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Original article

Expressed proteins and activated pathways in conditioned embryo culture media from IVF patients are diverse according to infertility factors

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ABSTRACT

Objective: Objective: Given that the embryo culture medium secretome reflects the embryo development, we hypothesize that protein profiles are affected according to infertility factors, which can be responsible for detrimental embryonic developmental competence. The aim of this study was to screen the protein profile of conditioned embryo culture media in patients presenting deep infiltrating endometriosis (ENDO) and polycystic ovarian syndrome (PCOS) undergoing IVF, by proteomics approaches. The control group was constituted by tubal factor patients.

Methods: Patients underwent *in vitro* fertilization (IVF) treatment as routine and oocytes were fertilized by ICSI. The embryos were group cultured until day 3 of development, and after transfer the culture media were collected. For the proteomics analysis, two pools of samples were prepared for groups CONTROL and PCOS, and 4 pools of samples for group DIE. Samples were prepared to deplete high abundant proteins and followed evaluated by high throughput proteomics approach.

Results: The embryonic organ and tissue development were physiological functions activated, based on proteins identified in the 3 study groups of samples. The samples coming from DIE patients presented a high calcium activity and on the other hand, embryos coming from PCOS patients showed a decreased calcium action. Other pathways as grow factors through the EGF signaling pathway overexpressed in ENDO culture medium and protein kinase A in PCOS were also observed.

Conclusions: Proteomic embryonic secretome will advance our knowledge of early embryogenesis and additionally could lead to improved selection of embryos for transfer warrants further investigation.

Keywords: embryo, conditioned culture media, secretome, IVF, endometriosis, ovarian polycystic ovarian syndrome

INTRODUCTION

In vitro fertilization (IVF) has gained attention since its introduction in 1978 and, to date, more than 2 million babies have been born worldwide through assisted reproductive technologies (ART). Developments in technologies for IVF, embryo culture and therapies for ovarian stimulation make ART relatively successful. However, it has its limitations with >50% of IVF embryos failing to implant. Selecting viable embryos for transfer is a key factor for the success of IVF treatment; and requires accurate pre-transfer assessment of embryo viability and morphology. However, the available methods to identify high implantation potential embryos are still limited (Gardner *et al*., 2015).

The field of human ART would therefore benefit from more quantitative methods of determining embryo viability and implantation potential to further improve the pregnancy rates. It is known that soluble ligands and its receptors mediate human pre-implantation embryo development and implantation (Thouas *et al*., 2015). Non-invasive analyses of the embryonic secretome, including proteins secreted by the embryo into the surrounding media, provide an alternative method for assessing an embryo's viability. These results may promote an understanding of the biological mechanisms, and potentially allow for the success of IVF developmental biomarkers.

A study evaluating conditioned media from non-manipulated human embryos cultured in vitro demonstrated that it contains extracellular vesicles and bearing the traditional microvesicle and exosome marker proteins CD63, CD9 and ALIX, which may suggested a way of communication at the maternal-fetal interface (Giacomini *et al*., 2017). A number of researchers has used the proteomics approach related to human reproduction in several subareas as ectopic pregnancy (Gerton *et al*., 2004), miscarriage (Liu *et al*., 2006), follicular fluid (Lo Turco *et al*., 2010; Kim *et al*., 2006; Silberstein *et al*., 2009; Estes *et al*., 2009; Twigt *et al*., 2012; Liu *et al*., 2007), oocytes (Ferreira *et al*., 2010), endometrium (Matorras *et al*., 2018) and embryos (Ferreira *et al*., 2010; Katz-Jaffe & Gardner, 2007; Katz-Jaffe *et al*., 2009; Dominguez *et al*., 2009). Recently, various proteins have been investigated as an embryo biomarker in the spent culture media (Butler *et al*., 2013; Mains *et al*., 2011; Ziebe *et al*., 2013; Cortezzi *et al*., 2013; Dominguez *et al*., 2008) given that the embryo culture medium secretome reflects the embryo development. We hypothesize that protein profiles are affected according with infertility factors, which can be responsible for detrimental embryonic developmental competence, ensuing on lower IVF success rates. Hence, we studied samples from

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patients presenting deep infiltrating endometriosis (ENDO) and polycystic ovarian syndrome (PCOS), which are the most frequent female factors of infertility. Endometriosis is present in until 50% (Missmer & Cramer, 2003) of infertile women and PCOS is present in the main cause of anovulatory infertility (Thessaloniki ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group, 2008). It is clear that the ovarian microenviroment is affected by the presence of endometriosis (Regiani *et al*., 2015; Singh *et al*., 2013; Karita *et al*., 2011) and PCOS (Huang *et al*., 2013; Ambekar *et al*., 2015; Roth *et al*., 2014)

Based on the knowledge that the potential of embryo development is an extension of oocyte quality, and the last is directly influenced by the microenviroment of ovary, possibly being affected by the presence of PCOS or ENDO, we hypothesized the embryo secretoma during its pre-implantation development could also vary reflecting the infertility factor condition. The aim of this study was to screen the protein profile of conditioned embryo culture media in patients with PCOS and ENDO undergoing IVF by proteomics approaches.

MATERIAL AND METHODS

Sampling

It was included in the study conditioned embryo culture media samples obtained from patients submitted to IVF cycle at the Huntington - Reproductive Medicine, Sao Paulo - Brazil. Institutional Ethical approval secured for the use of samples for the purpose of this research and the samples were donated by consenting patients whose signed the Informed Consenting Form as established by ethics for assisted reproduction treatment and research (CFM, 2013). Clinical characteristics and outcomes were obtained from patients' charts.

All women presented with the following inclusion criteria: infertile patients undergoing ICSI cycles with ejaculated sperm, presence of both ovaries, regular menstrual cycle, body mass index (BMI) lower than 35 Kg/m^2, no ongoing infectious disease, no uterus pathology, basal follicular stimulating hormone (FSH) measurement <14IU/L, and basal estradiol measurement <70pg/mL. The exclusion criteria were presence of gynecological bleeding, hydrosalpinx, allergy to gonadotropins or other medications used in the treatment, abusive use of any other medications during treatment, and male partners presenting with severe oligozoospermia.

Among patients included, three groups were analyzed according to infertility etiology: tubal factor patients who were considered the control group for this study (CONTROL; n=6), infertile patients presenting PCOS (PCOS; n=7) and infertile patients presenting endometriosis grades III and IV (ENDO; n=14). The diagnosis of infertility was carried out according to international patterns.

Patients were submitted to pituitary blockage and controlled ovarian stimulation as routine. The pituitary blockage was obtained with a GnRH agonist (Lupron kit™, Abbot SA Societé Française des Laboratories, France), and the ovarian stimulation was performed using recombinant FSH (rFSH, Gonal-F® Serono, Switzerland). When at least two follicles reached a diameter of 16 mm, the final follicular maturation was triggered with 250µg of recombinant hCG (rhCG, Ovidrel®, Serono, Switzerland). Oocyte retrieval was performed after 35 to 36 hours by transvaginal ultrasound-guided aspiration, and the luteal phase was supported by 90mg of daily progesterone (Crinone®, Serono, Switzerland) via vaginal approach.

After oocyte recovery and denudation, all of the mature oocytes were fertilised by ICSI (Palermo *et al*., 1992) as per the routine of the clinic. The normally fertilized oocytes were identified and cultured in groups until day 3 (D3) in 1 mL of cell culture medium (G-1 Plus, Vitrolife) under a layer of paraffin oil (OVOIL, Vitrolife), in incubators with 5% O_2 and 5% CO_2. From D3 until the blastocyst stage (D5), the embryos were cultured in 1 mL of medium containing 10% human albumin (CSCM, Irvine Scientific) under a layer of paraffin oil in triple gas incubators (90% N2, 5% O_2 and 5% CO_2). The blastocysts were morphologically classified and the highest grades ones were selected for transfer to the women's uterus using a catheter guided by ultrasound. The culture media was collected on day 3 of development and stored at -80ºC until use. For the proteomics analysis, two pools of samples were prepared for groups CONTROL and PCOS, and four pools for group ENDO.

MS analysis

All analyzes in this step of study were carried out at Proteomics and Mass Spectrometry Facility, Center for Drug Discovery and Innovation (CDDI), University of South Florida (USF), Tampa - FL, USA. Arbitrary samples were used for methods standardization, and clinical samples were pooled into 3 to 4 samples pools according to pre-established groups (Control, PCOS and ENDO).

Samples were prepared by using two steps of purification in order to eliminate the excess of albumin. First, samples were submitted to microfiltration using a 30KDa pore filter (Amicon® Ultra-0.5, Centrifugal Filter Devices - 30 kDa, Millipore, USA) and the filtrated material was collected. The material retained in the filter (higher than 30 KDa) was albumin depleted using affinity chromatography columns (Albumin & IgG Depletion SpinTrap, GE Healthcare Life Sciences, USA) according to manufacturer's protocol. The two aliquots of each sample were joined and protein concentrations were measured by Bradford method (Pierce 660nm Protein Assay Reagent, Thermo Scientific), using pre-diluted albumin standard curve (Pre-Diluted Protein Assay Standards: Bovine Serum Albumin, Thermo Scientific) and the absorbance's were acquired at 660 nm. Two hundred and fifty micrograms (250µg) of protein for each sample were diluted in 8M urea buffer and digested using FASP kits (Expedeon, Inc, USA) as per vendor specifications. Tryptic peptides were acidified by adding trifluoroacetic acid (TFA 1%) to get the 0.1% of TFA concentration, and then desalted on solid phase columns (DSC-18, Solid Phase Extraction - SPE, Discovery® DSC-18 SPE Products, Sigma Aldrich). Samples were vacuum dried (Wiśniewski *et al*., 2009).

Tryptic peptides resulting from the preparation were fractionated by liquid chromatography mass spectrometry (HPLC-MS/MS). Five microliters injection of each sample loaded with aqueous solvent (0.1% formic acid in water) were separated by nano-flow reversed phase HPLC using a Nano-LC Ultra 2D+ (Eksigent, Dublin, CA) equipped with a Proteopep 2 Integra Fit trapping column (100 µm i.d. x 2.5 cm; C18, 5 µm, 300Å) and a Proteopep 2 Integra Fit analytical column (75 µm i.d. x 10 cm; C18, 5 µm, 300Å, New Objective, Woburn, MA). Samples (0.5 to 3 µg in 5 µL) were loaded onto the trap column at 2 µL/min (Solvent A) for 12 minutes, after which a valve was switched to include the analytical column. Peptides were then eluted with a gradient (300 nL/min) of 2% B to 35% B over 240 minutes (Solvent A: 100% H_2O, 0.1% formic acid, Solvent B: 98% acetonitrile, 0.5% formic acid). Eluates were delivered by electrospray ionization (ESI) at 2 kV and analyzed by data-dependent MS/MS on a LTQ XL (Thermo Scientific, San Jose, CA, USA) mass spectrometer equipped with XCalibur (version 2.0.7) data acquisition software. Full MS scans were set for centroid mode at normal resolution. MS/MS scans were performed on the top ten most intense ions from each full scan to acquire spectral data for peptide identification. Dynamic exclusion durations were set to 180s with one repeat and a list size of 500.

Raw data acquired were searched against the European Bioinformatics Institute's (EBI) universal protein resource database (UniProt, November 2014) using Mascot (version 2.2). Peptide and protein validations were performed using the Scaffold plataform (version 3.00.08). Search parameters used were as follows: precursor mass error tolerance of 20 ppm; fragment mass error tolerance of 0.1 Da; trypsin as a protease with one missed cleavage allowed; and carbamidomethylation of cysteine as fixed modification and oxidation of methionine as a variable modification. Identified peptides and proteins were validated and visualized with Scaffold 3.6 (Proteome Software, Portland, OR).

The proteins identified in the previous analysis were analysed using Ingenuity™ Pathway Analysis software (IPA™, QIAGEN, Redwood, USA). We considered peptide threshold of 20% and the biological processes were staggered according to the IPA™ Knowledge Base. The association between the identified proteins and canonical pathways of the database was also accessed with IPA™ software using Fisher's exact test (significance of $p<0.01$). From 2880 proteins identified, decoy proteins that are considered false identification were excluded. Two sets of analysis were carried out with remaining proteins using the IPA™. First of all we identified proteins exclusive in each study group (PCOS or ENDO) compared with CONTROL. We considered exclusively expressed those protein expressed in at least two sample pools of each group and absent on the other comparison groups. Then, we evaluated proteins differentially expressed in the study groups (PCOS or ENDO) compared with CONTROL and considered fold change higher than two.

RESULTS

Table 1 describes the demographic data regards the patients included in this study. In the ENDO group, 17 proteins were exclusively expressed (present in at least two of four samples pools), and two were over expressed compared to CONTROL (Table 2). The canonical pathways identified which were related to proteins exclusively expressed in ENDO group were associated with calcium metabolism [calcium signaling ($p=0.0057$) and transport ($p=0.0058$) and calcium induced T -lymphocyte apoptosis ($p=0.041$)] and EGF signaling ($p=0.035$) (Figure 1).

On the other hand, in the PCOS group presented 284 proteins exclusively expressed and one overexpressed with fold change higher than 2 compared to CONTROL, which were associated with the following pathways: Protein Kinase A signaling and calcium signaling were downregulated, and GADD45 signaling, hydrocarbon receptor signaling and GDP-L fucose biosynthesis II were upregulated (Figure 2). The PCOS group had two pools of samples analysed and from 284 proteins exclusively expressed in the PCOS group, six were present in both pools of samples (Table 3).

Based on proteins identified, the CONTROL group had the following cellular and molecular function highlighted: cellular development, cellular movement, amino acid metabolism, small molecule biochemistry, cellular assembly and organization, which were function associated to general cellular development. Also, the embryonic organ and tissue development were physiological functions activated based on proteins identified in the 3 study groups of samples.

DISCUSSION

In the last decade, the application of proteomics high throughput methodologies to human reproductive fluids and cells have delineated novel biochemical functional profiles and molecular processes that characterize and may affect folliculogenesis, oocyte maturation and quality, and as consequence the embryo development potential and clinical outcomes. Embryos are programmed to produce soluble ligands and receptors, which elicit changes in embryo developmental phenotype and also modulating local responses in the receptive endometrium determining an embryo maternal cross talk during peri-implantational period. These signaling pathways are highly complex and it understanding has been gradually improved (Thouas *et al*., 2015). The embryos from *in vitro* fertilization also produce soluble factors that are secreted in the spent culture media. Proteomics in spent culture media involved the measurement of amino acids (Brison et al., 2004; Sturmey *et al*., 2008), proteins (Katz-Jaffe *et al.*, 2006a;b; 2009; Nyalwidhe *et al*., 2013) and metabolomics evaluates how the embryo alters its microenvironment (Scott *et al*., 2008; Leese *et al*., 2008). Authors have been trying to find if changes in the levels of some molecules are associated with implantation potential of embryos and clinical outcomes of IVF cycles.

Different approaches were necessary based on the nature of the specimens and the types of analyses being done. The standardization of proteomics approaches embryo culture medium was developed for this study, there we could obtain data available to a general protein profile in the samples. Depletion of abundant proteins combined with multidimensional protein fractionation was instrumental in allowing the study of middle- and lower-abundance proteins. Our study demonstrates that the technology can provide a consistent result given the restriction of starting material and time to analysis. These proteins with their regulatory pathways may play a vital role in reproductive process.

In embryos derived from ENDO group, we observed over expression of EGF signaling pathway compared to CONTROL. EGF is a growth factor family which has been already identified to be expressed by the human preimplantation embryo (Chia *et al*., 1995) and *in vitro* studies also showed the EGF treatment increases early human embryo development and blastocyst formation (Yu *et al*., 2012). Animal studies have shown that EGF enhanced developmental competence of cat embryos by stimulating cell proliferation and modulating the EGFR expression at

Table 1. Demographic characteristics of women included in this study

	CONTROL (mean±SD)	ENDO (mean±SD)	PCOS (mean±SD)	*p**
N	6	14	6	
Age (years)	33.8±3.3	34.4± 2.1	33.7±1.9	0.798
Time of infertility (years)	1.7±0.4	2.6±1.9	2.0±1.0	0.483
Basal FSH (IU/ml)	7.3±2.3	7.2±2.0	5.6±1.3	0.199
Number of MII collected	11.8±7.3	10.1±6.0	18.7±8.2	0.057
Number of embryos on D3	4.7±3.0	7.5±4.9	10.0±3.0	0.108

*ANOVA

Table 2. Proteins exclusively expressed and over expressed in the ENDO compared to CONTROL group

Group ENDO compared to CONTROL	Protein name	ID	Molecular weight (KDa)	Protein identification probability	Mascot identity score
Exclusive	GIGYF2 protein OS=Homo sapiens GN=GIGYF2 PE=2 SV=1	A6H8W4_HUMAN (+5)	152	98%	45.2
Exclusive	Uncharacterized protein OS=Homo sapiens GN=DST PE=4 SV=1	E7ERU0_HUMAN (+6)	616	74%	46.0
Exclusive	Isoform 2 of U4/U6 small nuclear ribonucleoprotein Prp4 OS=Homo sapiens GN=PRPF4	sp|O43172-2|PRP4_HUMAN (+1)	58	76%	45.0
Exclusive	C1q-related factor OS=Homo sapiens GN=C1QL1 PE=2 SV=1	C1QRF_HUMAN	26	74%	40.7
Exclusive	Uncharacterized protein OS=Homo sapiens GN=MFF PE=4 SV=1	C9JHF5_HUMAN	16	55%	45.2
Exclusive	Isoform 2 of Neuron navigator 1 OS=Homo sapiens GN=NAV1	sp|Q8NEY1-2|NAV1_HUMAN (+4)	202	57%	41.1
Exclusive	Uncharacterized protein OS=Homo sapiens GN=ATP2C1 PE=4 SV=1	D6REJ1_HUMAN (+6)	12	54%	43.8
Exclusive	Uncharacterized protein OS=Homo sapiens GN=MEN1 PE=4 SV=1	E7EN32_HUMAN (+3)	61	82%	44.8
Exclusive	Olfactory receptor 6B2 OS=Homo sapiens GN=OR6B2 PE=2 SV=2	OR6B2_HUMAN	35	65%	46.3
Exclusive	Angiopoietin-related protein 6 OS=Homo sapiens GN=ANGPTL6 PE=1 SV=1	ANGL6_HUMAN	52	57%	45.3
Exclusive	Arginine-glutamic acid dipeptide repeats protein OS=Homo sapiens GN=RERE PE=1 SV=2	sp|Q9P2R6|RERE_HUMAN	172	54%	43.7
Exclusive	Uncharacterized protein OS=Homo sapiens GN=ADAR PE=4 SV=1	E7ENU4_HUMAN (+5)	141	97%	45.2
Exclusive	Inositol 1,4,5-trisphosphate receptor type 2 OS=Homo sapiens GN=ITPR2 PE=1 SV=2	sp|Q14571|ITPR2_HUMAN	308	64%	44.9
Exclusive	Isoform 2 of Transcriptional-regulating factor 1 OS=Homo sapiens GN=TRERF1	sp|Q96PN7-2|TREF1_HUMAN (+3)	106	97%	44.7
Exclusive	Isoform 2 of Ubiquitin-conjugating enzyme E2 W OS=Homo sapiens GN=UBE2W	UBE2W_HUMAN	19	93%	45.0
Exclusive	Uncharacterized protein OS=Homo sapiens GN=LAMB2 PE=4 SV=1	E7EMH6_HUMAN	40	79%	44.0
Exclusive	Uncharacterized protein OS=Homo sapiens GN=MDM1 PE=4 SV=1	E7EPQ3_HUMAN (+2)	77	84%	44.7
Over expressed (FC=2.90;*p*=0.601)	Uncharacterized protein OS=Homo sapiens GN=BRCA1 PE=4 SV=1	E7EMP0_HUMAN (+9)	181	68%	45.4
Over expressed (FC=2.31;*p*=0.483)	Histone H1.4 OS=Homo sapiens GN=HIST1H1E PE=1 SV=2	H14_HUMAN	22	88%	45.8

FC=Fold Change. KDa=kilodaltons

various developmental stages (Thongkittidilok *et al*., 2015). On the other hand, an *in vitro* study cultured mice embryos with human tubal fluid from endometriosis patients and observed that the levels of embryonic EGF, IGF-I, and their receptors were increased, and it attenuated embryo development by impairing embryonic growth factor/receptor/signal transduction (Ding *et al*., 2010). Endometriosis has been associated with impaired IVF outcomes (Harb *et al*., 2013; Somigliana & Garcia-Velasco, 2015). Based on that, we can speculate the higher expression of EGF in endometriosis group may have an ovarian origin and it can be associated with the mechanism of embryo development of those patients.

Other pathways highlighted were associated with calcium signaling. Studies have shown that mitochondria has an enormous capacity to regulate Ca^{2+} (Giacomello *et al*., 2007). The embryo development is strongly correlated with the activity of mitochondria, and the abnormal

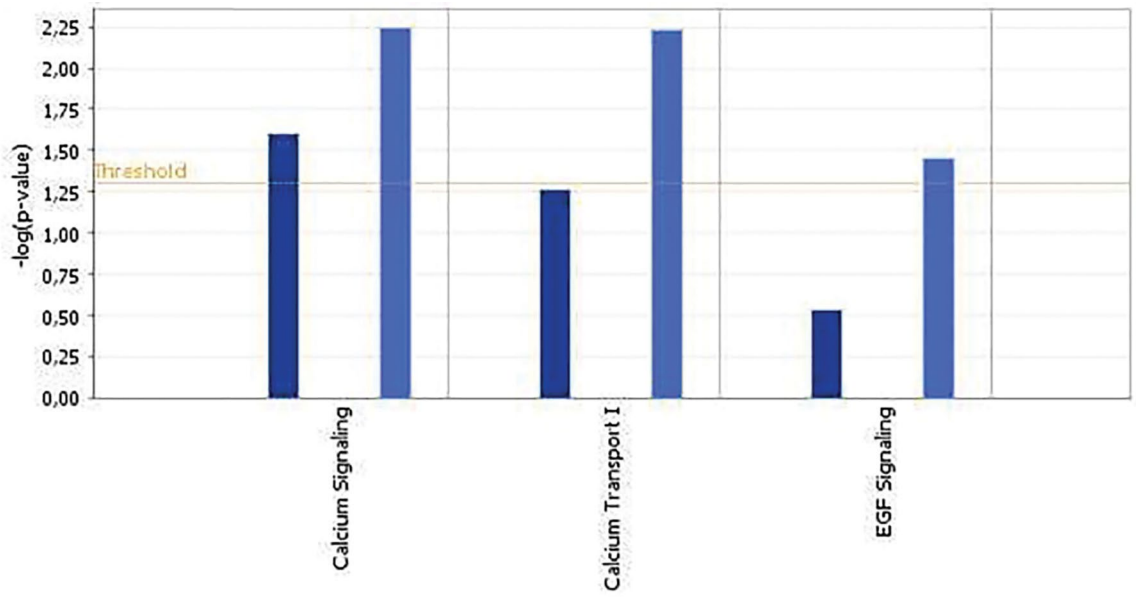

Figure 1. Comparative analysis of canonical pathways based on proteins over-expressed in ENDO group compared to CONTROL. ■ CONTROL ■ ENDO.

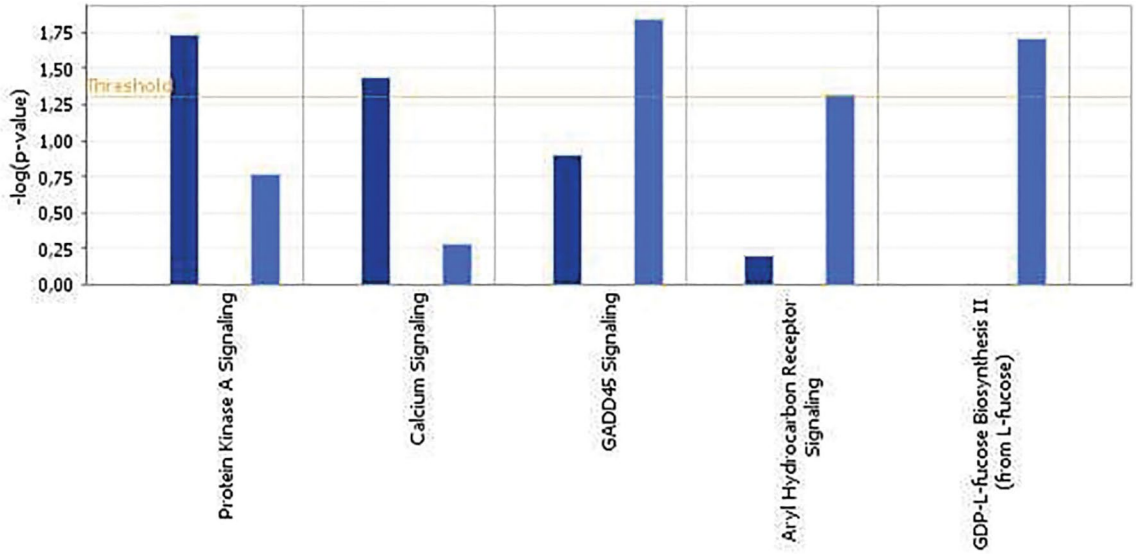

Figure 2. Comparative analysis of canonical pathways based on proteins over-expressed in PCOS group compared to CONTROL. ■ CONTROL ■ PCOS.

distribution of mitochondria exert negative effects on the embryogenesis due to the abnormal ATP distribution (Nagai *et al*., 2006), because high energy supply around nucleus is very important during embryonic development (Wang *et al*., 2009), and may perform an important function in embryonic cell-cycle transition and embryonic axis establishment (Whitaker, 2008). A study as shown that conditioned medium from human embryos also trigger calcium oscillations in human endometrial epithelial cells, and importantly, the endometrial responses are affected by developmentally competency of embryos as low-quality human embryos trigger prolonged and disorganized calcium oscillations, leading to a uterine stress response (Brosens *et al*., 2014). In our study, while samples coming from endometriosis patients presented calcium-signaling upregulated, the PCOS samples showed it downregulated. In spite of most of publication find similar clinical outcomes after IVF in PCOS patients, it is related to alterations in oocyte quality and consequently in embryo quality, may be due to endocrine and intra-ovarian environment (Sermondade *et al*., 2013). The calcium metabolism downregulated in the PCOS embryos might reflect the poorer oocyte quality observed in those patients.

Protein kinase A signaling is also down regulated in PCOS samples. A study in non-human primate showed that mechanisms underlying adrenal hyperandrogenism

Table 3. Proteins exclusively expressed and over expressed in the PCOS compared to CONTROL group

Group PCOS compared to CONTROL	Protein name	Accession Number	Molecular Weight (KDa)	Protein identification probability	Mascot identity score
Exclusive	SHANK2 protein OS=Homo sapiens GN=SHANK2 PE=2 SV=1	B7ZKU9_HUMAN (+3)	29	64%	41.4
Exclusive	Uncharacterized protein OS-=Homo sapiens GN=HIVEP1 PE=4 SV=1	F5H212_HUMAN (+3)	62	73%	44.0
Exclusive	Putative uncharacterized protein DKFZp434N071 OS=Homo sapiens GN=DKFZ-p434N071 PE=2 SV=2	Q68CU6_HUMAN (+1)	58	57%	40.0
Exclusive	Isoform 2 of Protein strawberry notch homolog 1 OS=Homo sapiens GN=SBNO1	sp|A3KN83-2|SB-NO1_HUMAN (+2)	154	72%	41.6
Exclusive	Collagen alpha-1(III) chain OS=Homo sapiens GN=CO-L3A1 PE=1 SV=4	sp|P02461|-CO3A1_HUMAN	139	73%	41.5
Exclusive	Isoform 2 of Phosphofurin acidic cluster sorting protein 1 OS=Homo sapiens GN=PACS1	sp|Q6VY07-2|PACS1_HUMAN (+1)	109	69%	43.8
Over expressed (FC=2.91;*p*=0.598)	Isoform 3 of Cytosolic carboxypeptidase 1 OS=Homo sapiens GN=AGTPBP1	CBPC1_HUMAN	139	55%	41.6

FC=Fold Change. KDa=kilodaltons

seen in the human condition of PCOS are further considered in terms of the effects of altered relative expression of CYP17, HSD3B2 and CYB5, as well as the altered signaling responses of various kinases including protein kinase A (Abbott & Bird, 2009). GADD45, another protein over expressed in PCOS samples, is a protein that is often induced by DNA damage and other stress signals associated with growth arrest and apoptosis (Salvador *et al*., 2013).

The data on secretory activity can vary considerably suggest that embryo can intrinsically modulate the microenvironment and may reflect developmental plasticity rather than quality. *In vitro* studies provided evidences for a putative mechanism by which the decidualized stromal cells sense developmental embryos through the molecules secreted in the culture media (Brosens *et al*., 2014). Also, signals emanating from competent human embryos triggered a very specific transcriptional response in the mouse uterus, characterized by the induction of multiple metabolic genes (Teklenburg et al, 2010). Hence, focusing on exposures that infertility factors per se influence differential proteome profiles in the embryo culture media, can help understand its effects in the embryo developmental plasticity and competence, and consequently in IVF outcomes.

This study relies on the proteomic profile of conditioned embryo culture media in different infertility conditions. We did not correlated the proteins identified with the embryo characteristics as samples come from embryos cultured in groups and they were group cultured and we joined them into pools according to infertility factors to be analyzed. Also, the sample size is reduced and outcomes should be confirmed using immunodetection technique and in a higher number of samples. Due to high concentration of contaminants in the culture media, as albumin, samples were submitted to a number of process which might depleted other less abundant proteins. To date, the proteomic approach has proven to be a challenging task due to the complexity and diversity of the human embryo and heterogeneity across patients and within embryo cohorts.

In summary, the embryonic, organ and tissue development were physiological functions activated based on proteins identified in the three study groups of samples. The embryos coming from endometriosis patients present a high calcium activity and on the other hand, embryos coming from PCOS patients showed a decreased calcium action, which may be related to embryo developmental competence or plasticity. Other pathways as grow factors through the EGF signaling pathway overexpressed in endometriosis embryos and protein kinase A in PCOS were also observed. Characterizing the proteomic embryonic secretome will advance our knowledge of early embryogenesis and the embryo's role during the initial stages of implantation. Additionally, the activity in embryo culture medium could lead to improved selection of embryos for transfer warrants further investigation.

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CONFLICT OF INTERESTS

The authors have no conflict of interest to declare.

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