ARTICLE IN PRESS

Theriogenology xxx (2015) 1–12

Contents lists available at ScienceDirect

Theriogenology

journal homepage: www.theriojournal.com

Isolation and characterization of mesenchymal stem cells from the yolk sacs of bovine embryos

C.A.F. Mançanares [a], V.C. Oliveira [a], L.J. Oliveira [a], A.F. Carvalho [b], R.V. Sampaio [c], A.C.F. Mançanares [c], A.F. Souza [c], F. Perecin [a], F.V. Meirelles [a], M.A. Miglino [c], C.E. Ambrósio [a,*]

[a] *Department of Veterinary Medicine, Faculty of Animal Science and Food Engineering, University of São Paulo, Pirassununga, São Paulo, Brazil*
[b] *Morphology Laboratory of Veterinary Medicine College, UNIFEOB, São João da Boa Vista, São Paulo, Brazil*
[c] *Department of Surgery, Faculty of Veterinary Medicine and Animal Science, University of São Paulo, São Paulo, São Paulo, Brazil*

ARTICLE INFO

Article history:
Received 16 January 2015
Received in revised form 25 May 2015
Accepted 28 May 2015

Keywords:
Yolk sac
Mesenchymal
Stem cell
Bovine
Embryo

ABSTRACT

The yolk sac (YS) represents a promising source of stem cells for research because of the hematopoietic and mesenchymal cell niches that are present in this structure during the development of the embryo. In this study, we report on the isolation and characterization of YS tissue and mesenchymal stem cells (MSCs) derived from bovine YSs. Our results show that the YS is macroscopically located in the exocoelomic cavity in the ventral portion of the embryo and consists of a transparent membrane formed by a central sac-like portion and two ventrally elongated projections. Immunohistochemistry analyses were positive for *OCT4*, CD90, CD105, and CD44 markers in the YS of both gestational age groups. The MSCs of bovine YS were isolated using enzymatic digestion and were grown *in vitro* for at least 11 passages to verify their capacity to proliferate. These cells were also subjected to immunophenotypic characterization that revealed the presence of CD90, CD105, and CD79 and the absence of CD45, CD44, and CD79, which are positive and negative markers of MSCs, respectively. To prove their multipotency, the cells were induced to differentiate into three cell types, chondrocytes, osteoblasts, and adipocytes, which were stained with tissue-specific dyes (chondrogenic: Alcian Blue, osteogenic: Alizarin Red, and adipogenic: Oil Red O) to confirm differentiation. Gene expression analyses showed no differences in the patterns of gene expression between the groups or passages tested, with the exception of the expression of *SOX2*, which was slightly different in the G1P3 group compared to the other groups. Our results suggest that YS tissue from bovines can be used as a source of MSCs, which makes YS tissue–derived cells an interesting option for cell therapy and regenerative medicine.

© 2015 Elsevier Inc. All rights reserved.

1. Introduction

The bovine yolk sac (YS) begins its development within the first 20 days of gestation and regresses at approximately 50 days of gestation. The YS is located in the exocoelomic cavity in the ventral portion of the embryo near the umbilical cord [1,2].

The YS is an extraembryonic membrane that has an important function in the initial survival of the embryo because it acts as a source of nutrition until the placenta is completely formed. Microscopically, the YSs of species, such as canines [3,4], bovines [2,5], bubalines [6], and goats [7], are extrafetal membranes that are morphologically

* Corresponding author. Tel.: +55 (19) 35654113; fax: +55 (19) 35611689.
E-mail address: ceambrosio@usp.br (C.E. Ambrósio).

0093-691X/$ – see front matter © 2015 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.theriogenology.2015.05.031

composed of three layers: the endoderm, an intermediate layer called the mesoderm, and a single layer called the mesothelium [8,9].

The YSs have important functions that include the production and transport of proteins that are necessary for the development of the embryo [10]; participate in the exchange of metabolites [11]; facilitate the maternal transfer of materials, such as vitamins [12], amino acids [13], and immunoglobulins [14]; participate in the formation of the first blood cells [15–18]; and are responsible for the production of germ cells, which are the precursor cells that form sperm and oocytes [16,19]. The YS is a promising source of stem cells because it contains hematopoietic [7,20,21] and mesenchymal cell niches [4] during the development of the embryo and fetus.

The YS is a source of mesenchymal stem cells (MSCs) that are considered to be multipotent if they have the potential to differentiate into multiple cell lines. Mesenchymal stem cells have generated great expectations as a potential source of cells for the development of therapeutic strategies because of their intrinsic abilities to self-renew and differentiate [22].

The MSCs of YSs have been isolated from humans, and these cells have fibroblastoid morphologies, form colonies, and are immunopositive for pluripotency markers, such as *OCT-4* and *NANOG*, and mesenchymal markers, such as CD105 (SH2), CD73 (SH3), CD29, CD44, CD166, and HLA-ABC. Moreover, these cells are negative for the hematopoietic and endothelial markers CD45, CD14, CD19, CD34, and CD31. Regarding their differentiation potential in mesodermal tissues, mesenchymal stem cells from the YS can be differentiated into osteoblasts, adipocytes, and chondrocytes [21,22].

Mesenchymal stem cells have been optimistically applied to regenerative medicine and tissue engineering. Some potential uses of MSCs include developing muscle cells, aiding liver regeneration, and forming cells in the central nervous system [23].

The study of this extrafetal membrane is crucial in preventing fetal death during the early stages of pregnancy [8]. Because the YS is a promising source of MSCs, the aim of this work was to isolate and characterize the mesenchymal cells of the bovine yolk sac (bYS-MSCs) and analyze them with respect to their expressions of specific cell surface markers with the aim of establishing an experimental model for cell therapies for the treatment of diseases.

2. Materials and methods

2.1. Macroscopic analysis of the YS

The study protocol was approved by the research ethics committee (1.1656.74.3/2011) of the Faculty of Animal Science and Food Engineering, University of São Paulo, Brazil. The uteri were collected in a slaughterhouse and surgically examined. The embryos were analyzed and categorized into groups according to the crown rump (CR) measurements described in Table 1.

Table 1
Estimated embryo ages of groups I and II.

Groups	Yolk sacs (n)	Crown–rump length interval (cm)	Estimated embryonic age (days)
I	3	0.3–1.4	20–34
II	3	1.5–3.1	35–50

2.2. Histologic and immunohistochemistry analyses of the YS

For histologic analysis, the YSs were postfixed in 4% paraformaldehyde in Dulbecco's PBS (DPBS; Gibco Co., USA), dehydrated in a series of ethanol solutions of increasing concentration (from 70%–100%), diaphonized in xylene, and embedded in Histosec embedding media. The materials were cut with a Leica RM2145 microtome into cross sections with thicknesses of 5 microns. The sections were placed on glass slides, and after drying (oven at 37 °C), they were stained with hematoxylin and eosin [24].

For immunohistochemistry analyses, other samples were embedded in Tissue-Tek and frozen at −80 °C. Subsequently, the blocks were cut with a Leica CM1950 cryostat into 5-micron slices and placed in previously silanized slides (3-aminopropyltriethoxysilane; Sigma) with two cuts per blade.

Then, the sections were heated in citrate buffer (0.384 g of citric acid monohydrate, 2.352 g of sodium citrate tribasic dihydrate, 1-L distilled water, pH 6.0) for 15 minutes in a microwave oven. Blocking was performed by incubation in hydrogen peroxide solution at 3% in 1-M Tris-HCl buffer, pH 7.5 (60.57-g TBS Tris in 500-mL ultrapure water) for 30 minutes. The sections were incubated with 10% goat serum and in Tris-buffered saline (TBS) for 30 minutes.

Primary antibodies (OCT4, CD44, CD90, and CD105) were diluted to 0.2 mg/mL in TBS buffer containing 1% goat serum and incubated "overnight" in a humid chamber at 4 °C. In parallel, cuts were incubated with the same concentration of irrelevant control antibody isotype (immunoglobulin G), and the sections were washed with TBS containing 1% goat serum. According to the manufacturer's recommendation, the reaction was visualized by means of the multipurpose kit, Dako Advance HRP Link (cat. #K4069, Dako, USA).

The reaction was revealed by precipitation of 3,3′-diaminobenzidine (DAB Peroxidase Substrate Kit, cat. #SK-4100). Finally, the sections were counterstained with hematoxylin, dehydrated, diaphanized, and blades mounted for analysis by light microscopy (Table 2).

2.3. Transmission electron microscopy of the YSs

For the transmission electron microscopy (TEM) analysis, nine YS samples and six plates of the bYS-MSCs were fixed in 2.5% glutaraldehyde diluted in PBS (pH 7.4–0.1 M) for 24 hours. After fixation, the fragments and cells were washed in PBS, postfixed in osmium tetroxide (4% w/w solution in water; Polysciences, Inc., USA) for 1 hour, and finally washed in PBS. The fragments and cells were then dehydrated in increasing concentrations of ethanol (70%–100%) using propylene oxide (EM Grade; Polysciences) as the final dehydration reagent. The samples were incubated for 12 to 16 hours in a 1:1 mixture of propylene oxide and Spurr's resin (Spurr's Kit; Electron Microscopy Sciences) and then incubated in 100% Spurr's resin for an additional 4

Download English Version:

https://daneshyari.com/en/article/10891658

Download Persian Version:

https://daneshyari.com/article/10891658

Daneshyari.com