Enhancement of tooth eruption by using amniotic stem cells (Immunohistochemical study of VEGF marker)

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ABSTRACT

Background: Tooth eruption is a localized process in the jaws which exhibits precise timing and bilateral symmetry. Develop within the jaws and their eruption is a complex infancy process during which they move through bone to their functional positions within the oral cavity. For species with more than one set of teeth, eruption of the second set also accomplishes. The key to the successful clinical management of tooth eruption consists of understanding that this process consists largely of the local regulation of alveolar bone metabolism to produce bone resorption in the direction of eruption and shift and formation of bone at the opposite side.The amniotic sac contains a considerable quantity of stem cells. These amniotic stem cells are able to differentiate into various tissues, which used in many field. Vascular endothelial growth factor (VEGF) is an important angiogenic factor reported to induce migration and proliferation of endothelial cells, enhance vascular permeability, and modulate thrombogenicity. VEGF expression in cultured cells (smooth muscle cells, macrophages, endothelial cells) is controlled by growth factors and cytokines. The aim of this study was to study the administration of cell molecules of (Chorion, Amnion and Amniotic fluid) around developing mouse tooth and studying the expression of VEGF marker.

Materials and Methods: forty eight albino Swiss mice of one day old age injected with isolated amniotic stem cells in the anterior region of maxilla (incisors area) other 16 mice injected with saline represents control. Sacrifice 4 mice for each period (4, 7, 10, and 13) day old age. The result were studied histologically and immunohistochemistry.

Results: VEGF marker localized and identified in 3 areas; pulp, P.D.L, and Bone. In pulp. The mean value of positive VEGF expression showed to be highest in Amnion group in comparison to the other studied groups. The marginal mean value of all periods reported to be highest in Amnion groups followed by Chorion group. The period 10 day showed highest marginal means value for positive VEGF expression for all groups. In P.D.L. area Amniotic fluid records the highest mean and marginal mean value specifically at day-10 in comparison to other studied groups. In Bone area Amniotic fluid records the highest mean and marginal mean value among the studied groups followed by Chorion group. Period 7-day and 10-day shows high mean value for VEGF expression. Coincidence test for VEGF marker illustrates to be affected by Amniotic fluid application in P.D.L. and in bone area while Amnion and Chorion application showed to be concerned with pulp.

Conclusion. It reported that amniotic fluid application affected on expression of VEGF in P.D.L and bone while amnion and chorion showed to affect on expression of VEGF in pulp.The present study highlighted on clinical and researcher application of Amniotic fluid and Chorion for supplement of stem cell in dental tissue engineering or even in other body tissues.

Keywords: tooth eruption, amniotic stem cells, immunohistochemical study, VEGF marker. **(J Bagh Coll Dentistry 2013; 25(2):80-88).**

INTRODUCTION

Amniotic stem cells are multipotent stem cells of mesenchymal origin extracted from amniotic fluid; it can be transformed into a more versatile state similar to embryonic stem cells, that they can revert to being pluripotent just by adding a chemical reagent that modifies the configuration of the DNA so that genes that are expressed in the embryo get switched back on [(1)].

Amniotic stem cells are able to differentiate into various tissue types such as skin, cartilage, cardiac tissue, nerves, muscle, and bone, and may have potential future medical applications [(2)].

The deployment of amniotic fluid AF-derived stem cells (AFS) for tissue regeneration offers advantages over the use of embryonic or adult stem cells [(3)]

VEGF is a sub-family of growth factors, specifically the platelet-derived growth factor family of cystine-knot growth factors. They are important signaling proteins involved in both vasculogenesis (formation of the embryonic circulatory system) and angiogenesis (the growth of blood vessels from pre-existing vasculature [(4)]. Its normal function is to create new blood vessels during embryonic development, new blood vessels after injury, and new vessels (collateral circulation) to bypass blocked vessels[(5)] .

MATERIALS AND METHODS

Seventy nine Albino Swiss female mice were used in the present study. Those mice were divided into 3 main groups:

1. Experimental group: consisted of 16 mice of one day old of age injected with isolated amniotic stem cells in the anterior region of maxilla (incisors area). Sacrifice 4 mice for each period (4, 7, 10, and 13) day old age. Those 16

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mice injected with **amnionic** cells, 4mice for each scarifying periods.

2. Control group: consists of 16 mice of one day old age, injected with normal saline in the anterior incisors region of maxilla. Sacrifice 4 mice for each period (4, 7, 10, and 13) day.

3. Pregnant mice group: consists of 15 pregnant mice: 5 out of 15 were used to collect their autologous amniotic fluid at (13 day of gestation period), and stored to be used to their neonatal embryo. While other 10 pregnant mice were scarified to obtain amnionic and chorionic cells from their placenta at (17 day of gestation period).

Collection of amniotic fluid

Amniotic fluid was collected from each 5 pregnant mice at 13 day of gestation period (separately) , by using needle aspiration technique, cleaned their skin and wiped with alcohol, then aspirate the fluid using insulin syringe and preserved the amniotic fluid in sterile tube at -80°C until it used.

Isolation amniotic stem cells from the placenta

Samples were obtained from 10 pregnant mice at 17 day gestation period to isolate Chorion and Amnion, after sacrifice the pregnant mice by over dose anesthesia, the embryos inside amniotic membrane with their placenta will excluded immediately. Then isolate the embryo from the placenta, and carrying the following procedures:

1. The placenta was cleaned from blood clot with a sterile phosphate-buffered saline solution.
2. Removing of amniotic membrane from embryos and put in flask.
3. Take a pair of sterile scissors and carefully cut the outside epithelial layer off. The more cut the more stem cells get. The amnion layer is mechanically peeled off the Chorion.
4. Washing the amnion in Phosphate buffered saline solution (PBS) in several times (8-10X) to remove blood.
5. Mince the tissue thoroughly with a pair of another sterile scissors.
6. To release amniotic epithelial cells, incubate the minced amnion membrane with Trypsin (0.05%) for 10 minutes at 37°C.
7. Treating the remaining tissue in another tube of trypsin (0.05%) for 20 minutes at 37 °.
8. Pooling the cells from the digests.
9. Fuge the filtered cell suspension for 8 minutes at 1200 RPM.
10. Washing the cell pellet with PBS and fuge again.
11. Counting the cells with a hemocytometer and it is advisable to determine the viability of the cells by exclusion of trypan blue dye,
12. Resuspending the pellet in freezing medium by pipetting gently.
13. In order to freeze the cells gradually and safe, place the ampoules in -60°C or less and leave them there for 16-24 hours[6]. **(**All operation was done under sterile condition, using a laminar flow hood.

Monoclonal antibodies CD34 and their Detection kit.

Monoclonal antibody V2110-18T3 (Rabbit anti-Mouse): US Biological VEGF Receptor 2 (VEGFR2). Immunohistochemistry with Detection Kit, HRP, Mouse Tissue, BioAssay™, US Biological, IHC detection kit, HRP, Mouse Primaries (Catalog No.17506-06).

RESULTS

Histological and immunohistological tests for detection the expression of VEGF marker were performed on both experimental and control groups for all periods.

View of pulp for control group of mouse 13 days old shows positive expression of VEGF in the endothelial cell of blood vessels; they show strong DABstain (Fig. 1).

Immunohistochemical examination for pulp of mouse 13 days old treated with **Amnion** illustrate positive staining for VEGF (Fig.2).

Fig. 3&4 show positive expression of VEGF in the mesenchymal cell of pulp, dental sac and in new formed bone around tooth of mouse 13 days old treated with **Chorion.** Endothelial cell of blood vessels in pulp of tooth of mouse treated with **Amniotic fluid** shows positive expression of VEGF (Fig. 5), while (fig.6 &7) illustrate the expression of VEGF in dental sac area with angiogenesis.

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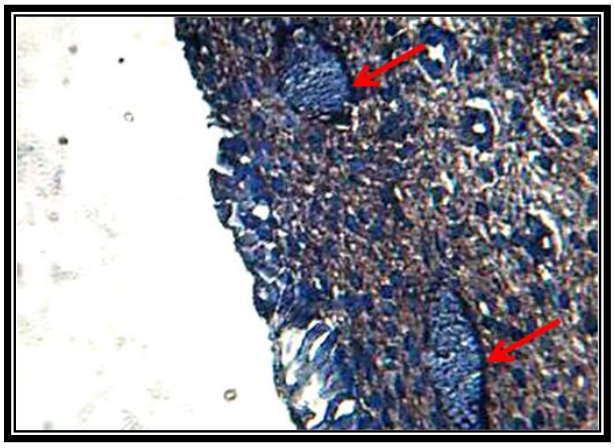

Figure 1: View for pulp of (Control) tooth mouse 4 days old shows positive VEGF (arrow). DAB stain with counter stain hematoxylin, X200

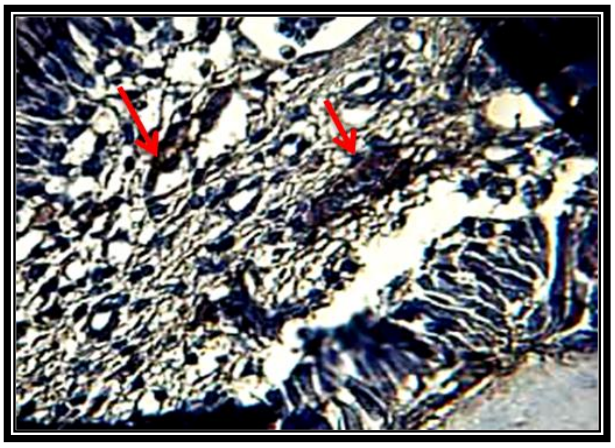

Figure 2: Positive VEGF demonstrated in pulp of tooth mouse 4 days old treated with Amnion. DAB stain with counter stain hematoxylin, X20

Figure 3: Positive VEGF view in endothelial cell (arrow) and in the Mesenchymal cell (MC) of pulp in tooth mouse 4 days old treated with Chorion.DAB stain with counter stain hematoxylin,X200

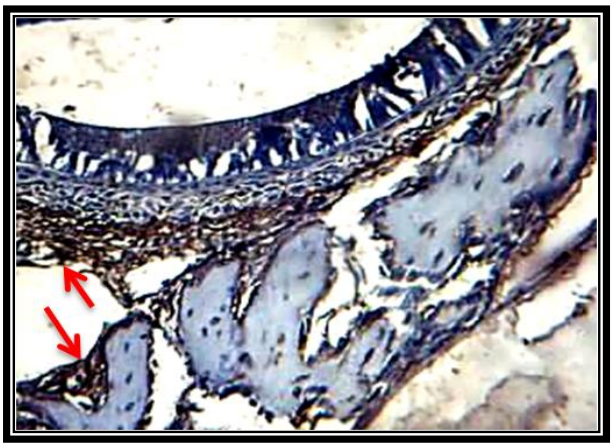

Figure 4: Positive VEGF demonstrated in dental sac area and in resorbed bone area (arrow).DAB stain with counter stain hematoxylin, X200

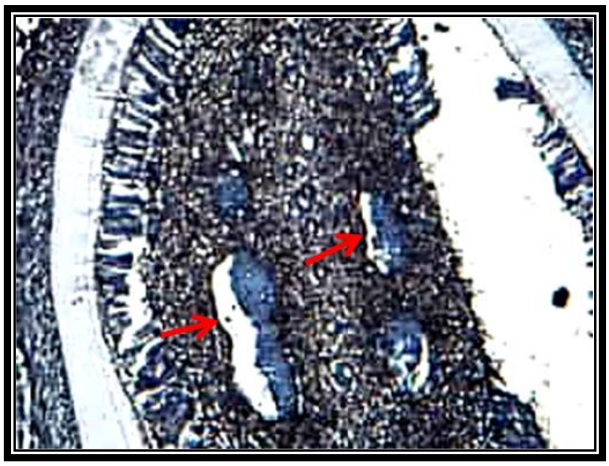

Figure 5: Positive expression of VEGF by endothelial cells of blood vessels in pulp of 4 days old mouse treated with Amniotic fluid (arrow). DAB stain with counter stain hematoxylin, X400

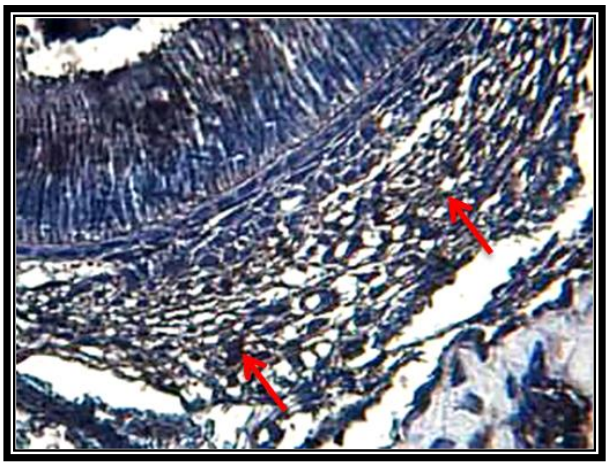

Figure 6: Positive VEGF in dental sac area (arrow) for previous figure (3-93). DAB stain with counter stain hematoxylin, X200.

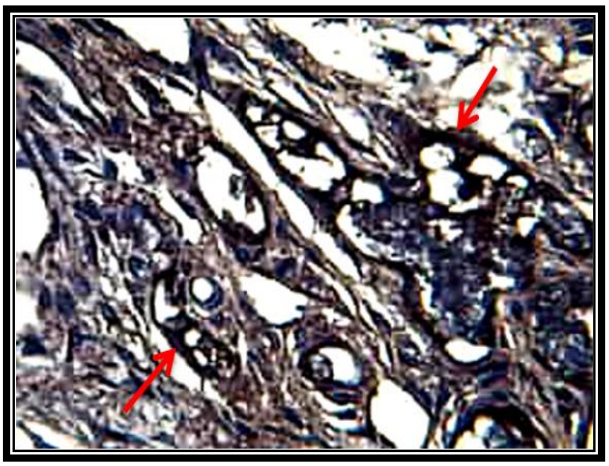

Figure 7: Angiogenesis view showing positive VEGF in dental sac of tooth mouse 4 days old treated with amniotic fluid. DAB stain with counter stain hematoxylin, X400

At 7 days old mouse

View of positive VEGF for stromal cell in new bone formed in maxilla of tooth mouse Control group (fig.8).Immunohistochemical view of mouse treated with **Amnion** shows positive VEGF expression of endothelial cells (fig 9).

Fig. (10&11) shows positive expression of VEGF in endothelial cells and stromal cells of new bone formed and in periodontal ligament of tooth of mouse treated with **Chorion**.While with **Amniotic fluid** shows positive VEGF expression of endothelial cell, Mesenchymal cell and dental sac tissue. Figure (12&13).

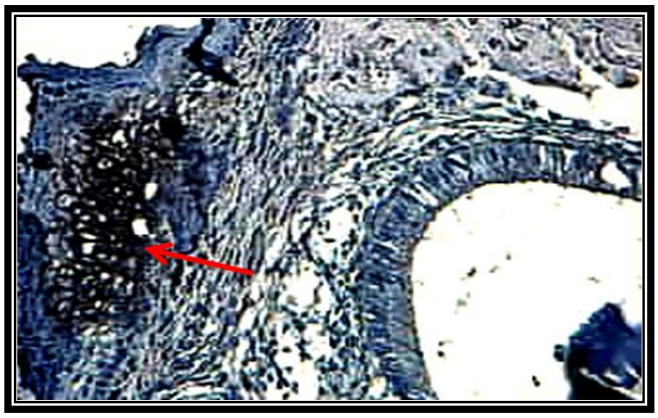

Figure 8: Stromal cell of new bone shows positive VEGF expression (arrow) of 7 days old mouse Control. DAB stain with counter stain hematoxylin, X400

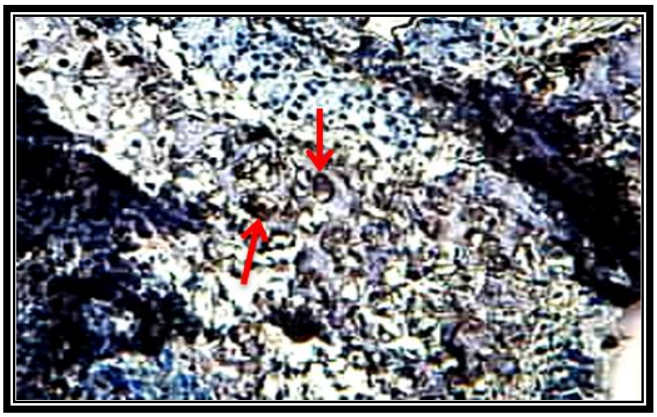

Figure 9: Pulp tissue of tooth germ (7 days old mouse) treated with Amnion, shows positive VEGF expression of endothelial cell (arrow). DAB stain with counter stain hematoxylin, X100

Figure 10: VEGF positive expressed by stromal cell in overlying bone (arrow) of tooth germ 7 days old mouse treated with Chorion. DAB stain with counter stain hematoxylin, X200

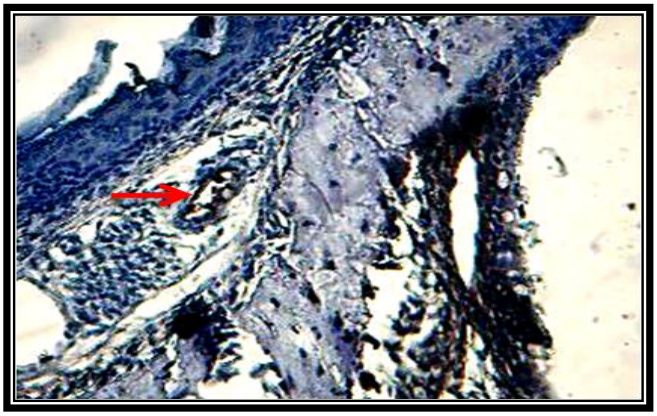

Figure 11: Lateral side view for periodontal ligament, and new bone formation of tooth germ of 7 days old mouse treated with Chorion, shows positive VEGF expression of endothelial cell (arrow). DAB stain with counter stain hematoxylin, X100

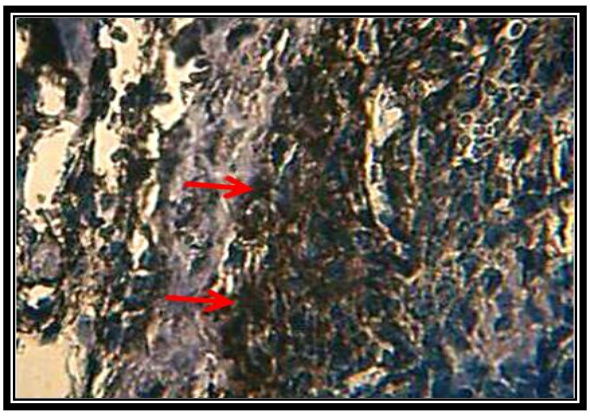

Figure 12: Pulp tissue of tooth germ (7 days old mouse) treated with Amniotic fluid shows positive VEGF expression of endothelial cell and Mesenchymal cell (arrow). DAB stain with counter stain hematoxylin, X400

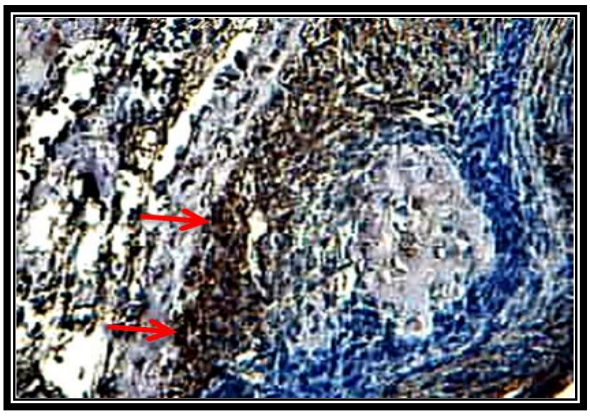

Figure 13: Dental sac tissue of tooth germ of treated 7 days old mouse with Amniotic fluid shows positive VEGF expression (arrow). DAB stain with counter stain hematoxylin, X200

At 10 days old mouse

Control group: Immunohistochemical localization for VEGF in endothelial blood vessels, pulp, and dental sac figure (14**).** Section in pulp of tooth of mouse 10 days old treated with **Amnion** illustrate positive VEGF expression in endothelial cells of blood vessels figure(15). In the apical area, VEGF marker localized in stromal cells between the bone figure (16). Microphotograph view illustrate expression of VEGF in endothelial cells of blood vessels in both of pulp and dental sac of tooth of mouse 10 days old treated with **Chorion**. Figure (17&18). Pulp of tooth treated with **amniotic fluid** shows positive expression of VEGF on endothelial blood vessels, new blood vessels and mesenchymal cell figure (19). The apical portion shows positive VEGF expression on proliferative dental sac area represented by periodontal ligament (P.D.L) and new bone formation area figure (20).

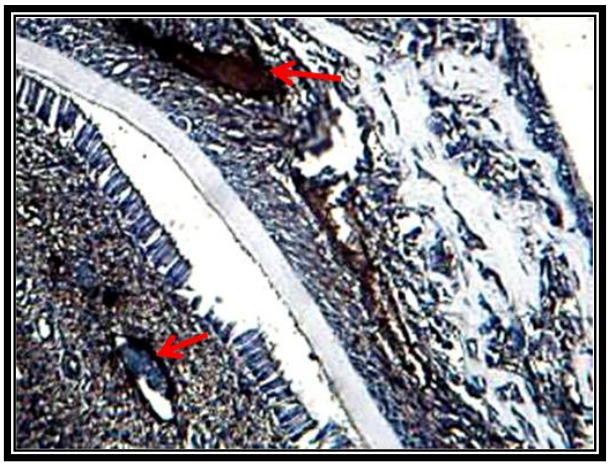

Figure 14: Immunohistochemical positive VEGF expressed in pulp, dental sac and endothelial blood vessels (arrow), of tooth germ mouse 10 days old Control. DAB stain with counter stain hematoxylin, X100

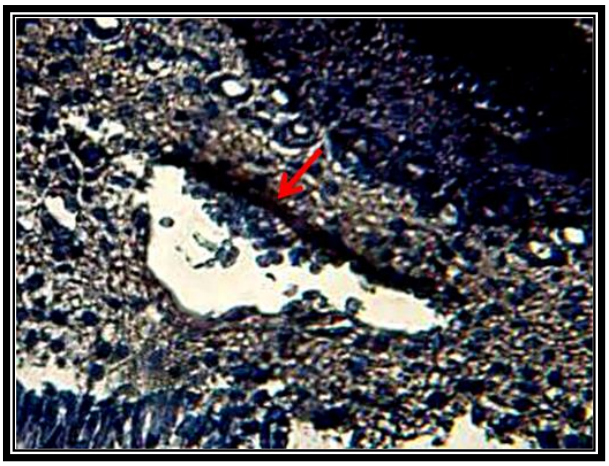

Figure 15: View for endothelial blood vessels expressed positive VEGF (arrow), section in pulp of tooth mouse 10 days old treated with Amnion DAB stain with counter stain hematoxylin, X400

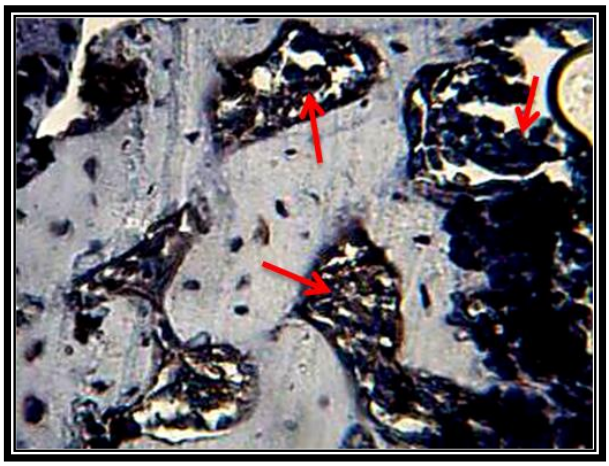

Figure 16: View for the bone formation area (apically) showing positive (VEGF) marker demonstrated in stromal cell (arrow). DAB stain with counter stain hematoxylin, X400

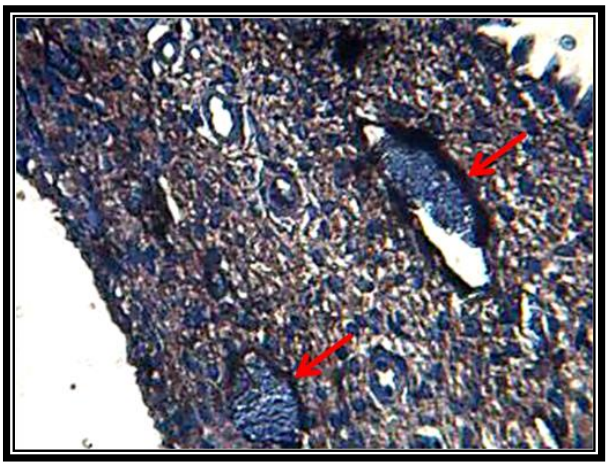

Figure 17: Immunohistochemical positive VEGF view expressed in endothelial cell (arrow) of blood vessels in pulp of tooth germ mouse 10 days old Chorion . DAB stain with counter stain hematoxylin, X400

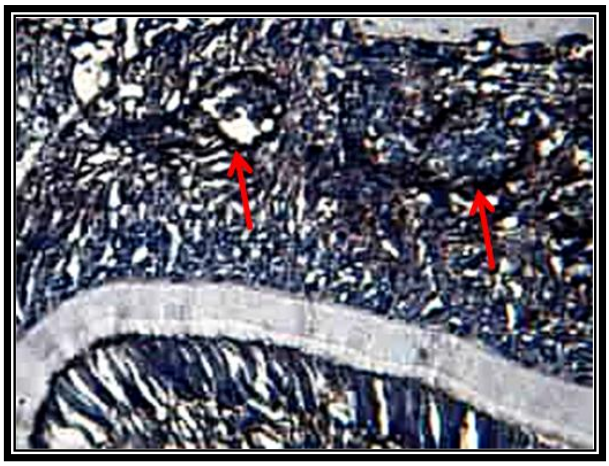

Figure 18: Other view for previous figure (3-105), showing positive VEGF in the endothelial cell of blood vessels in the dental sac. DAB stain with counter stain hematoxylin, X400

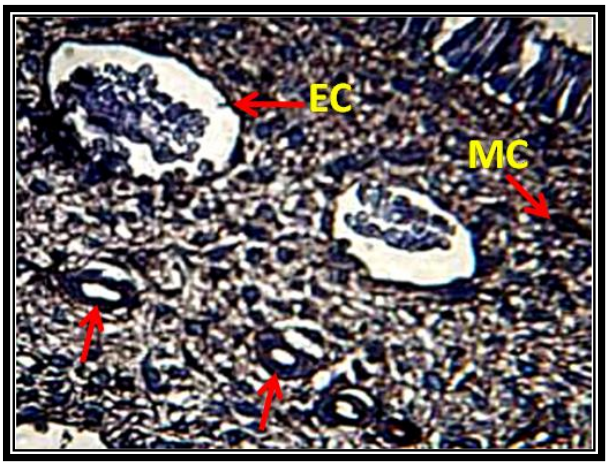

Figure 19: Positive expression of VEGF on endothelial cells (EC) of blood vessels in pulp, proliferative new blood vessels (arrow), even mesenchymal cell (MC). View of tooth germ of 10 days old mouse treated with Amniotic fluid. DAB stain with counter stain hematoxylin,X400

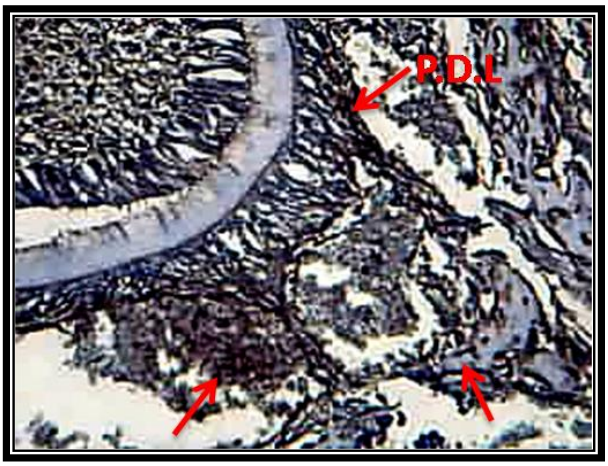

Figure 20: View of apical portion of previous figure (3-107) shows positive VEGF expression on proliferative dental sac area represented by periodontal ligament (P.D.L) and new bone formation area (arrow). DAB stain with counter stain hematoxylin, X200

DISCUSSION

The vascular endothelial growth factor (VEGF) is considered the most important GF controlling the vascular responses in the body. It is a mitogen for endothelial cells, and its expression is related to the phenomenon of angiogenesis. The present study illustrates positive VEGF in pulp, P.D.L and in bone in studied groups. Although all studied groups shows positive expression of VEGF, Amnion

group illustrates high difference value at 4 day in comparison to others. It can be explained that the dentin-pulp matrix is rich in growth factors (GFs) that, when diluted and diffused into the pulp tissue, aid the healing process. The angiogenic GFs participate in this event. Vascular endothelial growth factor (VEGF), a potent mitogen for endothelial cells, promotes endothelial cell survival and enhance new blood vessels formation.

Amniotic fluid group records the highest value for positive VEGF in P.D.L and in bone areas at day 10 in comparison to the others. The present result coincide with **Kaku et al** [7] who investigated whether recombinant human VEGF (rhVEGF) stimulated osteoclast differentiation during experimental tooth movement. For coincidence test of expression marker VEGF, it reported that Amniotic fluid application affected on expression of VEGF in P.D.L and bone while Amnion and Chorion showed to affect on expression of VEGF in pulp.

It seems that Amnion and Chorion affects the undifferentiated mesenchymal cell in pulp and enhances it or it may act as progenitor cell and differentiated to endothelial positive VEGF cells

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