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Homology based selection of cell surface markers for the purification of goat bone marrow derived Mesenchymal Stem Cells

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ABSTRACT

Adult stem cells are self-renewing cells and sustain for long period. Mesenchymal Stem Cells (MSCs) has been studied in a variety of species till date, however, there is limited information available for goats' MSC surface markers. Hence, as per the developmental scenario of mammals, homology of MSCs surface markers across the species can be explored for the enrichment of goat BM-MSCs. Accordingly, in present study a pure population of goat MSCs was isolated, while using diverse antibodies against MSC surface markers from other species including human, rat and mouse. Goat bone marrow was aspirated from the iliac crest, and cultured in DMEM. Further, MSCs were enriched using human CD90 antibodies through Magnetic-Activated Cell Sorting (MACS). Once, these cells exhibited fibroblast-like morphology, the expression of stem cell markers was analysed through FACS and Immunofluorescence. Pure MSCs were observed to be highly positive for CD90 and CD29 by 98-99% and turned out to be almost negative for CD45 and CD31, while using rat antibodies, hence proving the purity of this stem cell population. These goat bone marrow-derived MSCs are used for intra-ovarian transplantation, which seems to be crucial to restoring female fertility in aged infertile goats.

Keywords: Bone Marrow, CD markers, Characterization, Mesenchymal Stem Cells, Pluripotent

Mesenchymal stem cells (MSCs) are multipotent cells that can develop into several cellular lineages, including osteoblasts, cardiomyocytes, chondroblasts, endothelial cells, hepatocytes, myocytes, neuronal cells, and tenocytes; at the same time, still have the ability to self-renew (Ogliari *et al*. 2014, Arnhold *et al*. 2019, Dias *et al*. 2019). The anti-inflammatory, immunomodulatory, and regenerative properties of MSCs are the foundation of MSC therapy (Peroni and Borjesson 2011, Dabrowska *et al*. 2021). The most extensively researched cells are MSCs, and numerous studies have shown promising and secure outcomes. Although MSCs are traditionally isolated from bone marrow (BM), whereas, more recent reports have detailed the isolation of cells with MSCs characteristics from various tissues, including umbilical cord blood, chorionic villi of the placenta, Wharton's jelly, fatty tissue, and amniotic fluid (Hass *et al*. 2011). MSCs have also shown that cellular and gene-based therapies work better in a variety of congenital and degenerative disorders (Baksh *et al.* 2004). Furthermore, MSCs have been demonstrated in various species, whereas, data on, goats is limited. Hence, according to the available data for the rodent models, purified goat MSCs also can be used for various regenerative therapies (Azari *et al.* 2011) as well as for the restoration of ovarian function in case of aged goats. Therefore, before opting for such complicated procedure, it is a pre-requisite to standardise an affordable and easy purification procedure for the collection of goat MSCs.

Overall, this study provides comprehensive evidence for the successful isolation, culture, enrichment, and characterization of goat BM-MSCs which can provide great scope for future research to standardize the transplantation of goat BM derived MSCs for diverse therapeutic purposes. Moreover, this study suggests a significant advancement in understanding and harnessing the potential of goat BM-MSCs for regenerative medicine, tissue engineering, and reproductive therapies in veterinary science.

MATERIALS AND METHODS

The study was conducted according to the guidelines approved by the Institutional Animal Ethics Committee (IAEC), as per the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India. All the experiments were performed as per the ethical approval and approved protocols. Animals for bone marrow collection were obtained from Central Institute of Research on Goats, Mathura.

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Extracting BM from goat: BM was collected from healthy adult female Jamuna Pari goat aged 1-2 years in CIRG, Mathura. Animals were injected with Xylazine 1M (0.05 mg/kg body weight) for sedation. Subsequently, 2 mL of 2% lignocaine hydrochloride was injected around the iliac crest, and the BM was aspirated with a 16G BM biopsy needle and the BM was collected through a syringe with anticoagulant EDTA to prevent coagulation of BM. Furthermore, processing was carried out under a biosafety cabinet to avoid any microbial contamination.

Isolation and culture of goat BM-MSCs: The collected BM was transferred into a sterile 50 ml falcon tube with pre-warmed Dulbecco's Modified Eagle's Medium Low glucose (L-DMEM, 10567-014, Gibco) containing 10% Fetal Bovine Serum (FBS, 12662-029, Gibco), L-glutamine (35050-061, Gibco), sodium pyruvate (11360-070, Gibco), and 10% antimycotic antibiotic (100 IU/ml, 15240-062, Gibco) followed by centrifugation at 7000 rpm for 10 min. The cell pellet was dissolved in 5 ml PBS (D5652, Sigma Aldrich). The cell suspension was thoroughly mixed with 4 ml histopaque (10771, sigma) in a pre-sterilized 15 ml centrifuge tube and centrifuged at 2800 rpm for 30 min at room temperature. The buffy layer was carefully collected and washed with 2-3 ml of PBS following centrifugation at 7000 rpm for 10 min. The supernatant was discarded and the cell pellet was re-suspended in RBC lysis buffer to remove RBC followed by washing with PBS twice through centrifugation at 7000 rpm for 10 min. The cell pellet was re-suspended in 5 ml of complete L-DMEM medium. Cell viability and cell number were determined using the haematocytometer with 0.4% trypan blue solution. For this, 10 µl volume of cell suspension was mixed with 90 µl of 0.4% trypan blue, then applied a drop of the trypan blue/cell mixture to a haemocytometer and placed on the light microscope. The unstained (viable) and stained (non-viable) cells were then counted individually within the haematocytometer to determine cell viability and number. Following this, the nucleated cells were re-suspended in a complete DMEM medium (low glucose, L-DMEM) supplemented with 10 % FBS, and these cells were seeded at a density of 2.5 million cells/ml and incubated at 37°C with 5% CO_2. On the third day of incubation, non-adherent cells were removed, and fresh complete media was replaced to propagate the MSCs adhering to the surface. Upon confluency (70-80%), cells were detached by enzymatic digestion in 0.25% Trypsin-EDTA (25200-072, Gibco) in L-DMEM for the following passage.

Characterization of goat BM-MSCs: The goat BM-MSCs were characterised through morphology, magnetic-activated cell sorting (MACS), flow cytometry (FACS), and immunofluorescence (IF) analysis. Though real time PCR and western blotting are standard techniques for the charcterization of any gene expression, but the aim of this study was to confirm the purity of enriched MSCs. Hence, flow cytometry was observed to be the only technique to provide the exact percentage of the pure cell population of MSCs based on the expression of their cell surface markers where these results were further validated through immunofluorescence microscopy.

Enrichment of goat BM-MSCs through MACS: Goat BM-MSCs were enriched by two surface markers, i.e. CD90 Microbeads, a positive selection marker and CD45 Microbeads, a negative selection marker. Details of the antibodies used in present study is mentioned in Table 1. In this study both negative and positive selection were performed for the enrichment of MSCs by MACS at passage 1. The confluent monolayer of cells was harvested by 0.25% Trypsin-EDTA, and resuspended in buffer containing 1× PBS, 0.5% BSA (A3311, Sigma Aldrich), and 2mM EDTA (431788, Sigma Aldrich) at 10 million cells/ml. Firstly, the negative selection was done by adding 20 µl of CD45 microbeads per 10 million cells for 15 min while incubation on ice. Afterwards, cells were washed by adding 1-2 ml of buffer containing 1× PBS, 0.5% BSA, and 2mM EDTA per 10 million cells and centrifuged at 300 g for 10 min. The supernatant was completely aspirated and up to 10 million cells were re-suspended in 500µl of buffer containing 1× PBS, 0.5% BSA, and 2mM EDTA and further preceded for magnetic separation. MS column (BD) was used for the separation of the desired cellular population under the magnetic field of a suitable MACS separator (130-042-102, BD) followed by washing with the 500 µl

Table 1. List of antibodies used during MACS and FACS analysis

Primary antibody	Catalogue no.	Company	Conjugation
CD90 microBeads, human	130-096-253	Miltenyi Biotec	MicroBeads
CD45 microBeads, human	130-045-801	Miltenyi Biotec	MicroBeads
Nanog (1E6C4) mouse Monoclonal IgGI antibody	sc-293121	SantaCruz Biotechnology	FITC
Sox (E4) mouse monoclonal IgGI anitibody	sc-365823	SantaCruz Biotechnology	FITC
Oct 3/4 (C10) mouse monoclonal IgGI antibody	sc-5279	SantaCruz Biotechnology	FITC
Rat anti-mouse CD105	562761	BD Pharmingen	AF647
Hamster anti-rat CD29	562153	BD Pharmingen	AF647
Mouse anti-rat CD90	554897	BD Pharmingen	FITC
Mouse anti-ratCD45	554877	BD Pharmingen	FITC
Mouse anti-ratCD31	555027	BD Pharmingen	PE

of buffer containing 1X PBS, 0.5% BSA, and 2mM EDTA. The unbound cells were taken for positive selection and similarly 10μl of CD90 Microbeads antibody were added per 10 million cells followed by sorting with the magnet. Finally, these $CD90^+$ and $CD45^-$ cells were cultured in L-DMEM medium supplemented with 10% FBS. After the enrichment of MSCs through MACS, FACS analysis was performed to verify the surface marker expression for further confirmation of their purity.

FACS analysis using pluripotent and cell surface markers: FACS analysis was performed to investigate the expression of cell surface markers using Alexa Fluor/APC conjugated Hamster Anti-Rat CD29 and Rat Anti-Mouse CD105, FITC conjugated Mouse Anti-Rat CD 90 and Mouse Anti-Rat CD45, PE conjugated Mouse Anti-Rat CD31 and pluripotency-associated transcription markers using FITC conjugated Nanog mouse monoclonal IgG 1 antibody, Oct 4 mouse monoclonal IgG 1 antibody, and Sox 2 mouse monoclonal IgG 1 antibody. On the other hand, unstained cells were used as a negative controls for FITC, PE, AF647. Details of all the antibodies used during FACS analysis provided in Table 1. Briefly, goat BM-MSCs were harvested and aliquoted at a density of 0.5 million cells/ml for each staining and these cells were fixed in 500 μl of 4% PFA (15812-7, Sigma Aldrich) for 10 min at room temperature. Thereafter, these cells were permeabilised using 500 μl of 0.1% Triton×100 (T8532, Sigma) and 0.05% NP-40 (74385, Sigma) for 20 min followed by washing with 1 ml of 1X-DPBS twice. Cells were re-suspended in 100 μl of cell staining buffer (1X-DPBS containing 2% FBS and 0.05% sodium Azide and then incubated with fluorophore-conjugated primary antibody at a dilution of 1:50 for 30 min on ice. Following washing with 1ml 1X-DPBS twice, these cells were re-suspended in 400μl cell staining buffer and transferred into a FACS tube. Flow cytometry was performed using a FACS Aria (BD Biosciences) and the data were analyzed with cell quest software.The voltage settings used for FACS analysis were FSC-20, SSC-290, FITC-300, PE-330, APC/AF647-670 and sequential gating was performed to select cells of interest for analysis.

Immunofluorescence analysis: Immunolocalization experiments were done to further check the expression of cell surface markers and pluripotency related transcription markers in goat BM-MSCs at passage 3 after MACS which were first enriched by MACS at passage 1. Goat BM-MSCs (30,000 cells) were seeded in 12-well plate containing 18 mm diameter glass coverslips. After 24 h, once the cells confluency reached upto 80%, these cells were washed with 1X-DPBS twice and then fixed with 4% PFA for 10 min. The cells were then permeabilised with 0.1% triton X-100 and 0.05% NP-40 for 10 min. at room temperature, followed by washing with 1ml of 1X-DPBS twice. After blocking with PBS containing 0.1% Tween-20 (P1379, Sigma) and 2% BSA for 2 h followed by washing with 1ml of 1X-DPBS twice, these cells were incubated with primary antibodies such as mouse anti- tubulin (# A11126 used as a positive control), both positive markers viz. Alexa Fluor 647 conjugated Hamster Anti-Rat CD29 (562153, BD Pharmingen), Biotin conjugated Mouse Anti-Rat CD90 (554896, BD Pharmingen), Mouse Anti-Rat CD54 (554968, BD Pharmingen) and negative marker, Mouse Anti-Rat CD45 (554876, BD Pharmingen). Pluripotency-related transcription markers, viz. Rabbit Anti-Human Nanog (4903, Cell Signaling Technology) was also used at 1:150 dilutions for overnight at 4°C. After washing with 1ml 1X-DPBS twice, the cells were incubated with secondary antibodies, viz. PE-streptavidin, Mouse FITC and Rabbit Cy5 (Jackson) at 1:250 dilutions for 2 h. Finally, for nuclear staining, samples were further counterstained with DAPI (D9542, Sigma Aldrich) at 1μg/ml concentration for 20 min, followed by washing with 1ml 1X-DPBS twice and mounted with the glass cover slip using Prolong Gold Antifade Reagent (P36934, Invitrogen) and sealed with nail polish. Cells were observed and images were captured using confocal microscope (SP8, LEICA).

RESULTS AND DISCUSSION

MSCs have created enormous research interest due to their self-renewing capacity and multipotency, these cells can be isolated from variable sources such as bone marrow, adipose tissues, umbilical cord blood and matrix, synovial membranes, embryonic tissue, and amniotic fluid (Lee *et al.* 2013). The aspiration of bone marrow is more invasive technique than the placental tissue or amniotic fluid, but the availability of these sources can be a concern as they may not be feasible in various cases, at the same time placental tissue as well as amniotic fluid will always be allogenic, which may reduce the probability of their acceptance. Whereas aspiration of BM from the iliac crest is a standard procedure even in human case, where purified MSCs will be autogenous and can be accepted easily without any rejection. Simultaneously, the standardised technique for the purification of goat MSCs also can be applied to purify goat MSCs from the placental tissue and amniotic fluid as well. Although, few reports are available for the isolation and characterization of goat MSCs (Azari *et al.* 2011, Da Silva Neves *et al.* 2021, Mahajan *et al.* 2022) but available techniques so far have lesser growth rate, and purity. Present study has standardized a better technical method to isolate and purify goat MSCs through the homology based selection of MSCs surface markers across other mammalian species.

Cell culture and morphological cell analysis of BM-MSCs: Growth of MSCs from various species varies in different medium (Hagmann *et al*. 2013). According to previous study, L-DMEM was found to be a more appropriate medium for the growth of bone marrow derived MSCs (Martins *et al*. 2017). In present study, L-DMEM was used with some modifications, to isolate MSCs from the BM of the goat and cultured in vitro successfully. During primary culture, cells attached to the culture flasks (T25/ T75) revealed a non-fibroblastic, round, epithelial cell-like phenotype. During the first few

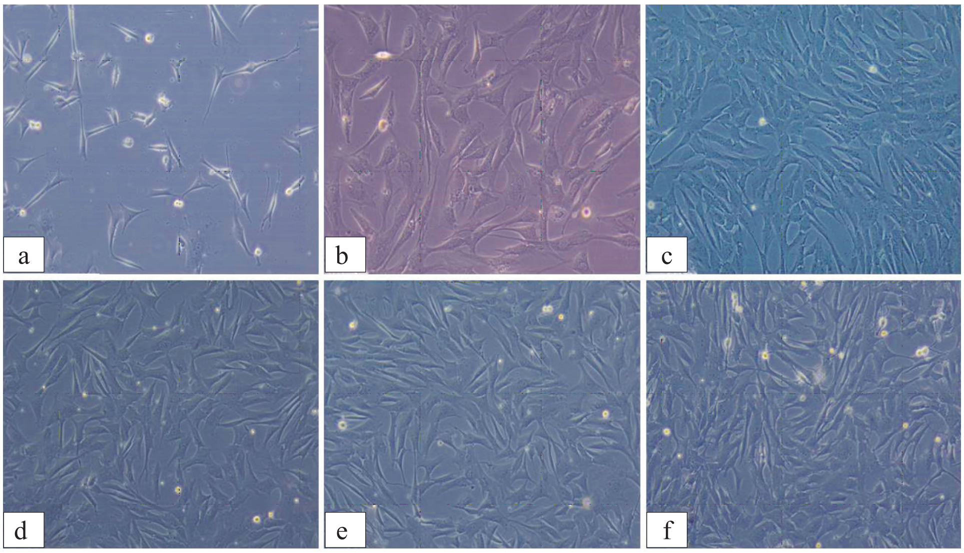

Fig. 1. Morphological appearance of goat bone marrow-derived mesenchymal stem cells (BM-MSCs) observed under a bright-field microscope. (a) On 3rd day after culture, BM-MSCs began to proliferate and demonstrated fibroblast-like morphology, with small non-adherent nuclear cells, (b) After 8 days, spindle-shaped cells presented 70-80% confluency, (c) BM-MSCs at Passage 2, showed homogenous spindle-shaped fibroblast-like morphology, (d) Passage 4, (e) Passage 5, (f) Passage 6 also showed similar morphology.

days of incubation, undesirable granulo-monocytic round cells were observed that initially remain suspended in the media and get eliminated with successive passages. On day 3 of the initial culture, adhered cells attained spindle-shaped morphology and began to proliferate (Fig. 1a). After 3–4 days of incubation, cells gradually expanded into tiny colonies over time. By 8-10 days following sowing, the number and size of the colonies had grown to 70-80% confluency as shown in Fig. 1b.

Goat BM-MSCs adhered to the surface of plastic culture dishes and consistently displayed a spindle-shaped fibroblast-like morphology without any morphological changes with subsequent passages (Fig. 1 c-f).

These cells were cultured up to passage three and co-stained with Tubulin antibody (FITC-tagged) and a nuclear stain DAPI, which reflected that enriched goat MSCs do have healthy cytoskeleton. (Supplementary Fig. 1).

Enrichment of MSCs from Goat BM-derived cells through MACS: Primary goat BM-MSCs are a heterogeneous mixture of cells with unwanted cells like haematopoietic stem cells (Mabuchi *et al*. 2021), but for efficient therapeutic purposes, a homogenous population of MSCs should be used. Hence, the purity of these stem cells is a real therapeutic concern. Present study demonstrated the complete strategy for the enrichment of MSCs and how to eliminate undesired cell population from the cultured cells. Therefore, to implement the enrichment, monoclonal antibodies against human stem cell surface markers were used, because not much is known about the goat cell surface markers. MACS was done at passage 1 to distinguish MSCs

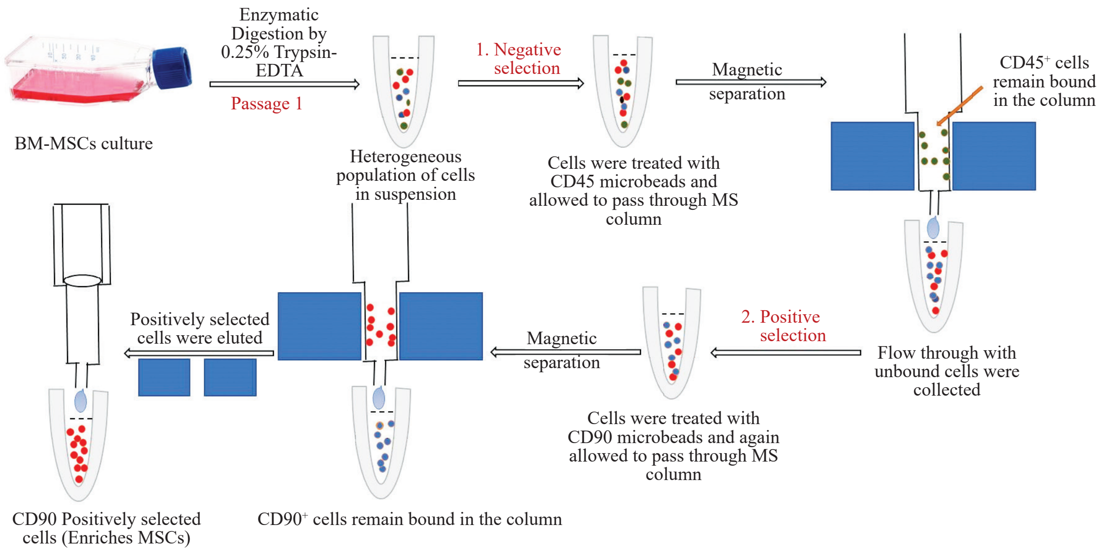

Fig. 2. Schematic representation of enrichment of BM-MSCs by negative selection (using CD45 Microbeads) and positive selection (using CD90 Microbeads) through Magnetic-Activated Cell Sorting (MACS).

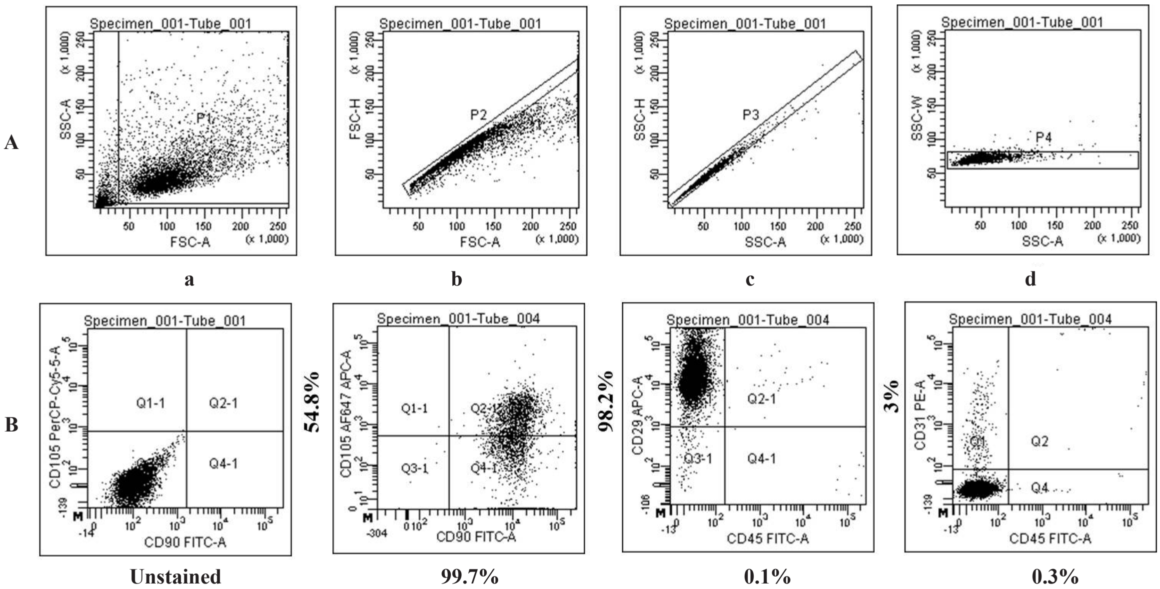

Fig. 3. Representative flow cytometry analysis of cell surface markers CD90, CD105 and CD29 for the enriched goat BM-MSCs: (A) Cell gating, to isolate cells of interest, after exclusion of debris (a) aggregates, (b) doublets, (c) and selected single cells, (d) for the analysis, (B) Unstained cells did not show any background for the selected surface markers.

from the heterogeneous population of cells isolated from the goat BM using both positive and negative markers. To select the antibodies for enrichment, the homology of cell surface markers CD45 and CD90 between goat and human was evaluated and shown in Table 2. Cells were counted before and after the enrichment, using a hemocytometer and out of 10×10^6 cells/ml, approximately 1×10^6 cells/ml were found to be positive for the CD 90 expression, which is a well-known marker for MSCs. Even though the homology of CD 45 between goat and human is around 69.38% and CD 90 is 73.29%; still these human antibodies were found to be good enough to enrich goat MSCs. Hence, it had been confirmed that human monoclonal CD90 Microbeads as positive marker and CD45 Microbeads as a negative marker can be explored for the enrichment of goat MSCs. After enrichment of MSCs; the purity of these enriched MSCs was confirmed by flow cytometry.

A schematic representation of enrichment of goat BM derived MSCs through both negative and positive selection is shown in (Fig. 2).

Purity testing of goat BM-MSCs through FACS analysis: To characterize the purity of MSCs, Fluorescence-Activated Cell Sorting (FACS) was used; where frequently expressed distinct cell surface markers as well as pluripotent markers were explored. According to prior information from the other mammalian species; CD29, CD90 and CD105 were selected to confirm the purity of goat MSCs, whereas, CD31 and CD45 surface antigens were considered as negative markers, in flow cytometry studies. At the same time, three pluripotent transcription markers, i.e. Nanog, Sox2, and Oct4 were also checked to confirm the purity of these MSCs at passage 3. To assess the percentage of purity of the enriched Goat BM-MSCs through the FACS analysis,unstained cells were used as negative control for cell surface markers (Fig. 3B) as well as for the pluripotency-associated transcription markers (Fig. 4B). To isolate cells of interest, sequential gating was performed as shown in Fig. 3A (a-d); forward Scatter (FSC) parameter is associated with cell size and side scatter parameter (SSC) is associated with granularity and complexity of cells. FSC-A vs SSC-A represents the selection of cells of interest out of the debris, FSC-A vs FSC-H denotes isolation of cells of interest from cell aggregates, SSC-A vs SSC-H describe the exclusion of doublets from the analysis and lastly, SSC-A vs SSC-W define the isolation of single cells for the analysis. For cell surface markers, anti-rat CD29, CD90 antibodies were used, as the homology of CD29 between goat and rat is quite high (93.51%) and the FACS data showed 98.2% purity of goat MSCs, while using this antibody across the species. Similarly, CD90 in goat exhibited 70.19% homology with rat CD90, and the FACS analysis demonstrated 99.7% of goat MSCs through this anti-rat antibody. For CD105, anti-mouse antibody was used for the FACS analysis of goat MSCs and around 55% goat MSCs showed the expression of CD105. The homology of CD105 between goat and mouse was 66.25% and hence a moderate percentage of cells were found to be positive for this marker. Previous studies reported that CD45 and CD31 can be explored as negative markers for MSCs selection. In the present study, anti-rat antibodies for CD45 and CD31 were used; where homology between goat and rat CD45 is 66.89% and for CD31 is 61.09% has been assessed through the sequence homology analysis. Obtained results showed only 0.1-0.3% positivity for CD45 and 3% goat MSCs were observed to be positive for CD31. Hence, the results of these negative cell surface markers further authenticated the purity of goat MSCs. Overall, this study reflected that anti-rat antibodies of CD90, CD29,

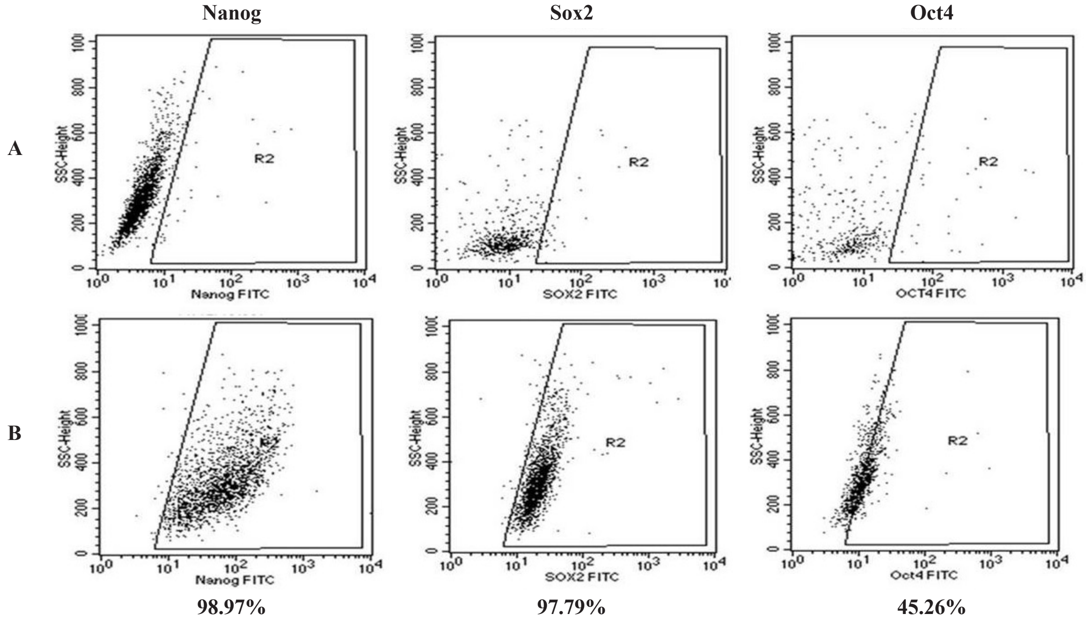

Fig. 4. Flow cytometry analysis of pluripotency-associated transcription markers Nanog, Sox2 and Oct4, in enriched BM-MSCs'. (A) Unstained cells of enriched BM-MSCs were used as negative control for fluorophore-tagged pluripotency-associated transcription markers, including Nanog FITC, Sox2 FITC and Oct4 FITC, (B) Stained cells of enriched BM-MSCs demonstrated 98.97% positive for Nanog, 97.79% for Sox2 and 45.26% for Oct4.

CD45 and CD31 can be explored for the purification of goat MSCs. FACS results for the cell surface markers are shown in Fig. 3B and Table 2. Out of selected cells for FACS analysis, 99.7% cells showed expression for CD90, 54.8% cells were found to be positive for CD105 whereas, 98.2% cells were observed to be positive for CD29. Double confirmation was also done for the purity of these MSCs through the expression of negative markers, where just 0.1 to 0.3% cells showed expression for CD45 and 3% cells were found to be positive for CD31.

Enriched and cultured goat MSCs were also checked for the expression of pluripotent stem cell markers, including Oct4, Sox2 and Nanog (Takahashi and Yamanaka 2006). In the present study anti-human antibodies were used to check the expression of these pluripotent stem cell markers in the enriched goat MSCs. FACS analysis of these permeabilized cells showed more than 98% positivity for the expression of Nanog; where the homology of goat vs human Nanog is around 70.57%; Sox2 also showed around 98% positivity of goat MSCs, while having homology of 96.85% with human Sox2; expression of Oct4 in these cells was surprisingly slightly low with 45.26%, where the homology of goat Oct4 with human was 97.50%. Unstained cells were used as negative control for these pluripotency-associated transcription markers shown in Fig. 4A and the percentage of cells showing expression for these markers has been elaborated in Fig. 4B and Table 2.

Immunofluorescence analysis of goat BM-MSCs: FACS results of these MSCs were validated further through immunofluorescence (IF) staining; where all the positive markers; including CD29 (Fig. 5A.a), CD90 (Fig. 5A.b) and CD54 (Fig. 5A.c) demonstrated strong staining in almost 95-100% of purified goat MSCs. At the same time; staining of these cells for the negative cell surface marker CD45 indicated negligible expression of CD45 (Fig. 5A.d); which further authenticated the purity of these goat MSCs and the absence of differentiated cells. Furthermore, these MSCs were also stained for the nuclear stem cell marker Nanog; most of the cells were observed to be positive for Nanog (Fig. 5B), confirming the stemness behavior of these cells.

Table 2. Expression analysis of stem cell surface markers in goat BM-MSCs after 3rd passage of MACS sorted cells

Markers	Nature	Percentage (FACS analysis)
Nanog	Stem cell marker	98.97 %
Sox2	Stem cell marker	97.79 %
Oct4	Stem cell marker	45.26 %
CD90	Positive marker for MSC	99.7 %
CD105	Positive marker for MSC	54.8 %
CD29	Positive marker for MSC	98.2 %
CD45	Negative marker for MSC	0.1 %
CD31	Negative marker for MSC	3 %

Largely these results, including cell morphology, FACS and IF analysis confirmed the purity and homogeneity of the enriched goat BM-derived MSCs. Thus, current research has established an easy, accessible and affordable method for isolation, culture and characterization of goat BM-derived MSCs; which can be utilized further for various regenerative therapeutic purposes, including restoration of ovarian function in dysfunctional aged ovaries as well as consequent reproductive lifespan of female goats.

In conclusion, the present study successfully demonstrated the enrichment of goat MSCs and the purity validation of these cells through various techniques. So far,

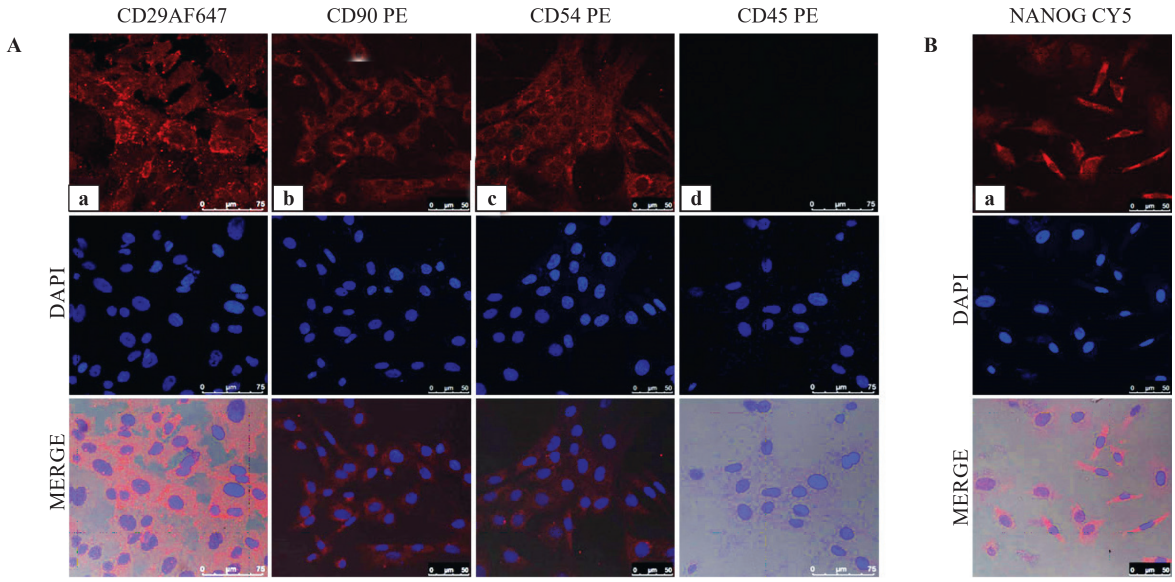

Fig. 5. (A) Immuno-fluorescent staining at passage 3rd after MACS for cell surface markers CD29, CD90 and CD54, further confirmed the purity of enriched MSCs: (a) Representing expression for cell surface marker CD29 in MSCs, (b) MSCs showing expression for CD90, (c) Demonstrating expression for CD54, (d) MSCs were observed negative for CD45; (B) Immuno-fluorescent staining of goat BM-MSCs at passage 3 after MACS for localization of stem cell marker Nanog. Expression of nuclear marker Nanog, further confirmed the purity of goat bone marrow-derived mesenchymal stem cells. *Original magnification 630×.

no customary antibodies are available for the isolation of goat MSCs, hence the technical standardization to purify goat MSCs is important, while exploring antibodies against the MSC surface markers across the species. Selection of these antibodies was done through the analysis of homologous MSCs surface markers across the species including human, rat and mouse. As pure population of MSCs is the key factor for efficient regenerative therapy, hence this concern for goat-MSCs has been addressed here technically and the homogenous population of MSCs was obtained, their purity was also authenticated further through the morphological appearance, Fluorescence-Activated Cell Sorting analysis and Immunofluorescence Staining. Obtained results demonstrated the expression of MSC-surface markers including CD29 and CD90 around 98%, whereas, cells were almost negative for the known negative markers of MSCs, i.e. CD45 and CD31. Expression of pluripotency markers of MSCs, including Nanog and Sox2 was also turned out to be around 98%. Hence, this investigation could provide an advancement in the research area of stem cell therapy as well as regenerative medicine applications for the livestock agriculture.

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