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AN *IN VIVO* STUDY OF THE IMPACT OF 4MT STATIC MAGNETIC FIELD TO MODIFY THE DIFFERENTIATION RATE OF RAT BONE MARROW STEM CELLS INTO PRIMORDIAL GERM CELLS

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ABSTRACT

Primordial germ cell (PGC) transplantation has become an assay to estimate the potential of germ cell development and a tool to determine whether the somatic environment or the germ cells are responsible for disturbed fertility of some transgenic animals. This investigation was performed to evaluate the differentiation capacity and the function of differentiated Bone Marrow Stem Cells (BMSCs) into PGCs using Static Magnetic Field (SMF) (4mT) and Bone Morphogenetic Protein 4 (BMP-4) (25 ng/ml) after transplantation to azoospermia rat. Suspension of the differentiated BMSCs was injected to the left testis of each of the recipient rat in three groups including: (i) control group (no SMF, no BMP-4), (ii) BMP-4 group (BMP-4 for 96 h, no SMF) and (iii) BMP-4 + SMF group (BMP-4 for 96 h and SMF for 48 h). The right testis in transplanted rat was considered as an internal control. The evaluated parameters were testicular mass weight, number of sperm in epididymis ducts and histological evaluation of the recipient's testes. A significant difference ($P \leq 0.05$) in the left testis mass and number of sperm and germ cells (spermatogonia, spermatocyte and spermatid) in epididymis ducts was observed after transplantation of cells in BMP4 and BMP4 + SMF groups in comparison to non-transplanted groups (right testis). For the first times in animal model, our results showed that in a synergistic manner, the combination of SMF with BMP4 exaggerates the differentiation potential of BMSCs to PGCs.

Keywords: Bone Marrow Stem Cell, Bone Morphogenetic Protein 4, Differentiation, Primordial Germ Cell, Static Magnetic Field

INTRODUCTION

Natural magnetic fields (MFs) vary over the earth's surface based on the geographic longitude and latitude of the locations between 0.035 and 0.07 mT. This natural MF is sensed by certain animals that use it for orientation (1). The interest in the variation biological effects of non-ionizing electromagnetic fields (EMFs) on the whole organism, as well as on cellular systems, has considerably increased in recent years in consideration of their probable health risk for humans (2). Several studies have shown that MF has an influence on a large variety of cellular functions; nevertheless its exact mechanism(s) is not still clear (3). There are many studies on the effects of static magnetic fields (SMFs) on living cells and tissues. Most of these studies dealt with the potentially genotoxic or oncogenic effects especially those that have a relationship with clinical applications of SMF. Some of them indicated that SMF play a critical role in activating and/or alternating the molecular mechanisms in eukaryotic cells (4-6). Bekhite et al. demonstrated that cardiomyogenesis of Flk-1^{+} cardiac progenitor cells derived from mouse embryonic stem cells (ESs) can be enhanced by application of static MFs (0.3 to 5mT). Moreover, the relationship between MF-mediated intracellular ROS

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generation and [Ca^{2+}] as second messengers in signal pathways leading to cardiomyocytes differentiation is not clear (1). Another study investigated the effects of MFs of 50 Hz and 1.1 mT on the differentiation and intracellular free calcium of bone marrow mesenchymal stem cells (7). They showed that MFs enhanced the cellular differentiation and the intracellular free Ca^{2+} concentration in MSCs. Based on these reports it seems that the primary site of action of magnetic fields is the plasma membrane (8, 9). The influence exerted by MFs (static or oscillating) on the plasma membrane has been described at different levels on: the plasma membrane surface (10), the distribution of membrane proteins and membrane receptors (11), cell-cell and cell-matrix junctions (12), cell membrane sugar residues (13, 14) and the trans-membrane fluxes of different ions, especially calcium (15). These perturbations were demonstrated through alteration in apoptotic rate, cellular and cytoskeleton shape (5, 14).

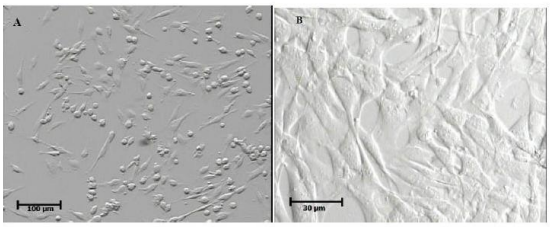

Fig. 1. Primary cell culture of bone marrow mesenchymal stem cells: A, the population of heterogeneous of cells after 4 h culture; B, a homogeneous population cells after a four-passage culture.

The common procedure in molecular research is to investigate the obtained results from different in vitro studies into the upper level of animal models. These complementary studies involving germ cell differentiation helps researchers to establish protocols for *in vitro* stem cell differentiation into the germ cell lineage which could be an alternative treatment for male infertility. Cell proliferation, survival, and differentiation in primordial germ cells (PGCs) are dependent on bone morphogenetic proteins (BMPs) -4, a member of the TGFb super family, secreted by extra embryonic tissue (16). PGC transplantation offers interesting strategies for research on germ cells. It has become an assay to estimate the potential of germ cell development and a tool to determine whether the somatic environment or the germ cells are responsible for disturbed fertility of some transgenic animals. Germ cell transplantation may also have important implications for the preservation of valuable animals, that is, rare animals and important livestock (17).

A common point within all the above-mentioned reports from the point of applied procedure was that all were carried out in vitro at cellular level. In fact no studies have been done on the effects of SMF on differentiation rate of bone marrow stem cells (BMSCs) into PGCs *in vivo*. As an advantage for *in vivo* studies it can be stated that any obtained results could be a proof or confirmation for the obtained results from *in vitro* studies. For the first time we aimed to evaluate the function of differentiated BMSCs into PGCs using SMF (4mT) and BMP-4 (25 ng/ml) after transplantation to azoospermia rat as an animal model.

MATERIALS AND METHODS

Isolation, culture and treatment of BMSCs

BMSCs were isolated from bone marrow, even though recent reports indicate that BMSC can be isolated from other sources, such as peripheral blood (18), fat (19), skin (20), vasculature (21), and muscle (22). BMSCs were isolated as described by Woodbury (23). Eight to ten week-old rats were killed and tibias and femurs were dissected and placed in PBS on ice. Under sterile conditions by cutting two ends of the tibias and using a syring filled with 10 ml αMEM (Invitrogen LT, Merelbeke, Belgium) marrow was flushed out. Cells were seeded in αMEM supplemented with 20% fetal bovine serum (FBS, Invitrogen LT, Merelbeke, Belgium), 100 U/ml penicillin (Gibco, Germany), 100/ml streptomycin (Gibco, Germany) in a 75 cm^2 flask and incubated at 37°C with 5% humidified. After 24h, non-adherent cells were discarded and adherent cells were thoroughly washed twice with phosphate buffered saline (PBS). BMSCs were grown until 70–80% confluent. Confluent BMSCs were passaged and plated at 1:2 or 1:3 dilutions every 5–6 days using 0.25% trypsin and 1mM EDTA (Invitrogen LT, Merelbeke, Belgium) medium was changed every other day. MSCs were purified based on their adherent properties from other cells. Very small embryonic-like (VSEL) stem cells were present in the specimens extracted from bone marrow. Ratajczak and Zuba-Surma in 2008 purified rare, small CXC chemokine receptor 4 expressing ($CXCR4^+$) stem cells from the murine bone marrow that express markers

characteristic for embryonic stem cells, epiblast stem cells, and primordial germ cells. They named these primitive cells very small embryonic-like (VSEL) stem cells (24, 25). Flow cytometry assay was performed to make sure that the isolated cells were free of any contamination of VSELs using CD90 and CD29 markers according to Chemicon protocol(26). Spindle-shaped cells were used from fourth passages for both physical (SMF) and chemical (BMP-4) treatments (Figure 1). The cells were frozen in αMEM with 93% FBS and 7% DMSO (MERCK, Darmstadt, Germany) in liquid nitrogen (Marandi et al. 2007). After four passages when the density of cultured BMSC was approximately 5 x 10^4 cells/cm^2, BMSC were ready to treat with BMP4 (23). Bone Morphogenic Protein (BMP)-4 (25 ng/ml; Chemicon, USA) was added to the fourth-passage BMSCs daily for 96 h based on the method described by Mazaheri et al. (27).

Magnetic field application

Exposure to MF was performed using a locally designed SMF generator (Figure 2). The electrical

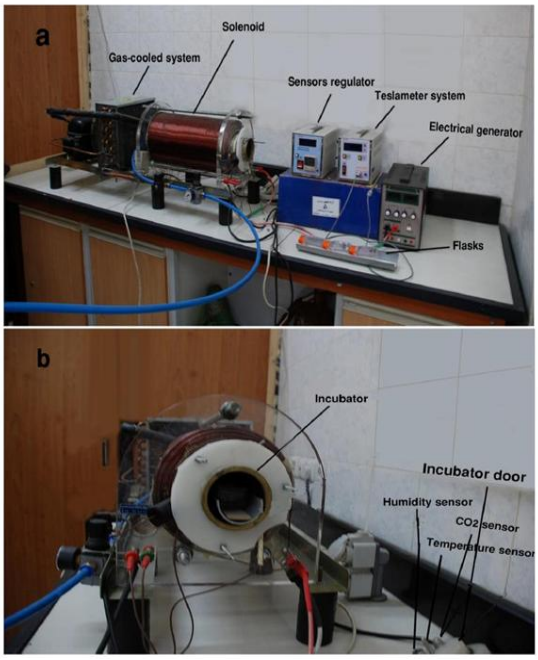

Fig.. 2 Photograph showing the apparatus used to generate static magnetic field (a) the whole body of the system (b) the incubator within solenoid.

power was provided using a power supply working in range of 0-50 V and 0-20 A with a maximum power of 1 kW. This system consisted of a 40 cm-long solenoid (1800 loops of 2.5 mm coated copper wire) equipped with an included incubator inside the solenoid (a copper container with 40 cm length, 8 cm diameter). Using three different sensors the controller system was able to control the temperature, humidity and CO_2 level. Heat was efficiently removed by a gas-cooled system using tetrafluoroethane. The circulation system consisted of a condenser, refrigerator engine and heat-exchanging pipe network of copper with 8 mm thickness which turned around both inner and outer sides of solenoid. This system designed to generate SMF in range of 0.5 µT to 90 mT with stable conditions. A stabilizer board was used to stabilize the system so that a uniform SMF inside the exposure unit was generated. Calibration of the system as well as tests for the accuracy and uniformity of the MFs were performed by a teslameter (13610.93, PHYWE, Gottingen, Germany) with a probe type of Hall Effect. A Hall Effect sensor is a transducer that varies its output voltage in response to a magnetic field. The accuracy of the teslameter was ± 0.1 % for MF and the range of measurements was 3 µT–30 mT. Presence of any pulsation in the efferent current was tested by an oscilloscope (40 MHz, model 8040, Leader, Japan). The predefined SMF of 4mT was generated by passing a 1 Ampere DC current according to calibration data. Temperature was routinely checked before and after all fields and control exposures. We evaluated the influence of different intensity of static magnetic field (4, 7 and 15mT) on the viability and proliferation rate of rat bone marrow stem cells. Increasing of intensity of static magnetic field-exposure decreased the viability and proliferation in treated groups compared with the corresponding control. With respect to this research, a 4mT static magnetic field was the optimized intensity (26). Three flasks were placed at the center of the incubator (10 cm distance from the center in each side) within solenoid generating a homogenous magnetic field, in each exposure. The duration of exposures was 48h. Before each exposure, the MF intensity was set to appropriate intensity (4mT) using a teslameter (26, 28-30). The value of geomagnetic field in our lab was 47 µM based on the measurement performed by Tehran geomagnetic observatory, institute of geophysics, university of Tehran.

Animals

Male adult Vistar rat (aged=4-6 weeks old; n=30),derived from the original stocks obtained from Razi Laboratory (Tehran, Iran), were maintained

under the standard conditions with free access to food and water at the Animal Facility of Tarbiat Modares University. To satisfy the moral concerns all experiments were conducted in accordance with the guidelines of the National Research Council (affiliated to the Tarbiat Modares University).

Transplantation technique

To destroy endogenous spermatogenesis, Busulfan (Sigma-Aldrich, Deisenhofen, Germany) wasadministered by intraperitoneal injection to rat at a dose of 35 to 40 mg/kg at least 4 weeks before transplantation. The compound was first dissolved in dimethyl sulfoxide (DMSO) and then an equal volume of sterile distilled water added to provide a final concentration of 35 to 40 mg/ml. The limit of solubility for busulfan is near this concentration, and precipitation is delayed by mixing the DMSO and water phase just before use and maintaining the solution at 35 to 40°C(31). Following busulfan treatment, the males were maintained for at least 4 weeks before cell transplantation to allow endogenous germ cells to disappear from the tubule lumen. After 4 weeks, the testes were fixed in 10% formaldehyde for 48 h, dehydrated, and embedded in paraffin. The 5 µm-thick sections were then visualized by H&E (Hematoxylin and Eosin) staining and optical microscope (MoticAE31, China). DiI dye (Vybrant DiI cell-labeling solution™, USA) with concentration of 0.1 mM in the culture medium was used for staining of donor cells according to the manufacturer's protocol (Fig 3).

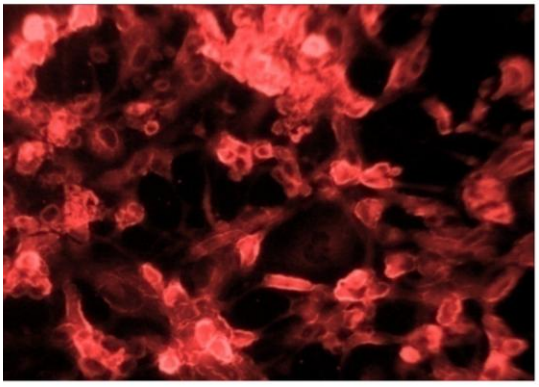

Fig.. 3 Differentiated cells that have been labeled whit 1 µM DiI dye before transplantation (Scale Bars: × 400).

The adult recipient rats were first anaesthetized using 10% ketamine and 2% xylazine (Alfasan International, Weorden, Netherlands). The differentiated cells (PGCs, 10^6 cells/100 µL αMEM for each testis) with 5 µL trypan blue as injection marker were transplanted into the rat seminiferous tubules through the efferent ducts (Fig. 4).

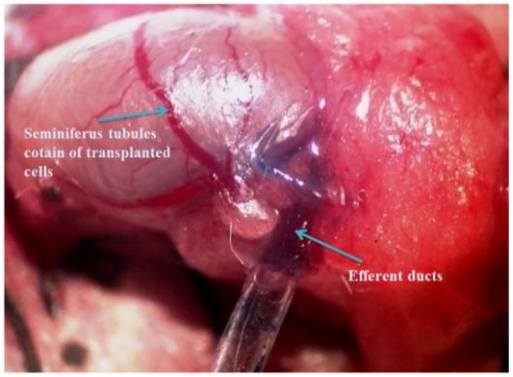

Fig.. 4 Transplantation of labeled cells into the azoospermia rat seminiferous tubules through the efferent ducts.

In this stage, to evaluate the effects of SMF (4 mT) on differentiation of BMSCs to PGC, suspension of the differentiated BMSCs was injected to the left testis of each of the recipient rat in three groups including: (i) control group (no SMF, no BMP-4), (ii) BMP-4 group (BMP-4 for 96 h, no SMF) and (iii) BMP-4 + SMF group (BMP-4 for 96 h and SMF for 48 h).The right testis in transplanted rat was considered as an internal control.

Efficiency of transplantation

Three parameters used to determine the efficiency of transplantation are as follows: testicular mass weight, number of sperm in epididymis ducts and histological evaluation of the recipient's testes. The transplanted testes of the recipient rats were examined 4 and 8 week after transplantation. The testes were weighted, fixed in 4% paraformaldehyde, dehydrated, and embedded in paraffin. The 5 µm-thick sections were then visualized under Olympus phase contrast microscope (BX51, Olympus, Tokyo, Japan) so that donor cells-derived spermatogenesis could be seen. To obtain the number of germ cells per volume (ml), the following formula was used:

$$Nv = \frac{K(N\overline{A})^{\frac{3}{2}}}{B(\overline{V}v)^{\frac{1}{2}}}$$

In this formula, K is constant coefficient and is equal to 1.1-1.2. B is the ratio of large diameter of cell to small diameter of cell. N_A is the average

number of cells per area and V_v is average volume density.

Statistical analysis

Different samples with same treatment were analyzed for the means and standard deviation (SD) calculations. One-way analysis of variance (Tukey test) was used to estimate the difference arose due to experimental conditions that were employed. The probability value which is regarded as statistically significant is $P<0.05$.

RESULTS

Differentiation of BMSCs to osteogenic and adipogenic lineages and characterization of mesenchymal stem cells were reported elsewhere (26). MSCs are positive for CD90 and CD29 markers, whereas very small embryonic-like (VSEL) stem cells are negative for these markers(24). Further information was reported in our previous published article (26). Analysis of the cell surface antigens CD90 and CD29 markers revealed that the isolated cells from bone marrow did not contain a significant number of contaminating VSEL stem cells.

Also, our other published article(32) was showed that increasing of the exposure time of the 4 mT SMF (24 and 48 h) and treatment time with 25 ng/ml BMP4 (48 and 96 h) caused a marked decrease in expression of pluripotency genes (Oct-4, Nanog and C-Myc) and Oct4 protein and increase in primordial germ cell-specific genes (Fragilis, Mvh and Stella) and Mvh protein compared with the control group *in*

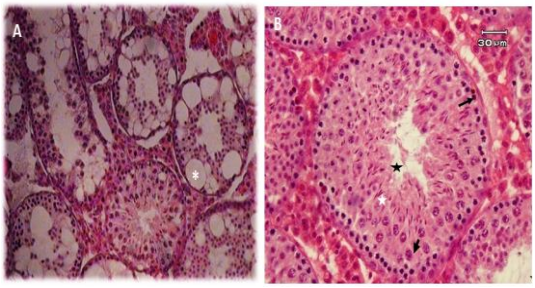

Fig.. 5 Histological evaluations of seminiferous tubules in male azoospermia (A) and normal rat (B).*; Spermatogonia cells disappear in the section after using 40 mg / kg busulfan. Arrowhead; spermatocyte cells, White star; spermatid cells, Blackstar; spermatozoa.H&E staining (Scale Bar A: × 400).

vitro. With respect to this research, exposed to 48 h SMF and treated with 96 h BMP4 were chosen as the optimized times (32).

Destruction of endogenous spermatogenesis

To destroy endogenous spermatogenesis, recipient rats were treated with busulfan for 4 weeks before transplantation. The 5 µm-thick sections were then visualized by H&E staining (Fig.5, A). This picture was compared with testis of normal rat (Fig. 5, B). Spermatogonia cells disappear in the section after using 40 mg / kg busulfan that showed endogenous spermatogenesis destroyed in testis.

Success of cell transplantation to azoospermia rat

Transplanted cells in seminiferous tubules of rat testis were assessed by introducing DiI-incorporated cells. To evaluate the success of transplantation of differentiated stem cells, tissue sections were prepared from testes at 4 and 8 weeks after cell transplantation in defined three groups. Results showed that transplanted cells presented on the basement membrane of the seminiferous tubules (Fig. 6 and 7).

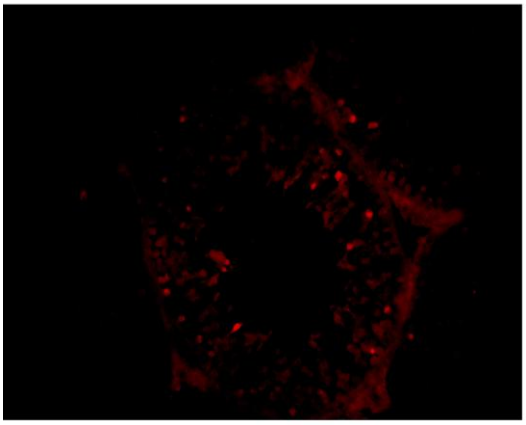

Fig.. 6 Cell homing evaluation after 4 weeks from transplantation to azoospermia rat. Labeled cells located on the basement membrane of the seminiferous tubules in left testis (Scale Bars: × 400).

Efficiency of transplantation

Testicular mass weight: Testicular mass weight was obtained during the 8^{th} weeks after transplantation. A significant increase ($P\leq0.05$) in the left testis mass was observed after transplantation of cells in BMP4 and BMP4 + SMF groups in comparison to non-transplanted groups (right testis). While, a significant decrease ($P\leq0.05$) in the left testis mass of control group was observed after transplantation of cells in comparison to BMP4 and BMP4 + SMF groups (Fig. 8).

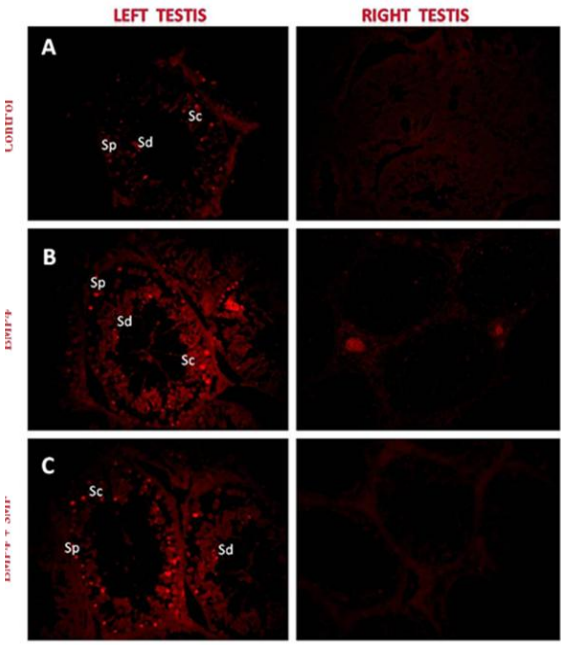

Fig.. 7 Assesment of spermatogenesis process development after 8 weeks transplantation. Sp; Spermatogonia cell, Sc; Spermatocyte, Sd; Spermatid cell (Scale Bars: × 400).

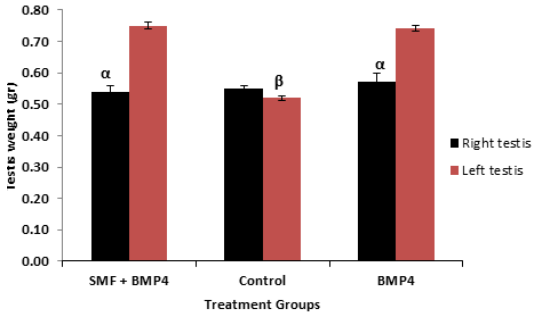

Fig.. 8 The mean testicular mass weight after cell transplantation in different experimental groups. Histogram shows mean values ± SD (n=3; P<0.05). α: significant difference with left testis in the same group. β: significant difference with left testis of other transplanted groups.

Number of sperm in epididymis ducts: Number of sperm in epididymis ducts was counted per volume during the 8th weeks after transplantation. Then these values were compared between transplanted groups. An increase was observed between number of sperm in epididymis ducts in right and left testis in all groups. This increase was significant (P≤0.05) in BMP4 and BMP4 + SMF groups. Also, results showed that there was an increase in number of sperm in epididymis ducts in left testes between BMP4 + SMF and BMP4 groups with control groups. But this increase was not a significant difference between BMP4 and BMP4 + SMF groups (Fig. 9).

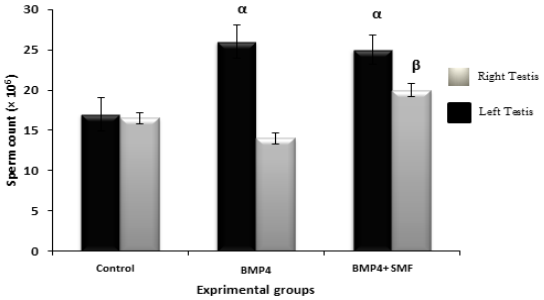

Fig.. 9 The mean number of sperms per each epididymis after cell transplantation in different experimental groups. Histograms show mean values ± SD (n=3; P<0.05). α: significant difference with right testis in the same group, β: significant difference with right testis of other transplanted groups.

Histological evaluation of the recipient testes: Histological studies were performed and number of germ cells (spermatogonia, spermatocyte and spermatid) in seminiferous tubules was counted in transplanted and non-transplanted testes during the 8th weeks after transplantation. A significant increase (P≤0.05) was observed between number of germ cells in seminiferous tubules of right and left testis in all groups. Results showed that there was a significant increase (P≤0.05) between BMP4 + SMF and BMP4 groups with control groups. Also, there was a significant increase (P≤0.05) between BMP4 + SMF and BMP4 groups (Fig. 10).

DISCUSSION

There is an ongoing intensive effort in the characterization of BMSCs to apply them in different clinical assessments due to its great potential usefulness (26). Efficient derivation of PGCs from different sources of stem cells *in vitro* and *in vivo* has been a subject of challenge in the treatment of male infertility (33). Previous reports suggested a plethora of chemical and physical stimulators for improving the biological condition of BMSCs. Hereby we report our investigation in which we evaluated the differentiation capacity and the function of differentiated BMSCs into PGCs using SMF (4mT) and BMP-4 (25 ng/ml) after transplantation to azoospermia rat.

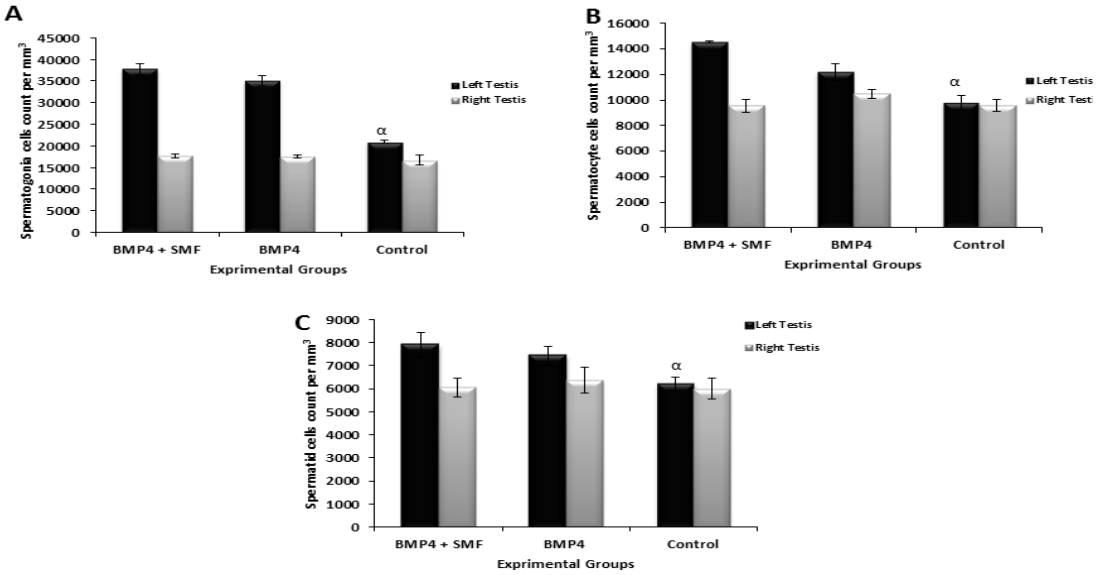

Fig.. 10 The mean number of Spermatogonia (A), Spermatocyte (B) and Spermatid (C) cells per volume (mm^3) in seminiferous tubules in different experimental groups. Histograms show mean values ± SD (n=3; P<0.05). α: significant difference with left testis of other transplanted groups.

Direct injection of donor cells into rodent seminiferous tubules is possible via the efferent ducts, which is feasible with mouth pipette and a stereomicroscope. We transplanted PGC cells into the testis of recipient using these simple tools. The DiI-labeled injected cells within recipient's testes resumed spermatogenesis which was evident by DiI staining. The efficiency of transplantation BMP4 + SMF group was higher compared to the BMP4 group alone. An explanation for this increase of efficiency may interpreted by the increased numbers of differentiated cells in the transplanted cell population (Fig. 8, 9 and10). Transplantation of large number of differentiated cells enhances their homing into tubules (34). We proposed that it also may result to restore the spermatogenesis in the arrested tubules. This point has also marked in previous autologous and homologues transplantation which showed a significant increase in the testis mass (3X) and in the percentage of tubules containing spermatogenic cells (≤80%)(35). Also, sperm arising from transplanted donor germ cells were capable of fertilization *in vivo*(36) and *in vitro*(37-39). We found that transplanted cells in BMP4 + SMF group were functional and produced more advanced germ cells in the recipient's testes (Fig. 9 and 10). These results proved our main hypothesis that SMF increased the rate of cell differentiation. The magnetic field can interfere with cell division, differentiation, and membrane voltage fluctuations, possibly by altering intracellular Ca^{2+} concentration. MF interacts with cell differentiation through two opposing mechanisms. MF can prevent the shift in surface charge potential promoted by differentiating agents. Simultaneously, MF stimulates the increase in intracellular Ca^{2+} in a dose-dependent manner. The increase in cytoplasmic divalent ions, by opening the K_{Ca} channels, acts as a rescue agent reestablishing cell's commitment to differentiation (40). Increasing the testicular mass weight, number of sperm in epididymis ducts and number of germ cells (spermatogonia, spermatocyte and spermatid) in seminiferous tubules (Fig 9 and 10) showed that SMF altered the differentiation rate. A possible explanation for these finding is the oscillation of Ca^{2+} concentration. A complementary mechanism for inducing differentiation using BMP4 is that it binds to its receptors and provokes phosphorylation of the BMP-specific smads (Smad1, Smad5, and Smad8). Each of these phosphorylated BMP-specific smads when associated with smad4 generates a complex that translocated into the nucleus and then activated the transcription of BMP-target genes. BMPs have also an important role in germ cell development and function. BMP2, BMP4, and BMP8b control the formation and early proliferation of PGCs (41). Furthermore, BMP4 acts through a non-Smad pathway such as ERK or p38

mitogen-activated protein kinase (MAPK) (42). The MAPK cascade is an essential signaling pathway through which extracellular stimuli are transduced into intracellular responses in all eukaryotic cells. Previous studies have shown static magnetic exposure modulates the gene expression (43-45).We have previously reported that SMF can induce the MAPK pathway in *vicia faba* (46). Activated MAPK can facilitate translocation to the nucleus, where it can phosphorylate and activate transcription factors, thereby modulating gene expression(32). Based on our results, we conclude that applying both physical (4mT SMF) and chemical (25 ng/ml BMP4) treatments extremely increase the differentiation rate of BMSCs into PGCs which is a proof for our obtained results through a synergistic manner.

There are several critical factors to achieve a successful transplantation of differentiated cells, including: a. the number of cells in donor cell suspension for autologous transplantation, b. suitable recipient's testes, c. an efficient transplantation procedure (47)and d. proximity in phylogenesis. To increase the number of differentiated cells in donor cell suspension, we applied both physical (4mT, 48 h SMF) and chemical (25 ng/ml, 96 h BMP4) treatments. Our previous results showed that both exposure to SMF and treatment with BMP4 have potential to enhance differentiation of BMSCs to PGCs(32). We took into consideration our previous experiences regarding cultural conditions in current study to transplant the differentiated BMSCs into azoospermia rat as an animal model. By doing so we found that these differentiated cells has function and capable to increase the number of germ cells in azoospermia model. Taking into consideration both results from *in vitro* and *in vivo* studies we reach to this conclusion that SMF can play an important role in clinical applications to treat the infertility in male through increasing the rate of differentiation.

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REFERENCES

1. Bekhite MM, Figulla H-R, Sauer H, Wartenberg M. Static magnetic fields increase cardiomyocyte differentiation of Flk-1+ cells derived from mouse embryonic stem cells via Ca 2+ influx and ROS production. International journal of cardiology. 2013;167(3):798-808.

2. Pagliara P, Lanubile R, Dwikat M, Abbro L, Dini L. Differentiation of monocytic U937 cells under static magnetic field exposure. European Journal of Histochemistry. 2009;49(1):75-86.

3. Marędziak M, Marycz K, Śmieszek A, Lewandowski D, Toker NY. The influence of static magnetic fields on canine and equine mesenchymal stem cells derived from adipose tissue. In Vitro Cellular & Developmental Biology-Animal. 2014:1-10.

4. Miyakoshi J. Effects of static magnetic fields at the cellular level. Progress in biophysics and molecular biology. 2005;87(2):213-23.

5. Fanelli C, Coppola S, Barone R, Colussi C, Gualandi G, Volpe P, et al. Magnetic fields increase cell survival by inhibiting apoptosis via modulation of Ca2+ influx. The FASEB Journal. 1999;13(1):95-102.

6. Lacy-Hulbert A, Metcalfe JC, Hesketh R. Biological responses to electromagnetic fields. The FASEB journal. 1998;12(6):395-420.

7. Zhao W, Ma W, Wu H. The Effects of Magnetic Fields on the Differentiation and Intracellular Free Calcium ofBone Marrow Mesenchymal Stem Cells. Sept. 28 2008-Oct. 2 2008; Hawaii: Automation Congress, 2008. WAC 2008. World; 2008. p. 1-3.

8. Rosen AD. Mechanism of action of moderate-intensity static magnetic fields on biological systems. Cell biochemistry and biophysics. 2003;39(2):163-73.

9. Rosen AD. Effect of a 125 mT static magnetic field on the kinetics of voltage activated Na+ channels in GH3 cells. Bioelectromagnetics. 2003;24(7):517-23.

10. Paradisi S, Donelli G, Santini MT, Straface E, Malorni W. A 50-Hz magnetic field induces structural and biophysical changes in membranes. Bioelectromagnetics. 1993;14(3):247-55.

11. Bersani F, Marinelli F, Ognibene A, Matteucci A, Cecchi S, Santi S, et al. Intramembrane protein distribution in cell cultures is affected by 50 Hz pulsed magnetic fields. Bioelectromagnetics. 1997;18(7):463-9.

12. Somosy Z. Radiation response of cell organelles. Micron. 2000;31(2):165-81.

13. Bordiushkov I, Goroshinskaia I, Frantsiiants E, Tkacheva G, Gorlo E, Neskubina I. Structural-functional changes in lymphocyte ans erythrocyte membranes after exposure to alternating magnetic field]. Voprosy meditsinskoĭ khimii. 2000;46(1):72.

14. Chionna A, Tenuzzo B, Panzarini E, Dwikat MB, Abbro L, Dini L. Time dependent modifications of Hep G2 cells during exposure to static magnetic fields. Bioelectromagnetics. 2005;26(4):275-86.

15. Pierre TGS, Dobson J. Theoretical evaluation of cell membrane ion channel activation by applied magnetic fields. European Biophysics Journal. 2000;29(6):455-6.

16. Lawson KA, Dunn NR, Roelen BA, Zeinstra LM, Davis AM, Wright CV, et al. Bmp4 is required for the generation of primordial germ cells in the mouse embryo. Genes & Development. 1999;13(4):424-36.

17. Schlatt S. Spermatogonial stem cell preservation and transplantation. Molecular and cellular endocrinology. 2002;187(1):107-11.

18. Kuznetsov SA, Mankani MH, Gronthos S, Satomura K, Bianco P, Robey PG. Circulating skeletal stem cells. The Journal of cell biology. 2001;153(5):1133-40.

19. Zuk PA, Zhu M, Mizuno H, Huang J, Futrell JW, Katz AJ, et al. Multilineage cells from human adipose tissue: implications for cell-based therapies. Tissue engineering. 2001;7(2):211-28.

20. Mizuno S, Glowacki J. Chondroinduction of human dermal fibroblasts by demineralized bone in three-dimensional culture. Experimental cell research. 1996;227(1):89-97.

21. Brighton CT, Lorich DG, Kupcha R, Reilly TM, Jones AR, ROBERT A WOODBURY I. The pericyte as a possible osteoblast progenitor cell. Clinical orthopaedics and related research. 1992;275:287-99.

22. Lee JY, Musgrave D, Pelinkovic D, Fukushima K, Cummins J, Usas A, et al. Effect of bone morphogenetic protein-2-expressing muscle-derived cells on healing of critical-sized bone defects in mice. The Journal of Bone & Joint Surgery. 2001;83(7):1032-9.

23. Sarvestani AS, Abdolmaleki P, Mowla SJ, Ghanati F, Heshmati E, Tavasoli Z, et al. Static magnetic fields aggravate the effects of ionizing radiation on cell cycle progression in bone marrow stem cells. Micron. 2010;41(2):101-4.

24. Ratajczak MZ, Zuba-Surma EK, Machalinski B, Ratajczak J, Kucia M. Very small embryonic-like (VSEL) stem cells: purification from adult organs, characterization, and biological significance. Stem cell reviews. 2008;4(2):89-99.

25. Kucia M, Wysoczynski M, Ratajczak J, Ratajczak M. Identification of very small embryonic like (VSEL) stem cells in bone marrow. Cell and tissue research. 2008;331(1):125-34.

26. Javani Jouni F, Abdolmaleki P, Movahedin M. Investigation on the effect of static magnetic field up to 15 mT on the viability and proliferation rate of rat bone marrow stem cells. In Vitro Cellular & Developmental Biology-Animal. 2013;49:212-9.

27. Mazaheri Z, Movahedin M, Rahbarizadeh F, Amanpour S. Different doses of bone morphogenetic protein 4 promote the expression of early germ cell-specific gene in bone marrow mesenchymal stem cells. In Vitro Cellular & Developmental Biology-Animal. 2011;47(8):521-5.

28. Javani Jouni F, Abdolmaleki P, Ghanati F. Oxidative stress in broad bean (Vicia faba L.) induced by static magnetic field under natural radioactivity. Mutation Research/Genetic Toxicology and Environmental Mutagenesis. 2012;741(1):116-21.

29. Sahebjamei H, Abdolmaleki P, Ghanati F. Effects of magnetic field on the antioxidant enzyme activities of suspension-cultured tobacco cells. Bioelectromagnetics. 2007;28(1):42-7.

30. Javani Jouni F, Abdolmaleki P, Ghanati F. Study the effect of static magnetic field on chromosomal aberrations on Vicia faba in area with high natural radioactivity. The Environmentalist. 2011;31(2):169-75.

31. Ogawa T, Arechaga G, Avarbock M, Brinster R. Transplantation oftestis germinal cells into mouse seminiferous tubules. Int J Dev Biol. 1997;41:111-22.

32. Javani Jouni F, Abdolmaleki P, Behmanesh M, Movahedin M. An in vitro study of the impact of 4mT static magnetic field to modify the differentiation rate of rat bone marrow stem cells into primordial germ cells. Differentiation. 2014;87(5):230-7.

33. Mazaheri Z, Movahedin M, Rahbarizadeh F, Amanpour S. Generation of In-vitro Spermatogonial Stem Cells following Genetic Manipulation of Primordial Germ-like Cells. Avicenna journal of medical biotechnology. 2012;4(2):55.

34. Kanatsu-Shinohara M, Ogonuki N, Inoue K, Ogura A, Toyokuni S, Shinohara T. Restoration of fertility in infertile mice by transplantation of cryopreserved male germline stem cells. Human reproduction. 2003;18(12):2660-7.

35. Izadyar F, Den Ouden K, Stout T, Stout J, Coret J, Lankveld D, et al. Autologous and homologous transplantation of bovine spermatogonial stem cells. Reproduction. 2003;126(6):765-74.

36. Yuan Z, Hou R, Wu J. Generation of mice by transplantation of an adult spermatogonial cell line after cryopreservation. Cell Proliferation. 2009;42(2):123-31.

37. Brinster RL, Avarbock MR. Germline transmission of donor haplotype following spermatogonial transplantation. Proceedings of the National Academy of Sciences. 1994;91(24):11303-7.

38. Honaramooz A, Behboodi E, Blash S, Megee SO, Dobrinski I. Germ cell transplantation in goats. Molecular reproduction and development. 2003;64(4):422-8.

39. Goossens E, Frederickx V, De Block G, Van Steirteghem A, Tournaye H. Reproductive capacity of sperm obtained after germ cell transplantation in a mouse model. Human reproduction. 2003;18(9):1874-80.

40. Tonini R, Baroni M, Masala E, Micheletti M, Ferroni A, Mazzanti M. Calcium protects differentiating neuroblastoma cells during 50Hz electromagnetic radiation. Biophysical journal. 2001;81(5):2580-9.

41. Dudley BM, Runyan C, Takeuchi Y, Schaible K, Molyneaux K. BMP signaling regulates PGC numbers and motility in organ culture. Mechanisms of development. 2007;124(1):68-77.

42. Ying Q-L, Nichols J, Chambers I, Smith A. BMP induction of Id proteins suppresses differentiation and sustains embryonic stem cell self-renewal in collaboration with STAT3. Cell. 2003;115(3):281-92.

43. Goto Y, Taniura H, Yamada K, Hirai T, Sanada N, Nakamichi N, et al. The magnetism responsive gene Ntan1 in mouse brain. Neurochemistry international. 2006;49(4):334-41.

44. Hiraoka M, Miyakoshi J, Li YP, Shung B, Takebe H, Abe M. Induction of c-fos gene expression by exposure to a static magnetic field in HeLaS3 cells. Cancer research. 1992;52(23):6522-4.

45. Potenza L, Ubaldi L, De Sanctis R, De Bellis R, Cucchiarini L, Dachà M. Effects of a static magnetic field on cell growth and gene expression in Escherichia coli. Mutation Research/Genetic Toxicology and Environmental Mutagenesis. 2004;561(1):53-62.

46. Haghighat N, Abdolmaleki P, Ghanati F, Behmanesh M, Payez A. Modification of catalase and MAPK in Vicia faba cultivated in soil with high natural radioactivity and treated with a static magnetic field. Journal of Plant Physiology. 2014;171(5):99-103.

47. Koruji M, Movahedin M, Mowla SJ, Gourabi H, Pour-Beiranvand S, Arfaee AJ. Autologous transplantation of adult mice spermatogonial stem cells into gamma irradiated testes. Cell Journal (Yakhteh). 2012;14(2):82.