UNIVERSIDADE ESTADUAL PAULISTA "JÚLIO DE MESQUITA FILHO"
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VESÍCULAS EXTRACELULARES E MEIO CONDICIONADO DE CÉLULAS TRONCO MESENQUIMAIS E SEU POTENCIAL IMUNOMODULADOR EM EXPLANTES ENDOMETRIAIS BOVINOS

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Epígrafe

“Eu quero(...)
a leve palha de um pequeno sonho.
Quero o que antes da vida
foi o profundo sono das espécies,
a graça de um estado.
Semente.
Muito mais que raízes.”

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RESUMO

QUEIROZ, C.M. **Vesículas Extracelulares e Meio Condicionado de Células Tronco Mesenquimais e Seu Potencial Imunomodulador em Explantes Endometriais Bovinos** - Botucatu, 2017, 90f. Faculdade de Medicina Veterinária e Zootecnia, Campus de Botucatu, Universidade Estadual Paulista "Júlio de Mesquita Filho".

Palavras-chave: exossomos, microvesículas, inflamação, biotecnologia.

O presente estudo teve como objetivo isolar, cultivar e caracterizar as células tronco mesenquimais de tecido adiposo (CTM-TA) bovino, obtenção de vesículas extracelulares e meio condicionado do cultivo celular (MC) e avaliação do seu potencial imunomodulador em cultivo de explantes endometriais bovinos. Para isso, as CTM-TA foram coletadas (n=3) de tecido adiposo e sofreram isolamento, processamento e cultivo. As amostras foram submetidas à análise citogenética, imunofenotípica (CD44, CD29, vimentina, CD34 e MHCII), de viabilidade e de diferenciação (adipogênica, osteogênica e condrogênica), e então criopreservadas. Após descongelamento e expansão, o meio condicionado do cultivo celular foi submetido à ultracentrifugações para obtenção das vesículas extracelulares que foram caracterizadas por microscopia eletrônica de transmissão e Western Blot (Alix e CD9). Explantes de endométrios bovinos (n=7) provenientes de abatedouro foram submetidos a cultivo e desafio com LPS (3μg/mL) por 48 horas. Os grupos experimentais foram CN (controle negativo, sem desafio com LPS), CP (controle positivo, apenas desafio com LPS), V (desafio com LPS e adição de vesículas extracelulares) e MC (desafio com LPS e adição de meio condicionado). Avaliou-se secreção de PGF2α e IL-1β por Elisa, morfologia do tecido através de histopatologia, expressão tecidual de Fas-L, TLR-4, PGDH e PGF2α-R por Imunoistoquímica. No cultivo celular das CTM-TA foi obtida uma população celular homogênea e fibroblastóide, com aderência ao plástico, expressando baixos níveis de CD34 e MHCII, e altos níveis de CD29, CD44 e vimentina. As células foram cromossomicamente estáveis, com boa viabilidade antes e após congelamento, e secretaram vesículas de aproximadamente 10 a 200nm de

diâmetro, positivas para Alix e CD9 no Western Blot indicando a presença de exossomos (Exos) em uma população mista VEs. Não houve efeito de tratamento para secreção de PGF2α e IL-1β, nem na imunomarcação de TLR-4, Fas-L, PGDH e PGF2α-R. Houve mudanças morfológicas como hipertrofia, atrofia e hiperplasia celular, bem como dilatação do lúmen glandular, independente do tratamento. Houve diminuição na densidade das glândulas após cultivo, devido à inflamação. Conclui-se que foi possível isolar, cultivar, caracterizar e criopreservar CTM-TA de bovinos, e estas células secretam VEs no meio condicionado de cultivo. Contudo, os achados apontam para inflamação no modelo *in vitro* de cultivo que se sobrepôs aos efeitos inflamatórios do desafio com LPS. Ademais, os tratamentos com V ou MC não resultaram em efeito imunomodulatório.

ABSTRACT

QUEIROZ, C.M. **Extracellular Vesicles and Conditioned Medium from Mesenchymal Stem Cells and its Immunomodulatory Potential in Bovine Endometrial Explants** - Botucatu, 2017, 90f. Faculdade de Medicina Veterinária e Zootecnia, Campus Botucatu, São Paulo State University “Júlio de Mesquita Filho”.

Keywords: exossomes, microvesicles, inflammation, biotechnology.

The present study aimed to isolate, culture and characterize bovine adipose tissue mesenchymal stem cells (AT-MSC) and obtain extracellular vesicles (EVs) and conditioned medium (CM) from the cell culture in order to evaluate its *in vitro* immunomodulatory potential in bovine endometrial explants. For this, adipose tissue were collected (n=3) and underwent isolation, processing and culture. The samples were submitted to cytogenetic, immunophenotypic characterization (CD44, CD29, vimentin, CD34 and MHCII), viability and differentiation (adipogenic, osteogenic and chondrogenic), and then cryopreserved. After thawing and expansion, the CM of the cell culture was subjected to ultracentrifugation to obtain the EVs that were characterized by electron microscopy and Western Blot (Alix and CD9). Bovine endometrium from slaughterhouse (n=7) were cultured and challenged with LPS. The experimental groups were CN (negative control, no challenge with LPS), CP (positive control, challenge with LPS), V (challenge with LPS and addition of EVs) and MC (challenge with LPS and addition of CM). PGF2α and IL-1β secretion was evaluated by Elisa, tissue morphology and Fas-L, TLR-4, PGDH and PGF2α-R protein expression by Immunohistochemistry. A monolayer with fibroblastoid cell population having plastic adherence, expressing low levels of CD34 and MHCII, and high levels of CD29, CD44 and vimentin was obtained in CTM-TA cell culture. The cells were chromosomally stable, with good viability before and after cryopreservation, and secreted EVs of approximately 10 to 200 nm in diameter, positive for Alix and CD9 in Western Blot indicating the presence of exosomes (Exos) in a mixed EVs population. There was no treatment effect for PGF2α and IL-1β secretion, nor in the immunolabeling of TLR-4, Fas-L, PGDH and PGF2α-R. There were morphological changes such

as hypertrophy, atrophy and cellular hyperplasia, as well as dilation of the glandular lumen, regardless of the treatment. There was a decrease in the density of the glands after culture due to inflammation. It was concluded that it is possible to isolate, culture, characterize and cryopreserve CTM-TA from bovines, and these cells secrete EVs in CM. However, the findings point to inflammation in the *in vitro* culture model that overlapped the inflammatory effects of LPS challenge. In addition, the treatments with EVs or CM did not result in immunomodulatory effect.

LISTA DE ABREVIATURA

AA – Ácido aracdônico
AN – Anexina
BSA – Bovine serum albumins
CA – Corpos apoptóticos
CD – Cluster of differentiation
COX-2 – Cicloxigenase 2
CTM – Células tronco mesenquimais
CTM-TA - Células tronco mesenquimais de tecido adiposo
DMEM - Dulbecco's Modified Eagle Medium
DMSO – Dimetilsulfóxido
E. coli – Escherichia coli
Exos – Exossomos
FADD – Fas-associated death domain
FITC - Conjugação de isoticianato de fluotresceína
H&E – Hematoxilina e Eosina
Hsc – Heat shock cognate
IL – Interleucina
IP – Iodeto de propídio
IV – Intravenoso
LPS – Lipopolissacarídeo
MC – Meio condicionado
MET – Microscopia eletrônica de transmissão
MHC - Major histocompatibility complex
MVs – Microvesículas
P2 – Segunda passagem
PAMPS – Padrões moleculares associados a patógenos
PGDH – 15-hidroxi prostaglandina desidrogenase
PGF2α – Prostaglandina F2 alfa
PRR – Receptores de reconhecimento de padrões
RNAm – RNA mensageiro

SFB – Soro fetal bovino

TLR – Receptores do tipo Toll

TNFα – Fator de necrose tumoral

Tris – Tris Hidroximetil Aminometano

TSG - Tumor susceptibility gene

VEs – Vesículas extracelulares

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6. Considerações Finais

De acordo com os objetivos propostos e com base nos presentes resultados obtidos nas condições experimentais utilizadas, podemos considerar que:

- Houve sucesso no isolamento, cultivo e caracterização das células tronco mesenquimais de tecido adiposo (CTM-TA) de bovinos;

- As CTM-TA apresentaram boa viabilidade antes e após criopreservação, demonstrando ser uma boa opção para criação de banco celular;

- As CTM-TA secretaram vesículas extracelulares que foram isoladas por ultracentrifugações e caracterizadas por Western Blot e Microscopia Eletrônica;

- Não foi possível desenvolver um modelo *in vitro* de inflamação endometrial com desafio por LPS em explantes;

- No presente modelo, os tratamento com VEs ou meio condicionado (MC) não evidenciaram em efeito imunomodulatório significativo com base nas análises de morfologia celular, na secreção de PGF2α e IL-1β, e expressão de TLR-4, FAS-L, PGF2α-R, PGDH.