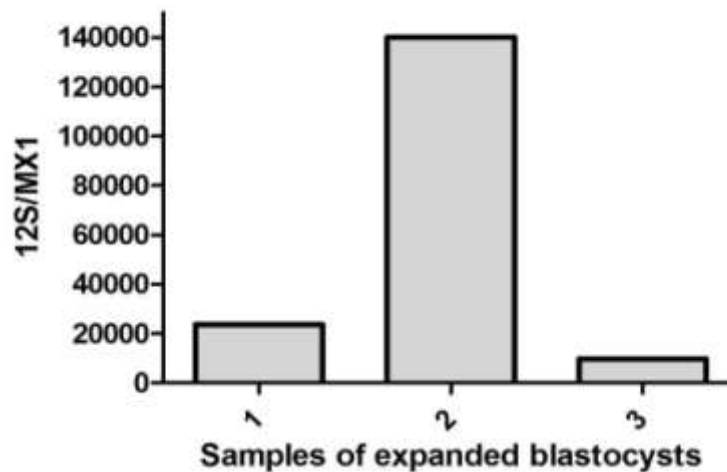
Mass spectrometry



Perspectives

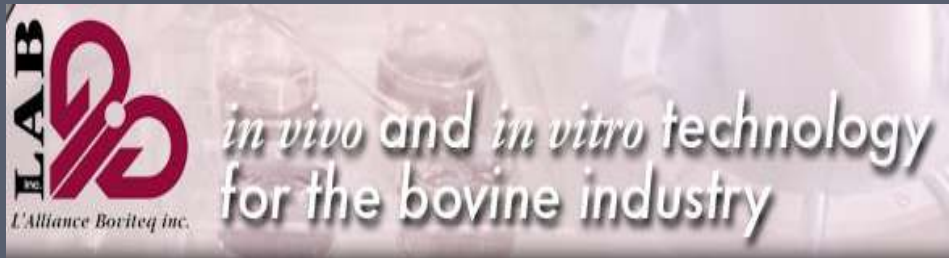
- Quantification of mitochondrial DNA(HO vs JE).

Number of mitochondrial genome per blastomere



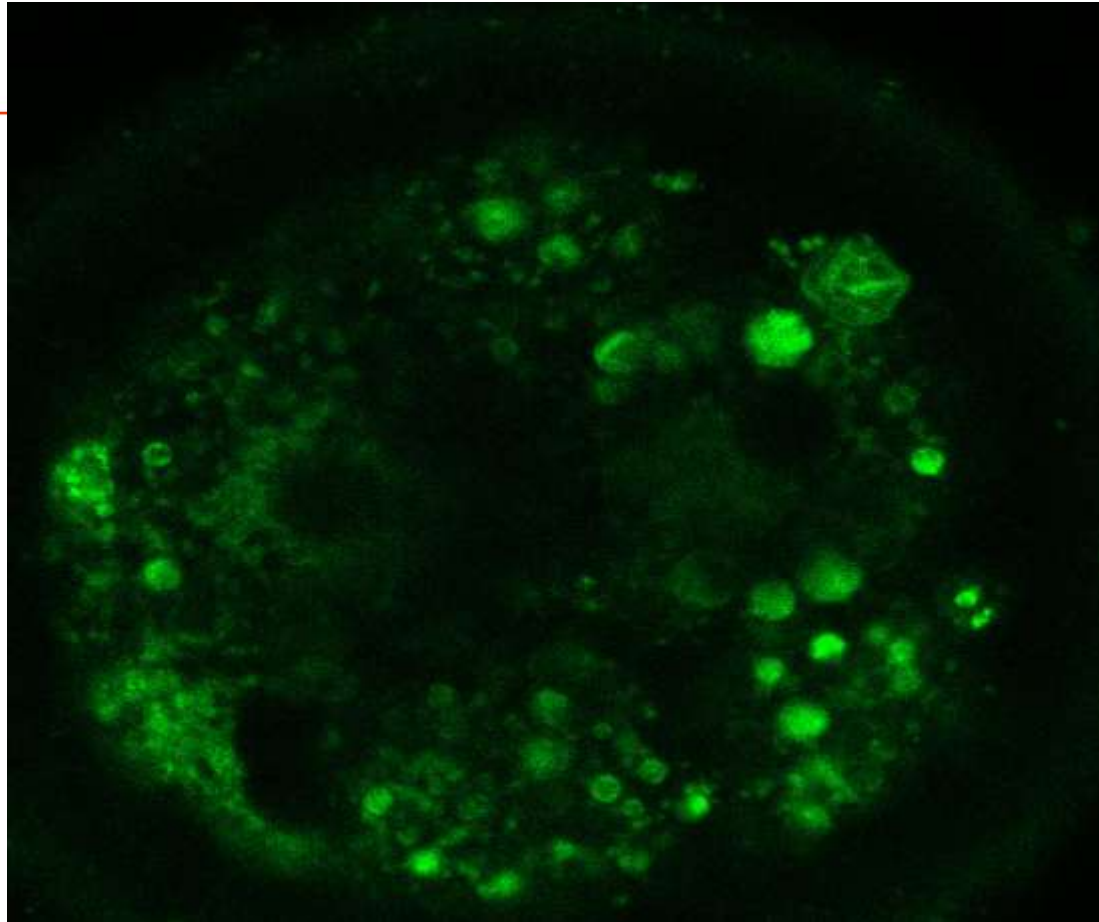
- Evaluation of the expression level of specific candidates involved in the lipid metabolism pathway using EmbryoGENE's microarray.

Acknowledgments



Muchas Gracias





MitoTracker FM Green (independant of membrane potential)