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Ovarian Extract Promotes Proliferation and Upregulates Pluripotency of Mesenchymal Stromal Cells

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Background and Hypothesis: Somatic cell nuclear transfer (SCNT) has been developed to reprogram differentiated cells into pluripotent cells. In SCNT, reprogramming is induced by introducing somatic DNA into an enucleated oocyte. We thus hypothesized that oocyte-derived factors could direct the reprogramming of somatic cells into pluripotent cells.

Methods: Mesenchymal stromal cells (MSCs) were isolated from murine bone marrow. MSCs were characterized by flow cytometry and *in vitro* differentiation into osteogenic and adipogenic lineages. MSCs were co-cultured with ovarian extract obtained after superovulation of female Swiss albino mice. Proliferation of cultured MSCs was measured by MTT assay, and the expression of pluripotency markers and epithelial-mesenchymal markers was measured by quantitative real-time PCR analysis, flow cytometry and confocal immunofluorescence staining.

Results: Co-culture of MSCs with ovarian extract caused significant increase in the proliferation rate of MSCs compared to control MSCs cultured alone. MSCs co-cultured with ovarian extract expressed Oct-4 and NANOG proteins, and showed significant increase in mRNA of the pluripotency markers Oct-4, NANOG and Rex1. A mesenchymal to epithelial transition (MET) phenotype change was also observed, and measured by an increase in EpCam expression and decrease in ZEB1 expression. This study provides evidence that ovarian extract contains factors that support survival and proliferation of MSCs, and upregulate pluripotency.

Keywords: oocytes, pluripotency, Oct4

1 Introduction

Reprogramming differentiated cells back into pluripotency can be achieved experimentally by nuclear transfer, cell fusion or induced pluripotent stem cell technology [1-5]. These manipulations provide an opportunity to create pluripotent cells from autologous adult cells and subsequently open the possibility for a relatively safe cell replacement therapy. Induced pluripotent stem cell (iPS) technology makes use of the overexpression of certain transcription factors such as Oct-4, Sox2, Klf4 and c-Myc [2, 6]. Nuclear transfer and cell fusion experiments on the other hand do not exact overexpression of new genes, but instead make use of natural components already present in oocytes and early embryos to initiate new transcription [7, 8].

Pioneering nuclear transfer experiments in amphibians have shown that the cytoplasm of the oocyte has the capacity to reprogram an already differentiated nucleus back to the embryonic state [9, 10]. The success of somatic cell nuclear transfer (SCNT) to generate cloned animals using enucleated oocytes [11, 12], and, recently, the successful derivation of SCNT human embryonic stem cells [13], have indicated that maternal factors present in the mature ooplasm are capable to sufficiently reprogram the nucleus of a differentiated cell to pluripotency. This process is known to involve a series of consecutive events involving protein exchange between the donor nucleus and ooplasm, donor nuclear chromatin remodeling, and pluripotency gene reactivation [14-21]. However, maternal factors in charge of this reprogramming process and the underlying mechanism(s) remain largely unknown.

iPS cells present a potentially useful source for drug discovery and cell transplantation therapies. However, attempts to date at generating iPS cells from a patient's own somatic cells are achieved using viral vectors, such as retrovirus and lentivirus. These transfection vectors integrate into their host genome and may increase the risk of tumor formation. To overcome the safety issues related to the generation of iPS cells, several methods have been reported such as transient expression of the reprogramming factors using adenovirus vectors or plasmids, and direct delivery of reprogramming proteins [8]. Despite the fact that these transient expression methods could avoid genomic

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modification of iPS cells, they were shown to be mostly inefficient. Thus suggesting the need for enhancing the reprogramming procedure itself, both quantitatively and qualitatively.

Since somatic cells could be reprogrammed by transferring their nuclear contents into oocytes [22], we examined the hypothesis that oocyte factors could direct the reprogramming of somatic cells into pluripotent cells. In this study, we tested the effect of ovarian extract on the pluripotency and proliferation of somatic mesenchymal stromal cells (MSCs).

2 Materials and Methods

Animals: Female Swiss albino mice (4-5 weeks old) were obtained from the National Research Center (Cairo, Egypt) and maintained in the animal facility at Zewail City of Science and Technology, according to a protocol approved by the Animal Ethics Committee. The mice were kept at a temperature of 24ºC in a 12-hour light/dark cycle facility. Animals were fed *ad libitum* in pathogen-free cages.

Isolation of murine bone marrow MSCs: Marrow MSCs were isolated from 4-5 weeks old female Swiss albino mice by dissecting the femur and tibia and flushing the dissected bones with DMEM (Lonza, USA) supplemented with 10% fetal bovine serum (Life Technologies, USA) to collect the bone marrow cells. The cells were prepared into single cell suspension, and the suspension was passed through a 100µm cell strainer (Greiner, Germany) to remove cell debris or bone particles. After centrifugation at 200 g for 10 min, the pellet was re-suspended in DMEM supplemented with 10% FBS and 1% penicillin/streptomycin (Life Technologies, USA) containing 1 g/L of glucose. The cells were plated in culture dishes (25 or 75 cm2) under standard conditions (5% CO2 and 37°C). After the adherent cells reached 70-80% confluency, they were passaged with 0.25% trypsin (Sigma-Aldrich, USA) and re-suspended in supplemented DMEM. In this study, we used BM-MSCs from passages 3 or 4.

2.1 Characterization of murine BM-MSCs

Differentiation capacity of BM-MSCs into adipogenic and osteogenic lineages: Briefly, MSCs were seeded into 6-well plates (Griener Bio-one, USA) and cultured for 21 days, in either adipogenic differentiation medium, or osteogenic differentiation medium. The former consists of DMEM (Lonza, USA), 10% FBS, 1% penicillin/streptomycin (Life Technologies, USA),1 µM Dexamethasone, 5 µg/ml Bovine insulin, 50 µM Indomethacin and 0.5µM 3-Isobutyl-1-methylxanthine(Sigma-Aldrich, USA), and the latter of DMEM (Lonza, USA), 10% FBS, 1% penicillin/streptomycin (Life Technologies, USA), 10 mMβ-glycerol phosphate, 50 ug/ml L-ascorbic acid and 100 nM Dexamethasone (Sigma-Aldrich, USA). After 21 days, Oil Red O (Sigma-Aldrich, USA) staining was performed to visualize lipid droplets and Alizarin Red (Sigma-Aldrich, USA) staining was performed to assay extracellular mineralization as previously described [23]. All images were taken using Leica DMi8 inverted microscope (Leica Microsystems, Germany).

Phenotypic characterization of BM-MSCs: BM–MSCs were characterized by analysis of phenotypic markers using flow cytometry. BM-MSCs from passage 4 were trypsinized using 0.25% trypsin/EDTA (Life Technologies, USA) and re-suspended in a blocking solution (PBS containing 1% FBS (Life Technologies, USA) for 10 minutes to prevent non-specific binding. After centrifugation at 200 g for 5 minutes, cells were re-suspended in the blocking solution and were stained with FITC anti-CD90, PE anti-CD29 and APC anti-CD45 (Becton Dickinson, USA) for 30 min at 4°C in the dark. Cells were then centrifuged for 2000 rpm for 10 min. to remove any non-bound antibodies. Cells were analyzed using a FACS-Calibur (Becton Dickinson, USA) following standard procedures and analyzed using CellQuest Pro Software (Becton Dickinson, USA).

Superovulation and oocyte retrieval: Female Swiss albino mice were induced to super-ovulate as previously described [24]. Briefly, mice were injected intraperitoneally with 30 IU of follicle stimulating hormone (FSH, Sigma-Aldrich, USA) and 50 IU of human chorionic gonadotrophin (hCG, Sigma-Aldrich, USA) 48 hours later. Oocytes were recovered 24 hours post-hCG injections. Ovaries and oviducts were dissected and the oviducts were flushed with 0.2 ml of DMEM/F12 containing 10% FBS. The collected oocytes were counted and cryopreserved at -80ºC until further use.

Ovarian extract and BM-MSCs Co-culture: The ovarian cytoplasmic factors extract was prepared by several rapid-freezing/thawing cycles of the oocytes. The oocytes were re-suspended by pipetting up and down in 2ml PBS. The sample was dropped at -80ºC to freeze (30 minutes). The sample was thawed in the water bath at 37ºC (10 minutes) followed by vortexing for 2 minutes. The rapid freezing and thawing was done three times. The sample was passed through a 40 µm cell strainer (Greiner, Germany) to remove any cell debris or crystals. Purified 1×10^5BM-MSCs, cultured (as described above) in 6-well plates were washed twice with 2 ml PBS and then 2 ml of the ovarian extract was added to the BM-MSCs and incubated for 7 days under standard conditions (5% CO2 and 37°C) until further analysis.

MTT assay: The proliferation of the co-cultured BM-MSCs was assessed using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay (Life Technologies, USA) as previously described. Briefly, after 7 days of co-culture with the ovarian extract, MTT (5 mg/ml) was added to each well of BM-MSCs and incubated in a humidified 5% CO_2 incubator at 37°C for 3 hours. In parallel, MTT was also added to BM-MSCs cultured alone with DMEM supplemented with 10% FBS and 1% penicillin/streptomycin. Formazan salts were dissolved with DMSO for 15 minutes and the optical density was measured at 570 nm with reference to 630 nm by using a

FLUOstar Omega-microplate reader (BMG Labtech, NC). The co-culture experiment was done in triplicates.

Table 1: Sequence of the used primers

Gene name	Primer Sequence
Mouse β-actin Forward	5' – TGACAGGATGCAGAAGGAGA – 3'
Mouse β-actin Reverse	5' – CTGGAAGGTGGACAGTGAGG – 3'
Mouse EpCam Forward	5' – GAGTCCCTGTTCCATTCTT – 3'
Mouse EpCam Reverse	5' – TCTCCTTTATCTCAGCCTTC – 3'
Mouse Zeb1 Forward	5' – CCATACGAATGCCCGAACT – 3'
Mouse Zeb1 Reverse	5' – ACAACGGCTTGCACCACA – 3'
Mouse Oct-4 Forward	5' – TAGGTGAGCCGTCTTTCCAC – 3'
Mouse Oct-4 Reverse	5' – GCTTAGCCAGGTTCGAGGAT – 3'
Mouse Nanog Forward	5' – TTGCTTACAAGGGTCTGCTACT – 3'
Mouse Nanog Reverse	5' – ACTGGTAGAAGAATCAGGGCT – 3'
Mouse Rex-1 Forward	5' – TGACAAAGGGGACGAAGCAAGAG – 3'
Mouse Rex-1 Reverse	5' – GCCATCAAAAGGACACACAAAG – 3'

Analysis of Oct-4 expression: Oct-4 (POU5F1) is critically involved in self-renewal and pluripotency of stem cells. After 7 days of co-culture with ovarian extract, ~1×10^6 BM-MSCs were harvested with 0.25% trypsin/EDTA (Life Technologies, USA) and fixed with 4% paraformaldehyde for 10 minutes. After centrifugation to remove the fixation buffer, cells were permeabilized by 0.1% saponin for 15 minutes. Cells were then re-suspended in PBS containing 1% BSA and incubated with FITC-conjugated anti-Oct-4mAb (R&D Systems, USA) for 60 minutes at 4°C in the dark. Cells were acquired using FACS-Calibur (Becton Dickinson, USA).

Confocal fluorescence microscopy immunostaining: To determine the expression of pluripotency markers, BM-MSCs were seeded after co-culture with ovarian extract on glass slides pre-coated with Poly-D-lysine (Sigma-Aldrich, USA). They were fixed in 4% paraformaldehyde, permeabilized with 0.1% Triton X-100, and blocked with 1% BSA. Cells were then stained with monoclonal antibodies to Oct-4 (Cell Signaling Technology, USA) and NANOG (Bioss antibodies, USA). Cells were labeled with the appropriate Alexa Fluor® secondary antibodies (Molecular Probes, USA) and counterstained with Hoechst 33342 (Molecular Probes, USA) to visualize the cell nucleus. Cells were imaged under a 63X objective with a Nikon A1R inverted laser scanning confocal microscope (Nikon microsystems, France).

Total RNA extraction and quantitative real-time PCR: RNA was extracted from both co-cultured oocyte and BM-MSCs by using PureLink® RNA Mini Kit (Thermo Fisher Scientific, USA), according to the manufacturer's instructions. RNA quality and quantity were checked with a NanoDrop ND-1000 spectrophotometer. The total RNA was quantified by optical density and the quality was evaluated by gel electrophoresis. Intact rRNA subunit of 28S and 18S were observed on the gel indicating minimal degradation of the RNA. RNA was converted to cDNA using High-Capacity cDNA Reverse Transcription Kit (Thermo Fisher Scientific, USA) and RNase inhibitor (Thermo Fisher Scientific, USA) according to the manufacturer's instructions. Synthesized cDNA corresponding to 100 ng total RNA was used for real time PCR. Mouse B-Actin was used as a normalizer for qPCR reaction. The primers used are purchased from Invitogen, USA.

qPCR reactions took place in 0.1 in a QuantStudio 12K Flex Real-Time PCR System (Applied Biosystem, USA), using SYBR Premix Ex TaqII Tli RnaseH Plus (Takara Bio Inc, Jaban), according to the manufacturer's instructions. For each 25 µl SYBR Green PCR reaction, 1 µl cDNA, 0.5 µl Forward primer (10 pmole), 0.5 µl Reverse primer (10pmole), 12:5 µl SYBR Green PCR Master Mix, and 11 µl nuclease free water were mixed together. The cycling conditions were 1 cycle of denaturation at 95°C/30 sec., followed by 40 three-segment cycles of amplification (95°C/5 sec, 59°C–55°C (gene depending, see table 1)/5 sec., 72°C/20 sec.) where the fluorescence was automatically measured during PCR and one three-segment cycle of product melting (95°C/15 sec., 59°C/1 min., 95°C/15 sec). Expression of Sox2, ZEB1 and EpCam genes was corrected with the constitutively expressed reference Actin gene using specific primers for all studied genes reported in table 1. The baseline adjustment method of the QuantStudio 12K Flex Real-Time PCR System (Applied Biosystem, USA) software was used to determine the Ct in each reaction. A melting curve was constructed for each primer pair to verify the presence of one gene-specific peak and the absence of primer dimer. All samples were amplified in triplicates and the mean was used for further analysis. Each PCR reaction also included a non-template negative control to check for primer-dimer.

Statistical analysis: All results are presented as mean ± SEM. Comparisons between groups were analyzed by use of Student *t* test.

3 Result and Discussion

BM-MSCs isolation and culture: Adherent cells were observed on the bottom of the flask 2 days after isolation. BM-MSCs proliferated in discoid, flat, triangular, and spindle shapes when examined by phase contrast microscopy. The BM-MSCs were uniformly distributed after passaging at day 0 (Fig.1-a), passage 2 at day 6 (Fig1-b), and confluency at passage 3 after 2 weeks (Fig1-c).

***In Vitro* Differentiation:** MSCs were cultured with adipogenic and osteogenic differentiation factors as mentioned in the Materials section. After incubation under adipogenic culture conditions for 3 weeks, they differentiated into adipocytes as determined by Oil Red O staining. Lipid droplets were observed after about 14 days of culture (Fig 2-a). MSCs cultured in the presence of osteogenic induction medium showed the presence of calcium deposits, and were positive for Alizarin Red

histochemical staining (Fig 2-b).

Surface marker characterization of BM-MSCs: Cell surface antigen phenotyping was performed by flow cytometry analysis to characterize the BM-MSCs. Our

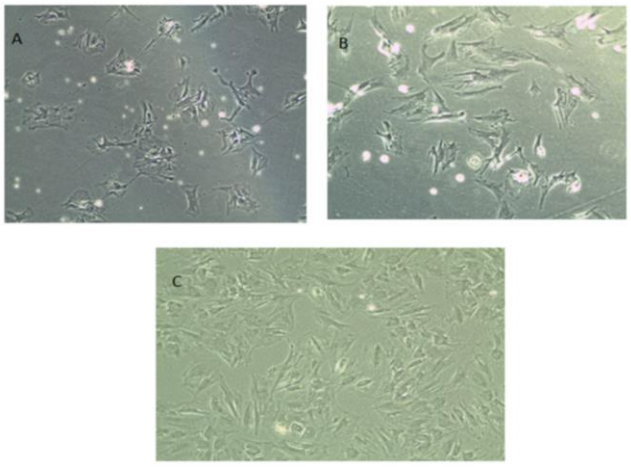

Figure 1: Phase contrast images of BM-MSCs. (A) passage 0 (B) passage 2, and 3 (C) at confluence. The cells exhibited an elongated spindle shape fibroblast-like morphology, and net and mesh formation (C). Scale bar, 100 µm. Magnification 10x.

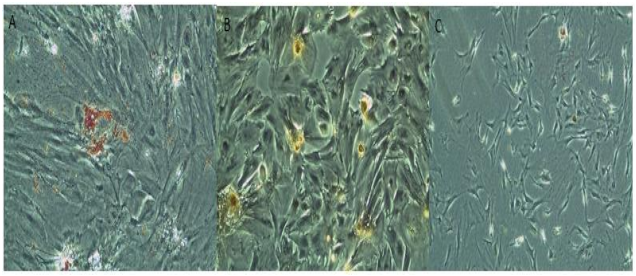

Figure 2: Phase contrast images of BM-MSCs differentiated into (A) adipocytes by oil red O staining. (B) Osteocytes by alizarin red staining (C) Control. Scale bar, 100 µm. Magnification 10x.

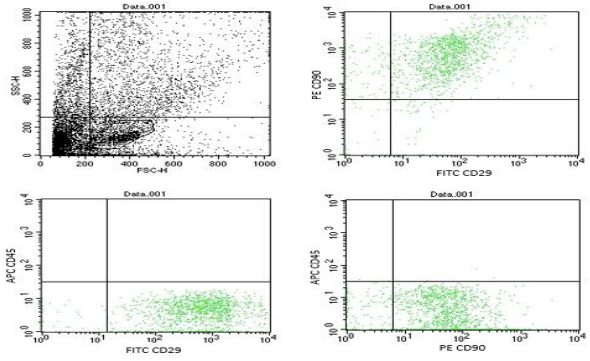

Figure 3: Flow cytometry characterization of BM-MSCs: CD45 leukocyte marker was weakly expressed, while the BM-MSC-specific markers, CD29 and CD90, were strongly expressed.

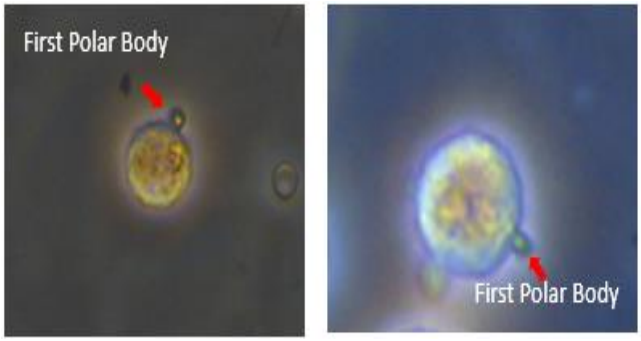

Figure 4: Phase contrast images of mouse oocytes. The red arrows indicate the polar body.

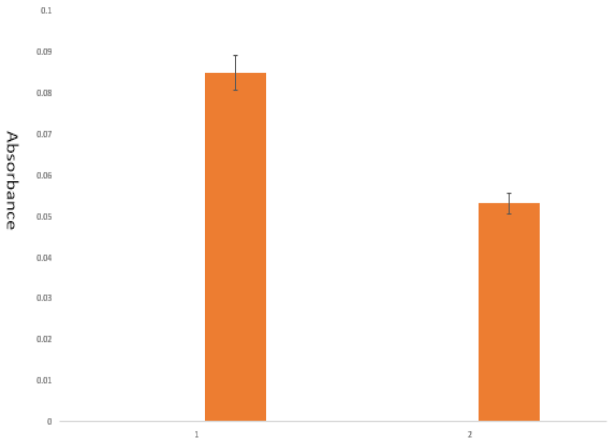

Figure 5: MTT assay evaluates the proliferation rate of BM-MSCs co-cultured with ovarian extract. Formosan absorbance is expressed as a measure of cell viability (1) BM-MSCs after co-culture with ovarian extract, and (2) BM-MSCs cultured alone. MTT (5 mg/ml) after 7 days of co-culture [*$p < 0.05$].

results demonstrate that ≥ 97% of the cells were positive for CD29 and CD90 surface markers, and were negative for CD45 leukocyte marker (Fig. 3).

Isolation of oocytes by superovulation: After superovulation following the protocol mentioned above, a total of ~10 oocytes per animal were isolated. Typical images of metaphase II (MII) oocytes are shown in Fig 4. The 1st polar body is clearly visible in MII oocytes derived using superovulation. Since our oocyte population contained contaminant cells from the ovarian tissue, the "extract" refers to the ovarian extract rather than the oocyte extract.

Ovarian extract increases BM-MSCs viability and proliferation: To examine the effect of the ovarian extract on BM-MSCs viability and proliferation, we used MTT assay after co-culture of the ovarian extract with BM-MSCs for 7 days. Phase contrast images (Fig. 5-a) and MTT results (Fig. 5-b) show that MSCs co-cultured with ovarian extract showed significant increase in proliferation compared to MSCs cultured alone. Viability of MSCs measured by Formosan absorbance was also superior when co-cultured

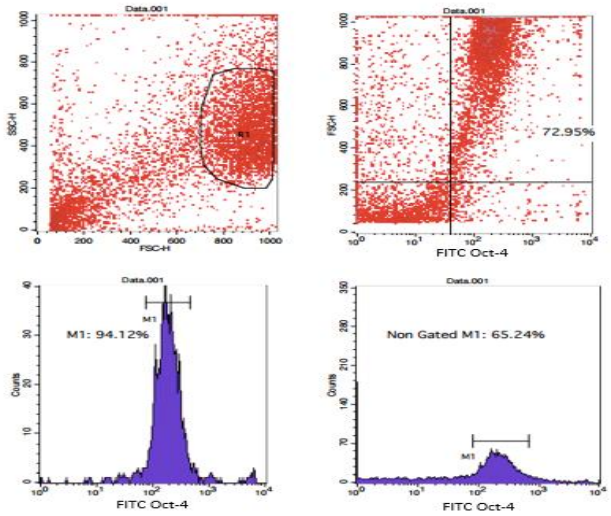

Figure 6: Flow cytometry analysis showing that 65. 24% of BM-MSCs co-cultured with oocytes the cells showed positive expression of Oct-4 surface marker.

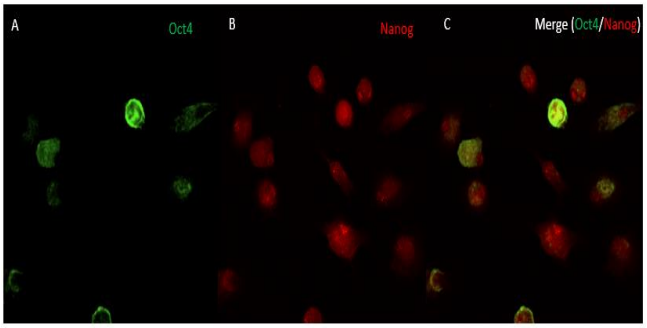

Figure 7: Confocal microscopy: BM- MSCs co-cultured with ovarian extract were immunostained with (A) Oct-4 (FITC), and (B) NANOG (TRITC) as described in the Methods section. Shown is a single optical section of confocal z-stack series for (A) Oct-4 (B) NANOG and (C) Merged (original magnification ×63).

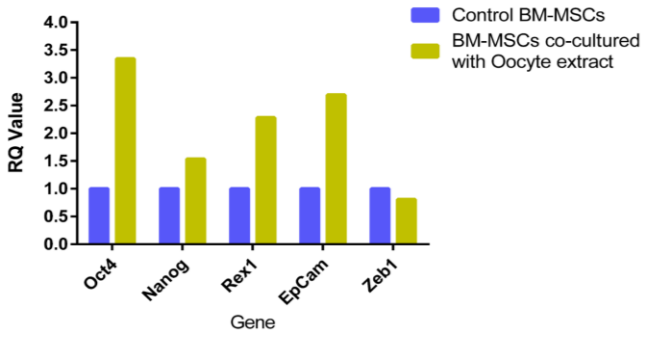

Figure 8: mRNA expression levels in BM-MSCs after co-culture with ovarian extract: qPCR analysis shows increase in pluripotency markers, Oct-4, NANOG, and Rex1, increase in the epithelial marker EpCam, and decrease in the mesenchymal marker ZEB1.

with ovarian extract compared to MSCs alone (Figure 5-b, *$p < 0.05$).

Ovarian extract induces expression of pluripotency markers: After co-culture with ovarian extract for 7 days, BM-MSCs were harvested by trypsinization, and Oct-4 expression was assessed by FACS analysis. BM-MSCs lack expression of Oct-4, however, after co-culture with ovarian extract, 65.24% of total MSCs cells were positive to Oct-4 expression by flow cytometry analysis (Fig. 6). We confirmed expression of Oct-4 by confocal microscopy (Fig. 7-a). We further determined expression of NANOG, another pluripotency marker using immunostaining. Figure 7-b shows expression of NANOG alone, while fig. 7-c shows co-expression of NANOG and Oct-4.

Levels of mRNA expression in BM-MSCs after co-culture with ovarian extract: Initially, the relative RNA expression level of 6 different genes was determined by a quantitative real-time RT-PCR procedure and compared between the BM-MSCs alone, and BM-MSCs with ovarian extract. Results were normalized to the level of expression in BM-MSCs using β-actin as a reference housekeeping gene. Significant up-regulation of Oct-4, NANOG and Rex1 genes was observed observed in MSCs cultured with ovarian extract compared to those cultured alone. As high as 3 fold up-regulation of the epithelial marker EpCam was also observed in the co-cultured MSCs, while the mesenchyamal ZEB1 marker was downregulated (Fig. 8). Oct-4 expression was confirmed using Flow cytometry and confocal immunofluorescence imaging. The four genes (EpCam, NANOG, Oct-4 and Rex1) showed a significant differential expression level in MSCs co-cultured with ovarian extracts compared to those cultured alone (Fig. 8).

4 Discussion

In this study, we examined the effect of ovarian factors on marrow MSCs. Bone marrow MSCs were first confirmed by plastic adherence and multilineage differentiation potential. Our cell preparation was highly pure as more than 97% of cells stained positive for CD29 and CD90 MSC surface markers, while lacked the expression of CD45 leukocyte marker. In addition, they differentiated into both adipogenic and osteogenic lineages. We have successfully identified metaphase II oocytes by super-ovulation followed by oocyte retrieval. However, we could not confirm the absence of contaminating ovarian cells. We thus concluded that our "oocyte" factors may include contaminating ovarian tissues, and thus are referred to through this manuscript as "ovarian" factors.

Oct-4 as it is considered one of the essential transcription regulators for maintaining pluripotency. In somatic cells, Oct-4 is inactive. MSCs co-cultured with ovarian factors showed a highly significant expression of Oct-4 (65%). In specific gated population, Oct-4 expression was as high as 94 % (Histogram 2, and Histogram 1), Figure 6. These data indicate that Oct-4 expression has been induced as an indication of the reprogramming abilities of the oocyte extract. This is in accordance with the work of Hansis et al. who showed that Xenopus egg extract was able to up-

regulate pluripotency markers such as Oct-4 [26]. Despite that fact that Oct-4 is considered the hallmark of pluripotency, other pluripotency markers that work in concert include NANOG and Rex 1. Our data also showed high expression of NANOG, Oct-4, and Rex1 in MSCs co-cultured with ovarian extract. In a previous study by Ganier et al., in 2011, incubation of mouse embryonic fibroblasts (MEF) with *Xenopus* egg extract could induce their reprogramming into pluripotent cells. After treatment of MEF with M-phase egg extracts, expression of Oct-4, Rex1, alkaline phosphatase and NANOG was detected by quantitative RT-PCR. A remarkable increase in cell proliferation rate was observed as well [27].

In another study by Mahapatra et al. in 2014, the group hypothesized that the egg extract of chicken can reprogram buffalo fetal fibroblasts into a less differentiated state, based on the claim that a less differentiated cell is easier to reprogram than a mature somatic cell [28]. iPSCs-like colonies appeared after co-culture with the egg extract. With the increase of the egg extract concentration, the reprogramming efficiency increased. The resulting cells were positive for the embryonic stem cell markers: alkaline phosphatase, TRA-1-60, TRA-1-81, OCT-4, NANOG, SOX2 and FoxD3. This indicated that the egg extract contains certain factors that promote reprogramming [28]. Oocyte extract of lower species can reprogram the differentiated cells of genetically unrelated higher species as observed in a study by Zhu et al., in 2009. Mouse fibroblasts underwent a reactivation of Oct-4 and NANOG upon exposure to fish-egg extract and they were able to differentiate into various types of cells [29]. In this study, expression of c-Myc and Klf4 was down-regulated in buffalo fetal fibroblasts.

Although c-Myc enhances the expression and maintenance of pluripotency factors by histone acetylation in multiple regions of the mammalian genome [30], it is not a necessary factor for reprogramming [31]. This indicates that up-regulation of Oct-4, Sox2 and NANOG expression in the reprogrammed fibroblasts might have been the result of factors present in the egg extract [28].

In our study, expression of Oct-4 and NANOG in somatic MSCs is the first report of possible reprogramming of adult somatic cells using co-culture with ovarian extracts. Reprogramming induced by oocyte extract was reported by Alex J. Rathbone et al. who showed that several factors in the oocyte extract, including transcription factors, transcription regulators and chromatin remodeling complexes interact together in complicated pathways. The group identified a total of 69 proteins which expression was modified after treatment with oocyte extract [32]. In another study by Biase et al. in 2014, the content of mRNAs expressed in metaphase II oocytes was found to influence the activation of the embryonic genome and enable further development to the blastocyst stage [33]. Another group has reported that bovine oocyte extract contains factors that improved epigenetic reprogramming of yak fibroblasts and enhanced cloned embryo development [34]. However, the molecular mechanisms and the complete proteome of these factors are still not fully understood.

During somatic cell reprogramming, mesenchymal-epithelial transition (MET), the reverse process of epithelial-to-mesenchymal transition (EMT), is a crucial step towards pluripotency. Mouse fibroblasts must undergo MET to successfully begin the initiation phase of reprogramming [35]. Epithelial-associated genes such as the epithelial cell adhesion molecule (EpCAM) is a type I transmembrane protein expressed in the majority of normal epithelial tissues, and is normally upregulated before NANOG and Oct-4 expression. Herein, we showed that the expression of EpCAM was upregulated by 3 folds in BM-MSCs co-cultured with ovarian extract. These data are in accordance with new discoveries in EMT research demonstrated that EMT induces stem-like properties to epithelial cells [36].

Identifying the oocyte factors underlying the reprogramming process may overcome many of the ethical and practical issues associated with somatic nuclear transfer. In comparison to induced pluripotency, introducing specific oocyte factors into differentiated cells may not confer genetic stress on the cells, as this process would mimic the natural reprogramming process during fertilization.

In summary, in this report, we provide evidence that specific factors in ovarian extract promote viability, proliferation and expression of Oct-4 and NANOG pluripotency factors, in somatic BM-MSCs. Further experiments are required to confirm the expression of these pluripotency factors at the molecular level, and to ensure that these factors are derived from pure oocyte extract. Further characterization of these factors may provide new insight into the process of reprogramming and offer new safer methods for induced pluripotency.

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Disclosure

The authors report no conflicts of interest related to this work

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