Veterinary Immunology and Immunopathology 187 (2017) 42–47

Contents lists available at ScienceDirect

Veterinary Immunology and Immunopathology

journal homepage: www.elsevier.com/locate/vetimm

Short communication

Shotgun proteomic analysis of the secretome of bovine endometrial mesenchymal progenitor/stem cells challenged or not with bacterial lipopolysaccharide

Carolina Nogueira de Moraes[a,*], Leandro Maia[a], Eliandre de Oliveira[b], Camila de Paula Freitas Dell'Aqua[a], Aspinas Chapwanya[c], Fernanda da Cruz Landim-Alvarenga[a], Eunice Oba[a]

[a] Department of Animal Reproduction and Radiology, UNESP – São Paulo State University, Botucatu, São Paulo, Brazil
[b] Proteomics Platform, Parc Cientific de Barcelona, Barcelona, Spain
[c] Ross University School of Veterinary Medicine, Department of Clinical Sciences, Basseterre, Saint Kitts and Nevis

ARTICLE INFO

Keywords:
Cow
nanoLC–MS/MS
Protein
Uterus
Endometrial mesenchymal cells

ABSTRACT

The use of the conditioned medium (CM) for diseases treatment is based on its enrichment with biomolecules with therapeutic properties and themselves have a beneficial effect. Secretome of bovine endometrial mesenchymal progenitor/stem cells (eMSCs) using a proteomics approach is until now unknown. This work aimed to evaluate the secretome of bovine eMSCs-CM challenged or not with lipopolysaccharide (LPS). For this, eMSCs characterized were challenged (TG) or not (CG). The CM was collected 12 h after stimulation and submitted to mass spectrometry analysis. The classification of identified proteins was done by PANTHER according to biological processes, molecular function, cellular component and protein class. 397 protein groups were identified in TG and 302 in CG. We observed positive enrichment for antibacterial response proteins, macrophage activation function, receptor-mediated endocytosis, hydrolase activity, inhibitory enzyme in TG, and for activity structural molecule and intermediate filament cytoskeleton in the CG. Our experimental model shows that eMSCs respond to LPS in the concentration used and can be used to study immune-inflammatory response, besides of the secretion of proteins mainly related to tissue remodeling, immune response and angiogenesis which is an interesting feature for use in cell therapy.

1. Introduction

In cattle, the endometrium is the main source of mesenchymal progenitor/stem cells (eMSCs), a small proportion of which are undifferentiated with high plasticity (Lupicka et al., 2015). Bovine eMSCs have been studied because of their biological properties, including the paracrine and immunomodulatory effects, which make them promising for use in cell therapy.

Besides the use of MSCs in therapies, the conditioned medium (CM) has a role in the cellular microenvironment, and can exert a therapeutic effect by accelerating organ regeneration processes (Lavoie and Rosu-Myles, 2013) and tissue repair (Ashiba et al., 2015). MSCs secrete bioactive molecules such as cytokines and growth factors (Ashiba et al., 2015) which are released as soluble molecules or through extracellular vesicles that together are responsible for paracrine (Lavoie and Rosu-Myles, 2013) and autocrine roles related to the regeneration, angiogenesis or modulation of immune responses (Skalnikova, 2013).

MSCs are sensitive to culture media and protein profiles may change in response to microenvironments to which they are subjected. The use of different immunological conditions evaluates the therapeutic potential of MSC-derived molecules and make the study of secreted soluble factors important for the understanding its therapeutic effects (Lavoie and Rosu-Myles, 2013). The use of an experimental *in vitro* model of inflammation using bacterial lipopolysaccharide (LPS) (Lange-Consiglio et al., 2015) makes it possible to evaluate the response of bovine eMSCs facing a stressful insult such as uterine disease.

Interaction of bovine eMSCs-CM is important for understanding how therapeutic approaches can be targeted at the mechanisms by which CM modulates the endometrium or use of eMSCs to treat reproductive pathologies.

The aim of this study was to evaluate the secretome of bovine eMSCs challenged with bacterial LPS by proteomic analysis

* Corresponding author.
E-mail address: carolnmoraes@hotmail.com (C.N. de Moraes).

http://dx.doi.org/10.1016/j.vetimm.2017.03.007
Received 22 June 2016; Received in revised form 6 March 2017; Accepted 23 March 2017

(nanoLC–MS/MS) using a shotgun strategy. To the best of our knowledge, this is the first report describing the secretome of bovine eMSCs-CM using proteomic analysis.

2. Material and methods

The study was approved and performed according to the ethical guidelines of the Institution's Animal Care and Experimentation Ethics Committee (Protocol Number 152/2014).

2.1. Isolation and culture of bovine eMSCs

Endometrial cells from bovine endometrial tissue (n = 3) in Phase II of estral cycle (Ireland et al., 1980; Chapawanya et al., 2013) were isolated as previously described (Fortier et al., 1988) with modifications. Briefly, samples were digested with 0.3% trypsin (Sigma®, USA) in HBSS (Thermo Fisher Scientific®, USA) for 3 h, at 22 °C under agitation. The samples were then filtered with 40 uM filter (Becton Dickinson® and Company, USA). For each sample, a second digestion step was performed with 0.05% mg trypsin, 0.05% collagenase, 0.1% bovine serum albumin and 0.01 mg DNase I (Sigma®, USA) in HBSS (Thermo Fisher Scientific®, USA) for 1.5 h at 37 °C. After filtration, the digesta was washed with HBSS medium and 10% fetal bovine serum (FBS) (Thermo Fisher Scientific®, USA) twice and centrifuged at 100 x g for 10 min. The sediment was plated and cultured at 37.5 °C in humid atmosphere containing 95% air and 5% CO_2. The culture medium consisted of DMEM high glucose/F12 (1:2), 20% FBS, 100IU/mL penicillin, 100 µg/mL streptomycin, 3 µg/mL amphotericin B (Thermo Fisher Scientific®, USA) and 11 µg/mL amikacin (Teuto®, BRA). The medium was changed within 18 h and every 2–3 days thereafter until the culture reached 90% confluence, and passaged three times.

2.2. Immunophenotypic characterization

The immunophenotypic characterization (n = 2) was performed by flow cytometry at a LSR Fortessa equipment (BD®, BR) using the antibodies anti–CD29 conjugated with Alexa fluor 647 (TS2/16, BioLegend®, USA), mouse anti-bovine CD-44 conjugated with fluorescein isothiocyanate (FITC) (IL-A118, AbD Serotec®, UK), mouse anti-horse MHC–II conjugated with FITC (CVS20, AbD Serotec®, UK), rabbit anti CD-34 conjugated with FITC (polyclonal, Biorbyt®, USA) and mouse anti-vimentin (v9, AbD Serotec®, UK). A secondary goat-anti mouse conjugated with FITC (abcam®, USA) was used for mouse anti-vimentin. All these antibodies cross-react with bovine antigens (Moraes et al., 2016). Fluorescence reactions were analyzed using the BD FACSDiva™ software and were accounted for 10,000 events. The debris population were excluded by gating FSC x SSC at FSC 5000 threshold. Markers with expression levels of ≥ 2% were considered positive. Data from immunophenotypic characterization is presented as mean and standard error of the mean.

2.3. Immunocytochemistry characterization

Immunocytochemistry (n = 3) was performed as previously described (Maia et al., 2013) and the reactions evaluated under an inverted light microscope (Leica® Microsystems, GER) using the software Leica Application Suite (LAS), version 4.3.0. The antibodies evaluated were vimentin (1: 200, V9, AbD Serotec®, UK), pan-cytokeratin (1: 100, C11, abcam®, USA) and CD-44 (1: 100, BAG40A, VMRD®, USA).

2.4. Assays for differentiation

After attaining 95% confluence, assays for differentiation of adipogenic and osteogenic lineages (n = 3) were performed by adding media (StemPro, Thermo Fisher Scientific®, USA) to the subcultures in triplicate, and also supplementing 5% rabbit serum (Maia et al., 2013) or 20% FBS.

Osteogenic differentiation was confirmed on the 14th day when calcium matrix deposits were noted on Alizarin red stains (Sigma®, USA). Confirmation of adipogenic differentiation on the 8th day was by presence of intracytoplasmic fat droplets after staining with 0.5% Oil red (Sigma®, USA).

2.5. Challenge of bovine eMSCs with LPS

For evaluating the protein profile of the secretome, bovine eMSCs were plated on 24 wells (2 cm^2) at a density of 1000 cells/cm^2 and cultured with complete maintenance medium (DMEM high glucose/F12 (1:2), 20% FBS, 100IU/mL penicillin, 100 µg/mL streptomycin, 3 µg/mL amphotericin B; Thermo Fisher Scientific®, (USA), 11 µg/mL amikacin Teuto®, (BRA)). After 60–70% confluence, the cells were cultured in maintenance medium without FBS for 24 h. The control (CG; n = 3) and LPS-stimulated (LPS treated, TG; n = 3) were cultured.

After 12 h, the conditioned medium was collected, filtered through 22 µm filter and centrifuged at 2000*g* for 5 min to remove cellular debris, and the supernatant stored at −86 °C for secretome analysis.

2.6. Secretome analysis by mass spectrometry and liquid chromatography (nanoLC–MS/MS)

Three biological replicates in both groups (treated vs. control) were analyzed. The samples were digested by initially denaturing in 8 M urea solution (Sigma 51459), followed by reduction with 50 mM dithiothreitol (32 °C/60 min, Sigma 9779). An alkylation step was then performed with 150 mM iodocetamida (25 °C/30 min in the dark) followed by digestion with trypsin sequence grade (35 °C, 16 h, Promega V511A). After digestion, the samples were clean-up with C18 reverse phase and strong cationic-exchange columns (C18, SCX, PolyLC). The samples were then analyzed in a nanoAcquity liquid chromatographer (Waters) coupled to a LTQ-Orbitrap Velos (Thermo Scientific) mass spectrometer.

2.7. Data analysis

Thermo Proteome Discover (v.1.4.1.14) was used to search with SequestHT search engine against Mammalia-SwissProt + Bos taurus-TREMBL protein database (v. april 2016). The search parameters used were: Enzyme: Trypsin; Missed Cleavage: 2; Precursor and Fragment Mass Tolerances: 10 ppm and 0.6 Da, respectively; Variable and Static: Oxidation methionine and Carbamidomethyl cysteine, respectively.

Gene ontology protein classification analysis according biological process (BP), molecular function (MF), cellular component (CC) and protein class (PC) was performed using PANTHER (Protein ANalysis THrough Evolutionary Relationships) Classification System (http://pantherdb.org/).

3. Results and discussion

Bovine eMSCs isolated from uteri in Phase II of the estral cycle were cultured and characterized by immunophenotyping and immunocytochemistry. The cells were also assessed for differentiation potential in addition to being challenged with LPS for secretome analysis using a proteomic approach.

The eMSCs adhered to plastic surfaces within six hours of culture, and showed fibroblastoid morphology after passaging (Fig. 1). Immunophenotypic evaluation by FC revealed high expression for the markers vimentin (94.35% ± 2.19), CD-29 (99.85% ± 0.07) and CD-44 (96.9% ± 2.40). Similar to previous studies (Xiong et al., 2014), there was low expression of the CD-34 (4.25% ± 1.06) marker, and no expression of the MHC-II marker (1.05% ± 0.78) (Fig. 2). After evaluating for osteogenic and adipogenic potential, the eMSCs cells

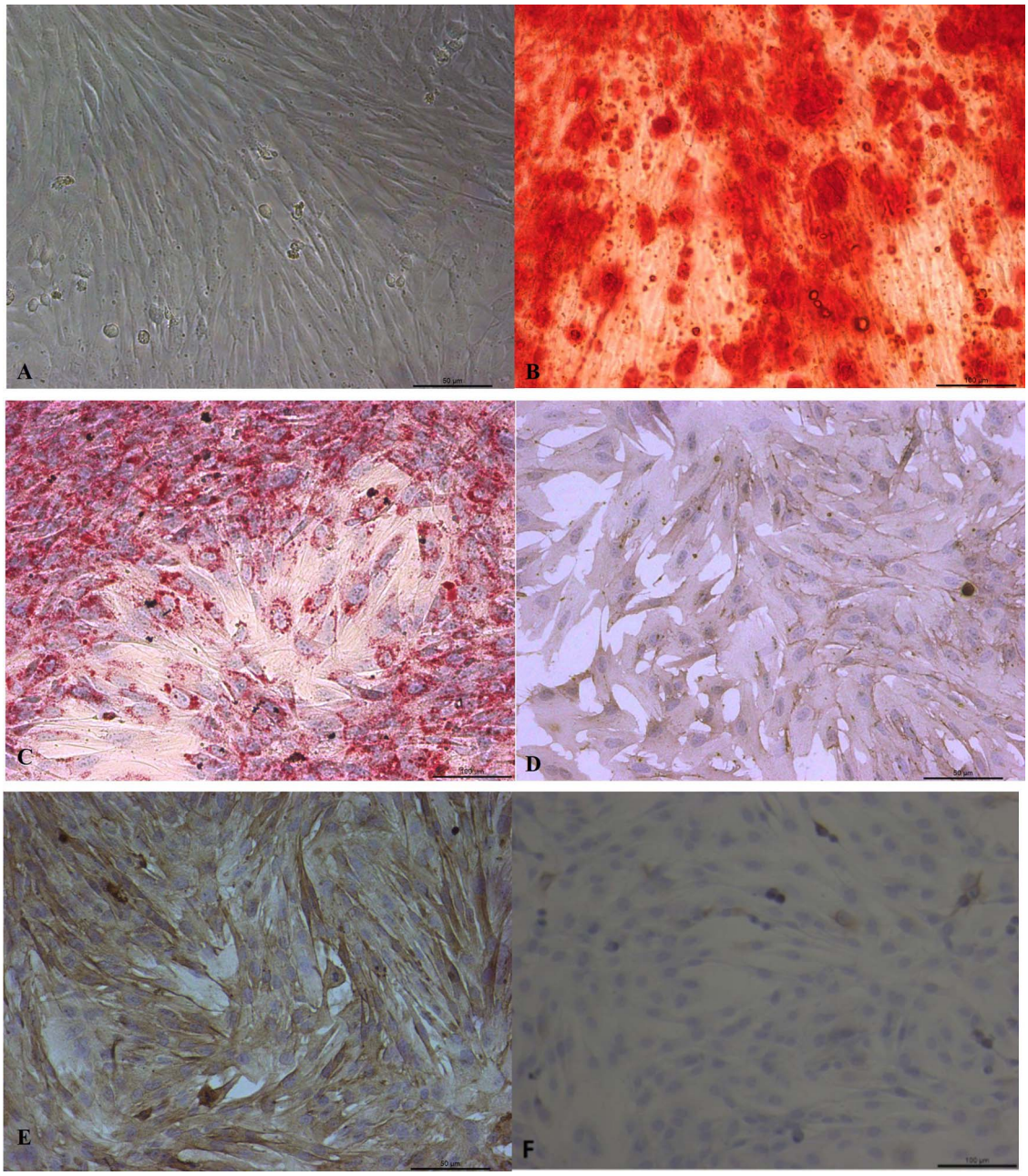

Fig. 1. Characterization of bovine eMSCs. A—Bovine eMSCs after first passage adhere to plastic surfaces and show fibrobastoid morphology. B—eMSCs differentiate into osteogenic lineage after the third passage. Calcium deposits stained with Alizarim are shown. C—eMSCs differentiate into adipogenic lineage of bovine eMSCs in third passage. Intracytoplasmic lipids droplets stained with Oil red are shown. Immunohistochemical staining of bovine eMSCs for CD-44 (D), Vimentin (E) and Pancytokeratin (F). Brown staining indicates positive. Hematoxylin stained cell nuclei. Size: 100 μm (A, D, F), 200 μm (B, C, E). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

stained positive, similar to previous finding (Lupicka et al., 2015). Similarly, other authors reported positive staining for the markers CD-44 in bovine endometrial putative mesenchymal cells on immunocytochemistry (Cabezas et al., 2014), and for vimentin in human eMSCs (Kato, 2012) (Fig. 1). Here, some endothelial epithelial cells were positive for pan cytokeratin, which showed that there were no cell morphology alterations after culture.

A proteomic analysis was performed in order to find more information on the protein content of the secretome of bovine eMSCs. A shotgun approach was used and the LC–MS/MS detected a total of 397 proteins groups on TG and 302 on CG with 242 commons between the groups (Fig. 3). Many proteins belonging to the immune system, angiogenic processes, antioxidant and tissue remodeling pathways were detected in the TG (Table 1).

Our *in vitro* experimental model showed that the cells respond positively and in a protective manner after stimulation with LPS. It can be inferred, for instance, from the presence of arginase I and heat shock proteins, that these proteins protect cells or tissues from stress (Frier and Locke, 2007) by stabilizing and repairing proteins (Fan, 2012). This response is characteristic of a defense mechanism via the secretion of crucial proteins for tissue restoration, or protection from tissue injury. This suggests that CM alone or together with MSCs can be an efficient alternative to assist in healing (Ashiba et al., 2015).

MSCs are sensitive to culture media and may change their proteomic profiles in response to the microenvironments in which they are subjected (Lavoie & -Rosu Myles, 2013). It is noteworthy that in the CM of CG, there were proteins with antimicrobial or antifungal activity, and also tissue remodeling (Table 1). The presence of these proteins in

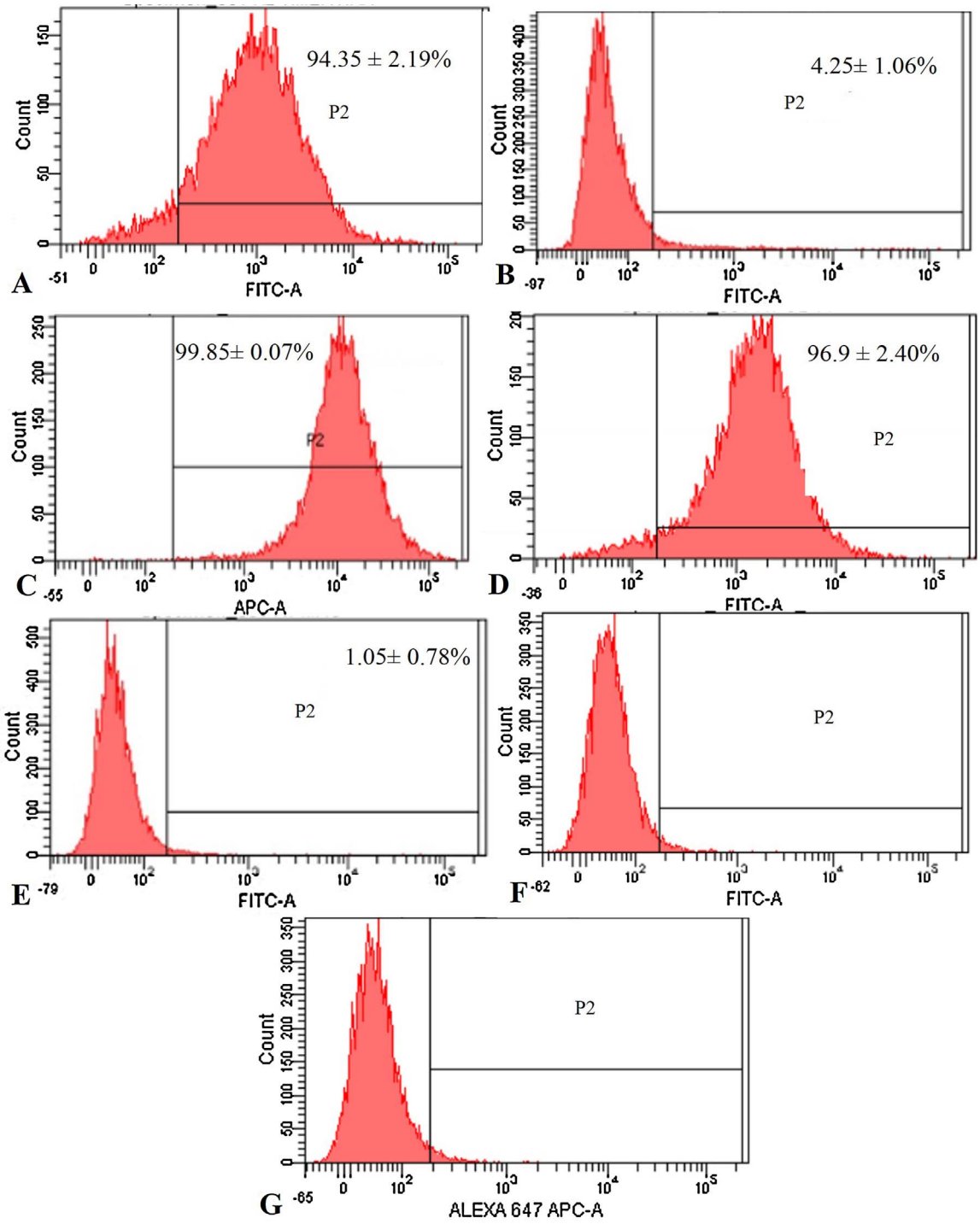

Fig. 2. Histograms of the bovine eMSCs cytomarkers analyzed by flow cytometry. A – Vimentin; B – CD34; C – CD29; D – CD44; E – MHC-II; F and G: controls.

the CM of CG, even without stimulation, could be promising to demonstrate the great potential and possibility of using this medium for use as therapeutics. In humans, the CM of MSCs from dental pulp was efficient in the experimental treatment of multiple sclerosis and even showed similar effects of treatment with their own MSCs (Shimojima et al., 2016).

A protein with antioxidant activity (peroxiredoxin-6, Table 1) was found in TG. This antioxidant capacity of the CM was previously reported for restoring or reducing retinal functions in diabetes animal model (Duarte et al., 2016).

Recently, Lange-Consiglio et al. (2015) showed in an experimental equine uterine inflammation model that horse amniotic MSCs-CM significantly reduce the expression of MMP-1 and MMP-13. These genes modulate inflammatory pathways. Also, in humans, CM of uterine cervical stem cells showed to have anti-inflammatory and bactericidal roles (Bermudez et al., 2015) and the eMSCs showed a potential to be used at pelvic organ prolapse once cells can induce the immune response and help at the tissue reorganization (Emmerson and Gargett, 2016). In our study, anti-inflammatory proteins (such as granulins, Table 1) were found in TG. In addition, *in vitro* use of the CM associated with endometrial cells resulted in improvement of the proliferation rate, showing the relevance of soluble factors produced by MSCs and its potential to increase cell replacement (Corradetti et al., 2014). Such studies support using CM as an alternative for treating

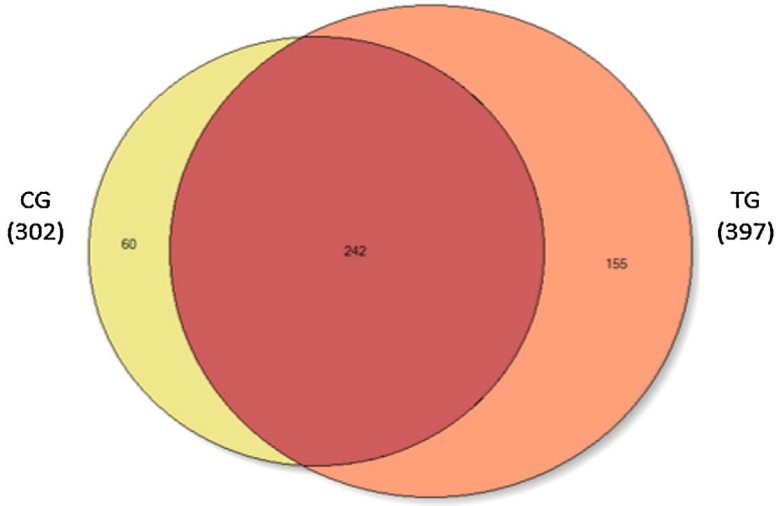

Fig. 3. Venn diagram of the proteins identified in the present study with at least one peptide sequence in the treated (TG) and control (CG) groups. The False discovery rate was ≤1%.

uterine diseases causing low fertility in cattle.

To better characterize the CM proteome, we performed some classification on the identified proteins using Gene Ontology tools (http://geneontology.org/). The Panther GO analysis identified positive enrichment (PE) for BP in macrophage activation function (GO: 0042116, fold enrichment, FE = 7.87) and receptor-mediated endocytosis (GO: 0006898, FE = 5.23) in TG. Regarding MF, there was PE for hydrolase activity (GO: 0016787; FE = 1.87) and inhibitory enzyme (GO: 0004857; FE = 5.15) in the TG, and activity structural molecule (GO: 0005198; FE = 2.73) in the CG. For CC, enrichments were verified

Table 1
Proteins with more than two PCMs differently expressed in the treated (TG) and control (CG) groups. These proteins have mainly tissue repair and immunomodulatory roles.

Biological Functions	Swiss-Prot accession	Protein name	Gene name	Group
Immune response	Q9UGM3	Deleted in malignant brain tumors 1 protein	DMBT1	Control
	KRT16	Uncharacterized protein	GP340	Control
	P01857	Ig gamma-1 chain C region	KRT16	Control
	A5A6M2	Annexin A1	IGHG1	Control
	P01876	Ig alpha-1 chain C region	ANXA1	Control
	P01834	Ig kappa chain C region	IGHA1	Control
			IGKC	
Antimicrobian/antifungal activity	P03973	Antileukoproteinase	SLPI	Control
	P59665	Neutrophil defensin 1	DEFA1	Control
	P06702	Protein S100-A9	S100A9	Control
	P01857	Ig gamma-1 chain C region	IGHG1	Control
	Q3T0Z0	Uncharacterized protein (WAP four-disulfide core domain 2)	WFDC2	Control
	P81644	Apolipoprotein A-II	APOA2	Treated
	G3N3P6	Cystatin	N/A	Treated
	F1MI18	Uncharacterized protein	N/A	Treated
	F1MNV5	Kininogen-1	N/A	Treated
	F1MVS9	Uncharacterized protein	KNG1	Treated
	P31944	Caspase 14	MASP1	Treated
	A2I7M9	Serpin A3-7	CASP14	Treated
	Q1JPB0	Leukocyte elastase inhibitor (LEI)	SERPINA3-2	Treated
			SERPINB1	
Anti-inflammatory activity	A5A6M2	Annexin A1	ANXA1	Control
	P06702	Protein S100-A9	S100A9	Control
	P28799	Granulins	GRN	Treated
Tissue remodeling	A5A6M2	Annexin A1	ANXA1	Control
	E2DI12	Syndecan-1 (Fragment)	SDC1	Control
	P28799	Granulins	GRN	Treated
	P50757	72 kDa type IV collagenase	MMP2	Treated
	E1B726	Plasminogen	PLG	Treated
	F1N2Y2	Uncharacterized protein	COL5A2	Treated
	E1B726	Plasminogen	PLG	Treated
Angiogenic activity	P80929	Angiogenin-2	ANG2	Treated
	Q2KIF2	Leucine-rich alpha-2-glycoprotein 1	LRG1	Treated
	P50757	72 kDa type IV collagenase	MMP2	Treated
Protective activity	E9RHW1	Heat shock 27 kDa protein 1	HSPB1	Treated
Antioxidant	P86215	Peroxiredoxin-6	PRDX6	Treated
Other functions	A0SXL6	Elongation factor 2 (EF-2)	EEF2	Control
Cell/cell adhesion	A7YWB6	Transforming growth factor-beta-induced protein ig-h3	TGFBI	Treated
Adhesion/cell aggregation	A5PJE3	Fibrinogen alpha chain	FGA	Treated
Platelet aggregation	P05997	Collagen alpha-2(V) chain	COL5A2	Treated
Extracellular matrix constituent	Q61245	Collagen alpha-1(XI) chain	COL11a1	Treated

in both groups to extracellular matrix (GO: 0031012) and extracellular region (GO: 0005576), as well as intermediate filament cytoskeleton (GO: 0045111) in the CG. Interestingly, with respect to PC we evidenced PE in antibacterial response proteins (PC00051; FE = 5.87) in the TG.

Here we observed proteins with anti-inflammatory, antibacterial properties and related to tissue remodeling in both CM which leads us to believe that these cells respond to many stimuli to defend the organisms. Thus, eMSCs and CM may have a role in treating reproductive tract diseases in cattle. Also, our results, especially based on protein enrichment to macrophage activation and identification of large number of proteins related to immune response on TG, allow us to infer that our *in vitro* model of stimulation with bacterial LPS of bovine eMSCs is effective to study immune and inflammatory response.

Acknowledgements

The authors thank the Proteomics Platform of the Barcelona Science Park. We are also indebted to PRB2, ProteoRed-ISCIII, the Spanish Proteomics network. This work was supported by Fundação de Amparo à Pesquisa do Estado de São Paulo [Proc.2014/20447-2, Proc. 2015/18964-1, Proc.2015/01057-1].

References

Ashiba, K., Terunuma, A., Terunima, H., Takane, T., Deng, X., Yamashita, Y., Watanabe, K., 2015. Immortalized mesenchymal stem cells producing conditioned medium in a large scale for −t, herapeutic useage. Inflamm. Regen. 35, 57–60. http://dx.doi.org/10.2492/inflammregen.35.057.

Bermudez, M.A., Sendon-Lago, J., Eiro, N., Trevino, M., Gonzales, F., Yebra-Pimentel, E., Giraldez, M.J., Macia, M., Lamelas, M.L., Saa, J., Vizoso, F., Perez-Fernandez, R., 2015. Corneal epithelial wound healing and bactericidal effect of conditioned medium from human uterine cervical stem cells. Cornea 56 (2), 983–992. http://dx.doi.org/10.1167/iovs.14-15859.

Cabezas, J., Lara, E., Pacha, P., Rojas, D., Veraguas, D., Saravia, F., Rodríguez-Alvarez, L., Castro, F.O., 2014. The endometium of cycling cows contains populations of putative mesenchymal progenitor cells. Reprod. Dom. Anim. 49, 550–559. http://dx.doi.org/10.1111/rda.12309.

Chapawanya, A., Meade, K.G., Doherty, M.L., Callanan, J.J., O'Farrelly, C., 2013. Endometrial epithelial cells are potent producers of tracheal antimicrobial peptide and serum amyloid A3 gene expression in response to *E. coli* stimulation. Vet. Immunol. Immunopathol. 151, 157–162. http://dx.doi.org/10.1016/j.vetimm.2012.09.042.

Corradetti, B., Correani, A., Romaldini, A., Marini, M.G., Bizarro, D., Perrini, C., Cremonesi, F., Lange-Consiglio, A., 2014. Amniotic membrane-derived mesenchymal cells and their conditioned media: potential candidates for uterine regenerative therapy in the horse. PLoS One 9, 1–9. http://dx.doi.org/10.1371/journal.pone.0111324.

Duarte, D.A., Papadimitriou, A., Gilbert, R.E., Thai, K., Zhang, Y., Rosales, M.A.B., Faria, J.B.L., Faria, J.M.L., 2016. Conditioned medium from early- outgrowth bone marrow cells is retinal protective in experimental model of diabetes. PLoS One 11, 1–15. http://dx.doi.org/10.1371/journal.pone.0147978.

Emmerson, S.J., Gargett, C.E., 2016. Endometrial mesenchymal stem cells as a cell based therapy for pelvic organ prolapse. World J. Stem Cell 8, 202–215. http://dx.doi.org/10.4252/WJSC.v8.i5.202.

Fan, G.C., 2012. Role of heat shock proteins in stem cell behavior. Prog. Mol. Biol. Transl. Sci. 11, 305–322. http://dx.doi.org/10.1016/B978-0-12-398459-3.00014-9.

Fortier, M.A., Guilbault, L.A., Grasso, F., 1988. Specific properties of epithelial and stromal cells from the endometrium of cows. J. Reprod. Fert. 83, 239–248.

Frier, B.C., Locke, M., 2007. Heat stress inhibits skeletal muscle hypertrophy. Cell Stress Chapereones 12, 132–141. http://dx.doi.org/10.1379/CSC-233R.1.

Kato, K., 2012. Stem cells in human normal endometrium and endometrial cancer cells: characterization of side population cells. Kaohsiung J. Med. Sci. 28, 63–71. http://dx.doi.org/10.1016/j.kjms.2011.06.028.

Lange-Consiglio, A., Perrini, C., Esposti, P., Derigibus, M.C., Camussi, G., Pascucci, L., Marini, M.G., Corradetti, B., Bizarro, D., Cremonesi, F., 2015. Effects of microvesicles secreted from equine amniotic-derived progenitor cells on in vitro lipopolysaccharide-treated tendon and endometrial cells. Reprod. Fertil. Dev. 28, 244–245. http://dx.doi.org/10.1071/RDv28n2Ab226.

Lavoie, J.R., Rosu-Myles, M., 2013. Uncovering the secretes of mesenchymal stem cells. Biochimie 95, 2212–2221. http://dx.doi.org/10.1016/j.biochi.2013.06.017.

Lupicka, M., Bodek, G., Shpigel, N., Elnekave, E., Korzekwa, A.J., 2015. Identification of pluripotent cells in bovine uterus: in situ and in vitro studies. Reproduction 149, 317–327. http://dx.doi.org/10.1530/REP-14-0348.

Maia, L., Landim-Alvarenga, F.C., Mota, L.S.L.S., Golim, M.A., Laufer-Amorim, R., De Vita, B., Barberini, D.J., Listoni, A.J., Moraes, C.N., Heckler, M.C.T., Amorim, R.M., 2013. Immunophenotypic, immunocytochemistry, ultrastructural and cytogenetic characterization of mesenchymal stem cells from equine bone marrow. Micros Res. Tech. 76, 618–624. http://dx.doi.org/10.1002/jemt.22208.

Moraes, C.N., Maia, L., Camargos, M.D., Freitas-Dell' Aqua, C.P., Mota, L.S.L.S., Chapwanya, A., Landim-Alvarenga, F.C., Oba, E., 2016. Bovine endometrial cells: a promising source of mesenchymal stem/progenitor cells. Cell Biol. Int. 40, 1332–1339. http://dx.doi.org/10.1002/cbin.10688.

Shimojima, C., Takeuchi, H., Jin, S., Parajuli, B., Hattori, H., Suzumura, A., Hibi, H., Ueda, M., Yamamoto, A., 2016. Conditioned medium from the stem cells of human exfoliated deciduous teeth ameliorates experimental autoimune encephalomyelitis. J. Immunol. 15, 4164–4171. http://dx.doi.org/10.4049/jimmunol.1501457.

Skalnikova, H.K., 2013. Proteomic techniques for characterization of mesenchymal stem cell secretome. Biochimie 95, 2196–2211. http://dx.doi.org/10.1016/j.biochi.2013.07.015.

Xiong, H., Bai, C., Wu, S., Gao, Y., Lu, T., Hu, Q., Guan, W., Ma, Y., 2014. Biological characterization of mesenchymal stem cells from bovine umbilical cord. Anim. Cells Syst. 18, 56–67. http://dx.doi.org/10.1080/19768354.2014.880370.