A new contribution to the improvement of human embryo culture media: a comparative study of low-abundance proteins of reproductive fluids and plasma of fertile women

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INTRODUCTION

The improvement of the embryo culture media is gaining relevance as demonstrated by the growing number of publications describing its influence on successful implantation rates, pregnancy, neonatal outcomes and potential effects in the adult life. The ideal conditions for embryo development are those naturally occurring in the female reproductive tract, i.e., the oviductal and uterine fluids. These fluids provide all the nutrients, hormonal and non-hormonal factors, electrolytes, macromolecules as well as precisely regulated volume, pH and osmolality required for the gametes, zygotes, and later, embryo development. Proteins are crucial components of these fluids but detailed studies about their presence and abundance are scarce. This study was designed to shed light on the differences between protein composition of reproductive fluids and plasma in fertile women in order to identify potential candidates to be used as additives in culture media.

OBJECTIVE

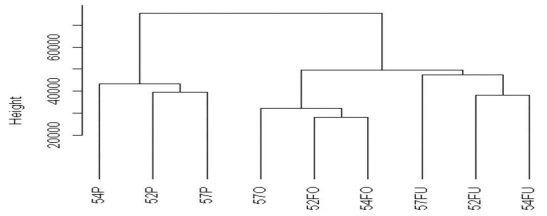
To perform the first comparative study of the low abundance proteins in plasma, uterine and oviductal fluid collected from healthy and fertile women that underwent a salpingectomy.

STUDY POPULATION

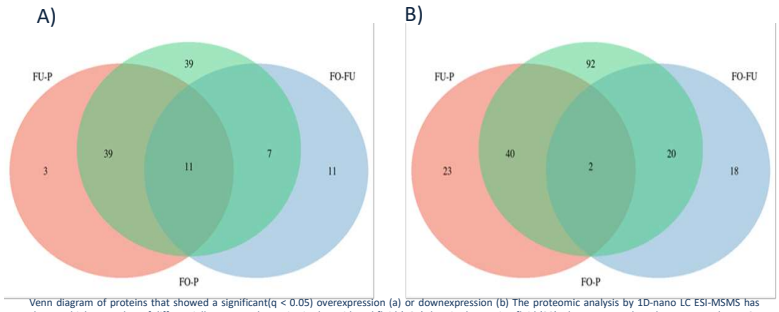
Demographic data of the recruited patients - "Virgen de la Arrixaca" University Clinical Hospital

	BRA-52	BRA-54	BRA-57
Age (years)	33	39	31
Menarch (years)	11	14	10
Parity	G4C4	G2P2	G2C1P1
Menstrual cycle duration	30 days	30 days	29/30 days
Sample collection day (Secretory phase)	Day 18	Day 17	Day 22

HIGH-THROUGHPUT ANALYSIS OF FEMALE REPRODUCTIVE TRACT FLUIDS 1D-NANO LC ESI-MSMS AND MRM VALIDATION

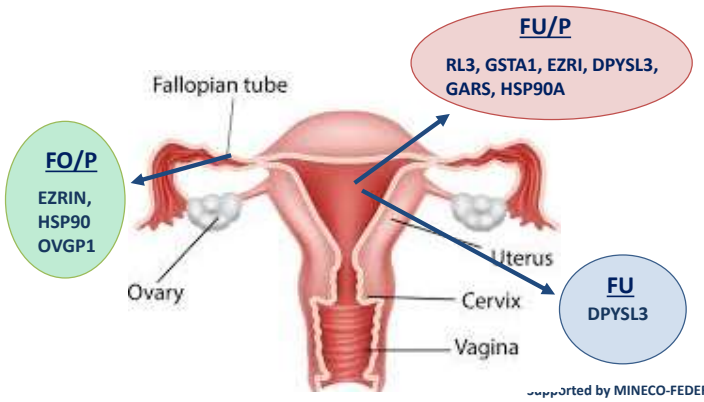


Hierarchical clustering of the samples based on label-free quantification (LFQ) values of protein identified by liquid chromatography-tandem mass spectrometry (LC-MS/MS) of proteolytic peptides from the samples P (plasma), O (oviductal fluid), and U (uterine fluid) of the three patients (52, 54 and 57)



Venn diagram of proteins that showed a significant(q < 0.05) overexpression (a) or downexpression (b) The proteomic analysis by 1D-nano LC ESI-MSMS has shown a higher number of differentially expressed proteins in the oviductal fluid (131) than in the uterine fluid (22) when compared to plasma. From these 131 proteins, 92 were upregulated and 39 downregulated.

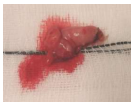
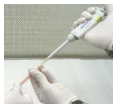
SUMMARY OF QUANTIFICATION OF 1D-NANO LC ESI-MSMS AND MRM VALIDATION



REPRODUCTIVE FLUIDS COLLECTION

Directive 2004/23 / EC of the European Parliament and of the Council of 31 March 2004
Law 14/2007, of July 3, Biomedical Research of Royal Decree 1716/2011, of November 18

FO - OVIDUCTAL FLUID

- Carrasco et al. 2008
- Oviducts separated from the tracts
- Dissection on ice
- Aspiration with an automatic pipette

FU - UTERINE FLUID


- Mucat® (CDD Laboratoires)
- Insertion of the device inside the extracted uterus, through cervix
- Aspiration with the integrated plunger, without a syringe.

	BRA-52	BRA-54	BRA-57
FO - oviductal fluid (µl)	57	70.9	49
FU - uterino fluid (µl)	260	100	59

CONCLUSIONS

- ✓ **Several proteins were detected in high amounts in OF when compared to UF and P samples (RL3, GSTA1, EZRI, DPYSL3, GARS, HSP90A).**
- ✓ **DPYSL3 has been detected in UF for the first time.**
- ✓ **OF is a rich fluid with essential proteins that could be a target to improve fertilization rates and early embryo development, if used in the culture media, namely EZRIN, HSP90 or OVGP1.**
- ✓ **Reproductive fluids represent an important source of biomarkers with potential interest in the development of an improved embryo culture media.**

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