METABOLOMICS APPLIED IN EMBRYO CULTURE MEDIA USING NUCLEAR MAGNETIC RESONANCE

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IX SIMPOSIO de Investigación EN CIENCIAS EXPERIMENTALES

NMR MBC Advanced NMR Methods and Metal-based Catalysts

INTRODUCTION

According to the *WHO*, infertility is a disease of the reproductive system which affects around 50 million couples in the world[1]. Nowadays, some biomedical techniques facilitate or try to solve some of the problems associated with this condition, where *In Vitro* Fertilization (IVF) is the most popular. One of its disadvantages is the low success rate that presents, which is around 26% in IVF cycles in Spain[2], and could be conditioned by the primary method applied to the selection of transferred embryos, which is a non-invasive technique based on morphology (as ASEBIR[3] criteria), where variables associated with the rate of cleavage and blastocyst formation are evaluated by the embryologist following standardized criteria that are somehow subjective. This morphological approach is therefore inadequate for the prediction of embryo quality, and several studies have focused on developing new non-invasive methods using molecular approaches based particularly on metabolomics, with the aim to improve the actual embryo selection method. Specifically, most of these studies are focused on Nuclear Magnetic Resonance (NMR) as it has the potential to become a very useful tool.

Infertility
50 million afected couples in the world (WHO)

***InVitro* Fertilization (IVF)**

IVF success rate around 26% in Spain (SEF)

More than 8 million babies borned from IVF (2018, WHO)
Europe main continent in IVF treatments (2018, WHO)

Embryo cycle:

Embryo quality depends on **Morphological criteria** *(ASEBIR)*

- **A class:** Greater quality
- **B class:** Good quality
- **C class:** Medium quality
- **D class:** Bad quality
- **E class:** Blocked embryos

PROCEDURE

1. *Sampling and sample preparation*

1. Placement of one of the ends of the plate on a slide, at 30º
2. **Removal of paraffin**
3. Take of 20 µL of sample
4. Cleaning of the disposable tip with paper
5. Ejection of the sample intro a 0.2 mL Eppendorf
6. Storage at -80 ºC

162 Vitrolife™ culture media samples collected after embryo removal.

Only 73 of them could me measured.

2. *^{1}H-NMR analysis*

15 µL of sample + 400 µL NaCl (0.9%) in D_2O with TSP (0.01%)

❖Bruker Avance III 600 MHz spectrophotometer equipped with cryoprobe

❖^{1}H CMPG sequence

❖TopSpin 3.2. (Bruker)

3. *Statistical Analysis*

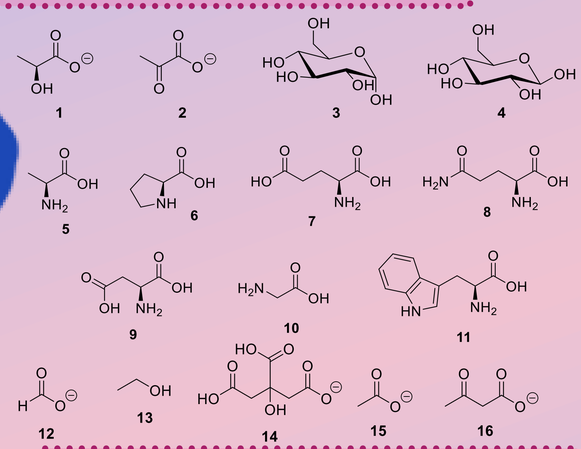
❖***Bucketing***
- AMIX (*v. 3.9.12*) → 0.04 ppm buckets
- Standarization to normal intensity (δ_H 0.2-10 ppm except δ_H 4.25-5.72 ppm)
- Other included data: women's age, oocyte number, transfer day (D+3, D+4, D+5), morphological embryo quality

❖***Metabolites and biomarkers asignation***
- CHENOMX (*v. 8.5*)

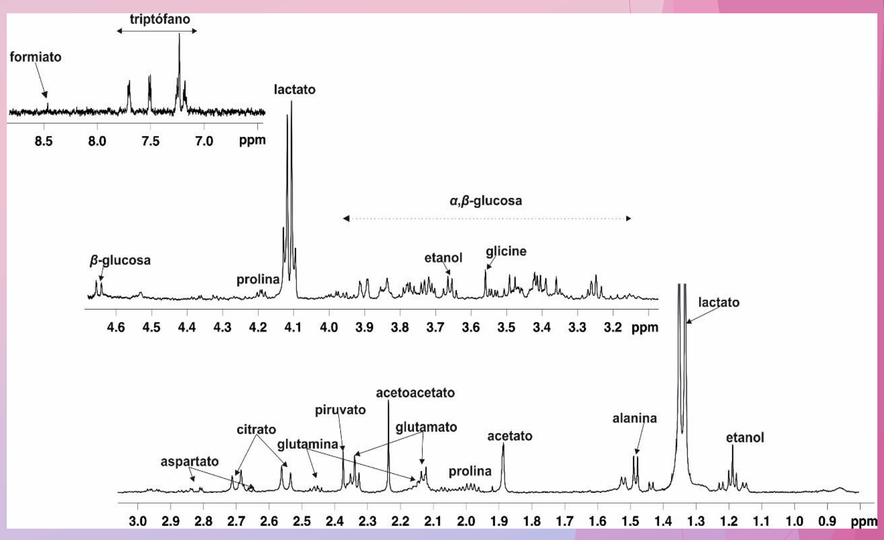
❖***Multivariate data analysis***
- SIMCA (*v. 14.0*)
- PCA – scaled to *Pareto*
- PLS – scaled to *Unit Variance*

RESULTS

Metabolites identification

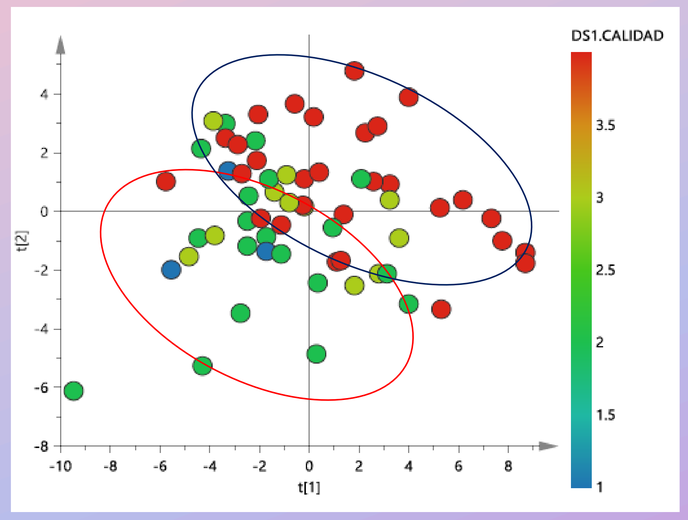


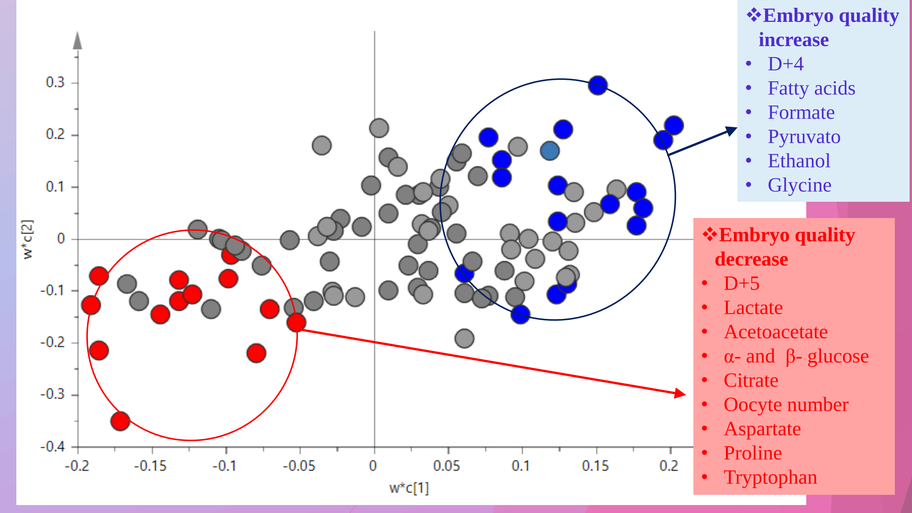
Metabolites	*Chemical shifts (ppm), multiplicity and coupling constant (Hz)*
Ethanol	1.19 (t, J = 7.0 Hz), 3.66 (c)
Lactate	1.33 (d, J = 7.3 Hz), 4.11 (c)
Alanine	1.46 (d, J = 7.8 Hz)
Acetate	1.89 (s)
Acetoacetate	2.24 (s)
Proline	1.98 (m), 2.02 (m), 2.03 (m), 3.35 (m), 3.40 (m), 4.20 (m)
Glutamate	2.08 (m), 2.13 (m), 2.33 (m)
Glutamine	2.14 (m), 2.41 (m)
Pyruvate	2.37 (s)
Citrate	2.54 (d, J = 16.1 Hz), 2.67 (d, J = 16.1 Hz)
Aspartate	2.80 (dd, J = 16.9, 3.8 Hz), 2.64 (m)
Glycine	3.56 (s)
α-Glucose	5.23 (d, J = 3.9 Hz)
β-Glucose	4.65 (d, J = 8.0 Hz)
Tryptophan	7.70 (d, J = 7.7 Hz), 7.51 (d, J = 7.7 Hz), 7.25 (m), 7.18 (m), 7.23 (s), 3.14 (dd), 3.36 (dd)
Formate	8.46 (s)



Statistical analysis

PLS-DA scores (left) and loadings (right) plots obtained from ^{1}H-NMR data and correlated with embryo quality (1- greater, 4- minor). Model scaled by Unit Variance. Quality parameters: . R^2X= 0.52, R^2Y=0.73, Q^2=0.13.





❖**Embryo quality increase**
- D+4
- Fatty acids
- Formate
- Pyruvato
- Ethanol
- Glycine

❖**Embryo quality decrease**
- D+5
- Lactate
- Acetoacetate
- α- and β- glucose
- Citrate
- Oocyte number
- Aspartate
- Proline
- Tryptophan

CONCLUSIONS

- Identification of 16 metabolites.
- Correlation between decreased morphological quality and D+5 transfers, less intake of lactate, acetoacetate, glucose, citrate, aspartate, proline, tryptophan and increase in the number of oocytes.
- Correlation between increased morphological quality and D+4 transfers, less intake of formate, etanol,, glycine, pyruvate and some fatty acids.
- PLS model presents Q^2= 0.126 (low predictive capacity).
- Would be of interest adding a larger number of samples and trying to find correlation with pregnancies.

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ACKNOWLODGMENTS

This research has been funded by the State Research Agency (projects RTC-2016-5239-2, CTQ2017-84334-R and PSI2017-86847-C2-1-R) of the Spanish Ministry of Science, Innovation and Universities and EU FEDER funds.

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