Immunohistochemical study of CD34 in tooth eruption by using amniotic stem cells

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ABSTRACT

Background: Tooth eruption is a more general process, however, which includes certain posteruptive tooth movements. There are two fundamental requirements for both tooth eruption to occur:

(1) Require soft tissue, intervening between tooth structure and alveolar bone, which plays an important role in regulating the remodeling of adjacent tissues.

(2) Require bone turnover that is temporally and spatially regulated to facilitate specific translocations of teeth through alveolar bone

These amniotic stem cells are multipotent and able to differentiate into various tissues, which may be useful for human application and recently it used in many medical branches. CD34 is an endothelial marker that is extensively used in immunohistochemistry and most vascular endothelial cells. Expression of the stem cell antigen CD34 is a defining hallmark of hemopoietic stem cells and progenitors. This study aimed to study the expression of CD34 by dental cells involved in tooth eruption after administration of amniotic stem cell

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: Immunohistochemical result revealed positive expression of CD34 in pulp (Vascular, Paravascular), Mesenchymal cell and in the Dental sac of different groups. Coincidence test of expression marker CD34 in various studied group shows that Chorion application affected on CD34 expression in pulp while Amniotic fluid affected on dental sac.

Conclusion Immunohistochemical study of expression marker CD34 in various studied groups show that chorion application affected on CD34 in pulp .While amniotic fluid affected on dental follicle.

Key words: amniotic stem cells, tooth eruption. (J Bagh Coll Dentistry 2013; 25(2):47-53).

INTRODUCTION

Stem cells are reprogrammed cells which were able to develop into many different types of functioning cells, including liver, bone and nerve cells. Amniotic fluid stem cells are intermediate between embryonic stem cells and adult stem cells. All over the world, universities and research institutes are studying amniotic fluid to discover all the qualities of amniotic stem cells. Amniotic fluid is a good source of stem cells. The advantages of generating pluripotent cells without any genetic manipulation makes them more likely to be used for therapy.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 [(3)].

CD34 is a 90- to 120-kDa cell surface sialomucin that is widely used for the enrichment of human hematopoietic stem cells (HSCs) because of its selective expression on progenitor cells and absence on mature hematopoietic cells.

CD34 molecule is a cluster of differentiation molecule present on certain cells within the human body. It is a cell surface glycoprotein and functions as a cell-cell adhesion factor. It may also mediate the attachment of stem cells to bone marrow extracellular matrix or directly to stromal cells. CD34 Marker is the commonly used marker of hematopoietic progenitor cells and endothelium [(4)].

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 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

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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[(5).] **(**All operation was done under sterile condition, using a laminar flow hood.

Monoclonal antibodies CD34 and their Detection kit.

Anti-CD34 antibody [MEC 14.7] - Hematopoietic Stem Cell Marker (ab8158) Monoclonal (Rat anti -Mouse), Isotype (IgG2a), applied dilution 1:50, Store at -20ºC. Abcam anti mouse HRP/ DAB Immunohistochemistry Detection Kit, (Catalog No. ab64259**)** was used**.**

RESULTS

Histological and immunohistological tests for detection the expression of CD34 marker were performed on both experimental and control groups for all periods.

1. At 4 days old mouse

In Control group, Dental sac only shows positive expression of CD34 in tooth of mouse 4 days old seen in figure (1).

In Experimental group (Amnion) **:**Pulp tissue of tooth in mouse 4 days old treated with shows positive e expression of CD34 in paravascular area. Figure (2)

With Chorion: positive CD34expression of vascular cell and Mesenchymal cell Figure (3). While Tooth of mouse treated with Amniotic fluid shows the expression of CD34 marker in mesenchymal cell and paravascular cell in the pulp. And in the area of new bone formation around the tooth view the CD34expression in stromal cell. Figures (4&5)

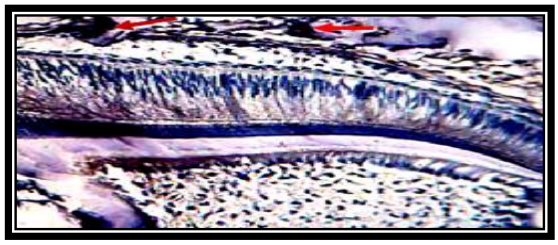

Figure 1: Positive CD34 demonstrated in Dental sac (arrow) area of tooth germ of

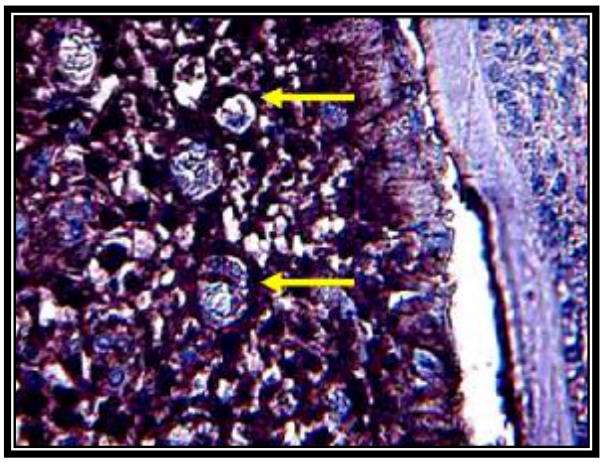

Figure 2: CD34 positive (arrow) paravascular location in pulp tissue of mouse 4 days old treated with Amnion, DAB stain with counter stain hematoxylin X400

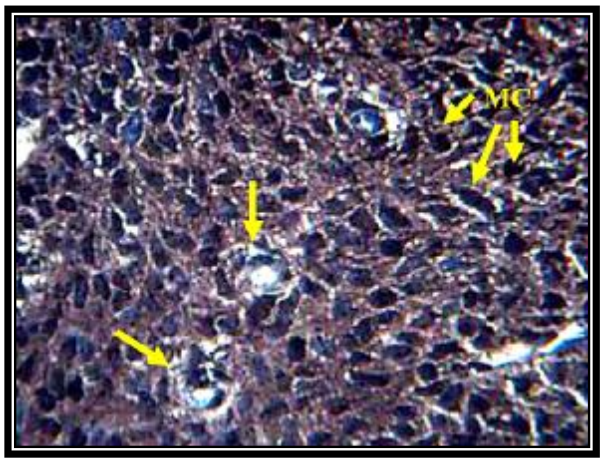

Figure 3: CD34 positive expression by vascular cell (arrow) and even in Mesenchymal cell (MC) in pulp of mouse 4 days old treated with Chorion. DAB stain with counter stain hematoxylin. X 400

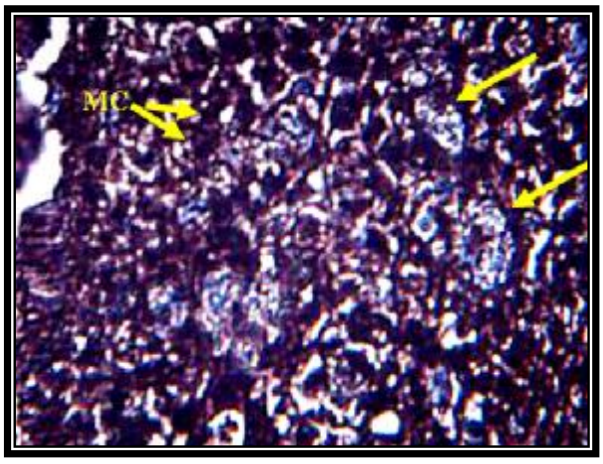

Figure 4: Positive CD34 expression by paravascular cell (arrow) and in Mesenchymal cell (MC) in pulp of mouse 4 days old treated with Amniotic fluid. DAB stain with counter stain hematoxylin. X400

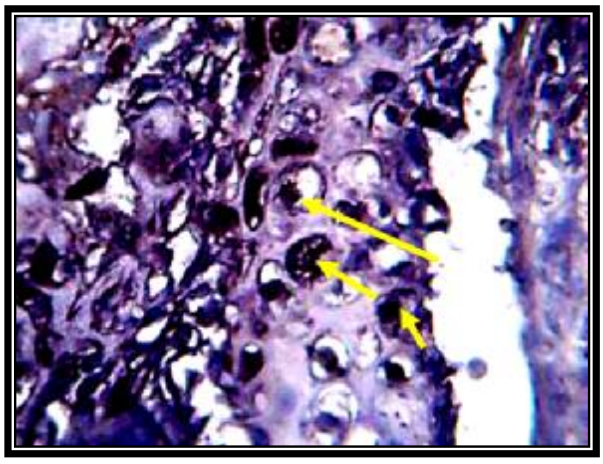

Figure 5: Positive CD34 demonstrated in stromal cell (arrow) of new bone formation around teeth of mouse 4 days old treated with Amniotic fluid. H&E X400

2- At 7 days old mouse

Figure (6) shows the endothelial cell of blood vessels with positive CD34expression in Control group. While Section in pulp of tooth mouse treated with **Chorion** illustrates expression of CD34 obviously in the endothelial cell (vascular cell, mesenchymal cell, and paravascular cell) in figures (7, and 8). With **Amnion** see the expression in pulp and dental sac as seen in figure (9).

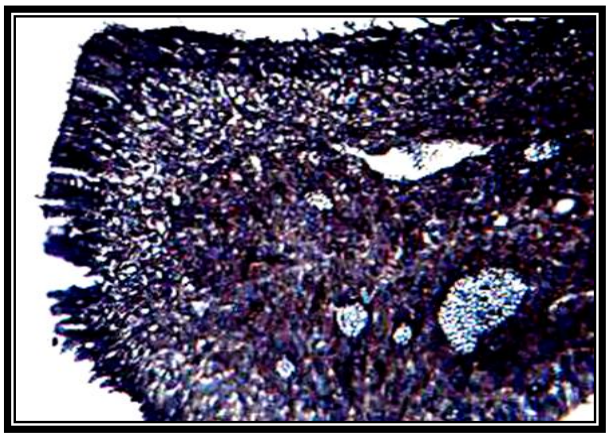

Figure 6: Positive CD34 in endothelial cell of blood vessels in pulp of mouse 7 days old (Control). DAB stain with counter stain hematoxylin , X200.

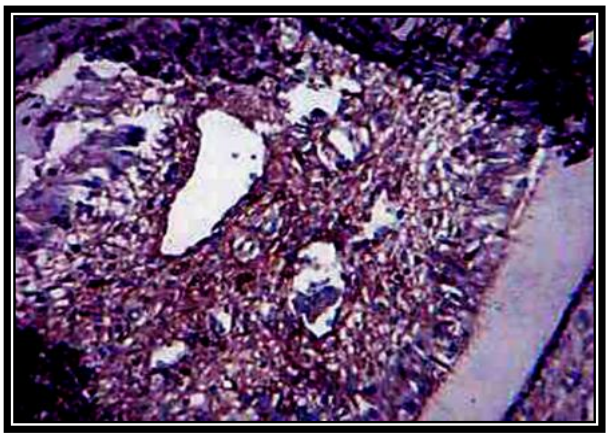

Figure 7: Positive CD34 expressed on endothelial cell, Mesenchymal cell in pulp of mouse 7 days old treated with Chorion. DAB stain with counter stain hematoxylin .X200

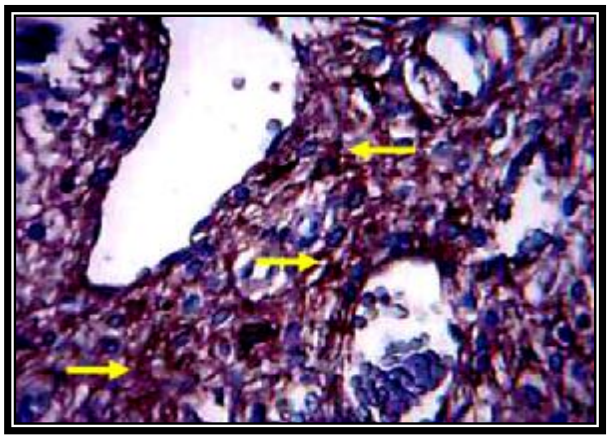

Figure 8: Magnifying view of figure (3-64) shows positive vascular, and paravascular cell (arrow). DAB stain with counter stain hematoxylin. X400.

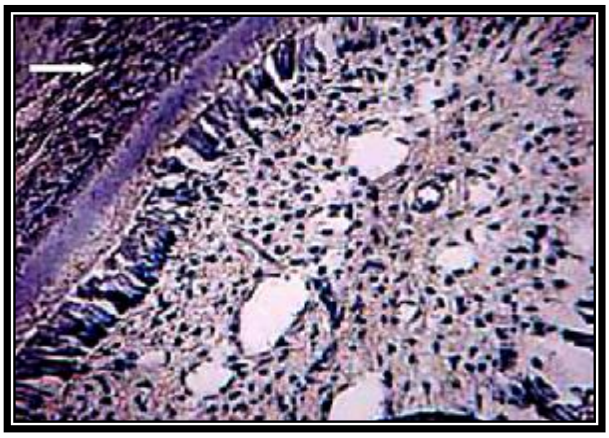

Figure 9: Positive CD34 expression in pulp and dental sac area (arrow) of tooth mouse 7 days old treated with Amnion. DAB stain with counter stain hematoxylin, X400.

3- <u>At 10 days old mouse:</u>

(Fig.10) Shows obviously positive CD34 expression in dental sac and pulp (vascular and paravascular) in Control group.

With Amnion, view expression of CD34 in root formation portion (Fig.11).

With Chorion illustrates expression of CD34 in pulp and dental sac area include periodontal ligament and stromal cell of newly formed bone (Fig.12).

With Amniotic fluid CD34 express in numerous blood vessels in pulp and dental sac area, as seen in (fig.13).

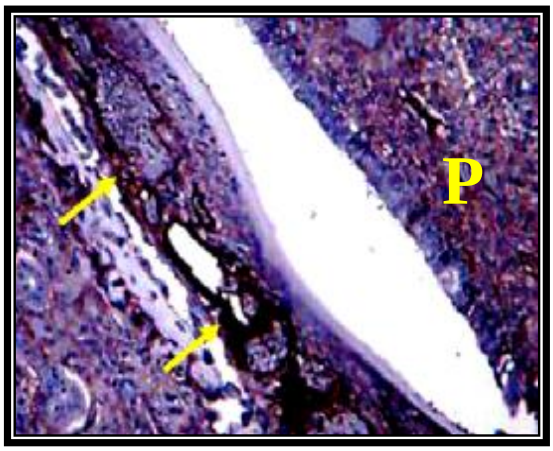

Figure 10: Positive CD34 expression in pulp (P) and dental sac (arrows) in tooth of mouse 10 days old (Control). DAB stain with counter stain hematoxylin, X200.

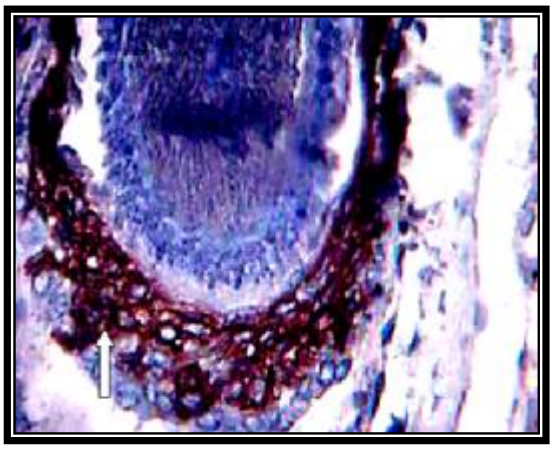

Figure 11: Positive CD34 expression in apical area (arrow) of root formation in tooth of mouse 10 days old treated with Amnion. DAB stain with counter stain hematoxylin ,X400.

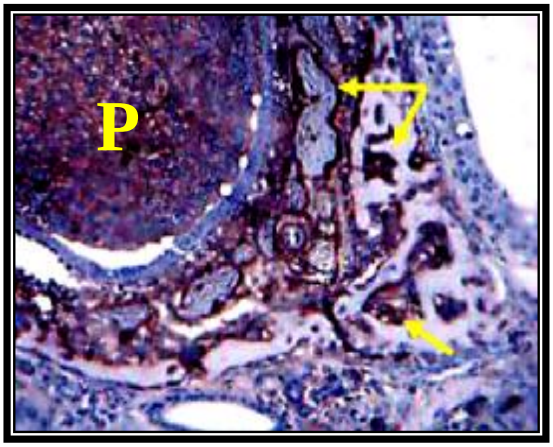

Figure 12: Positive CD34 expression in pulp (P) and dental sac area include P.D.L and in stromal cell of newly formed bone (arrow) in tooth of mouse 10 days old treated with Chorion. DAB stain with counter stain hematoxylin, X200.

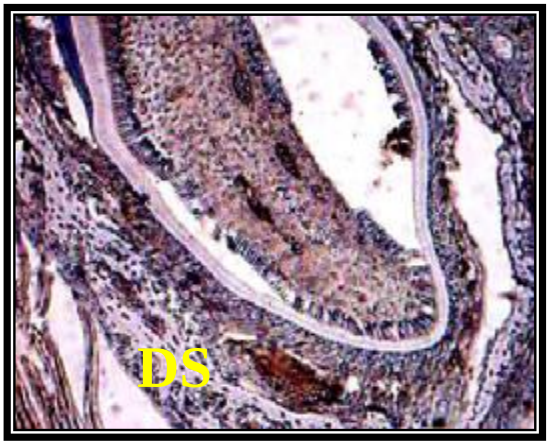

Figure 13: Positive CD34 expression in pulp and dental sac (DS) area include blood vessels in tooth of mouse 10 days old treated with Amniotic fluid. DAB stain with counter stain hematoxylin, X200.

4- At 13 days old mouse

Figure (14) shows positive expression of CD34 in blood vessels and endothelial cells. With Amnion and Chorion shows positive expression of CD34 as seen in figure (15, 16&17). Amniotic fluid shows expression of CD34 marker in the newly formed blood vessels and in vascular endothelial cells (Fig.18&19).

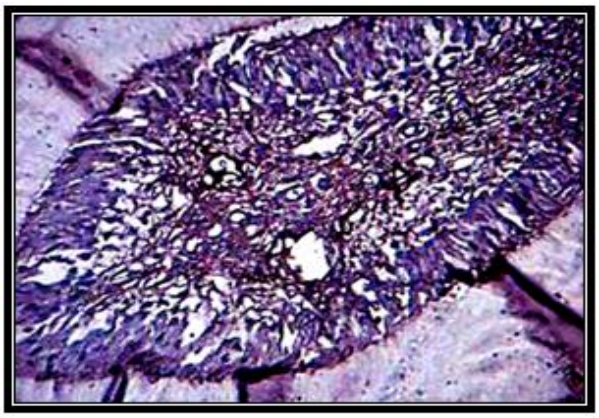

Figure 14

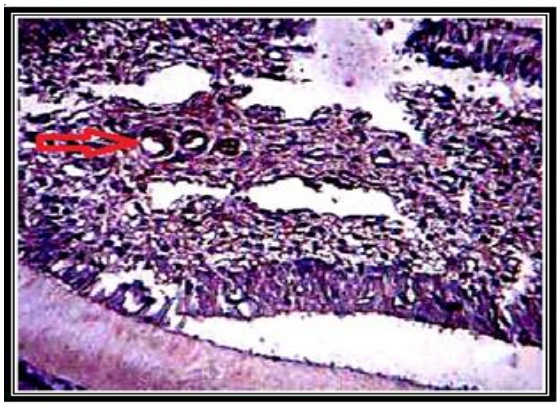

Figure 15

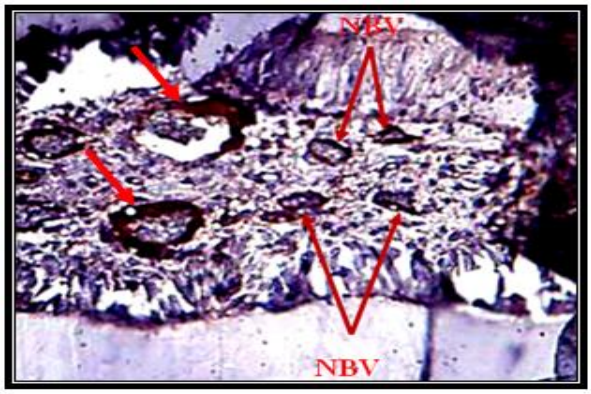

Figure 16

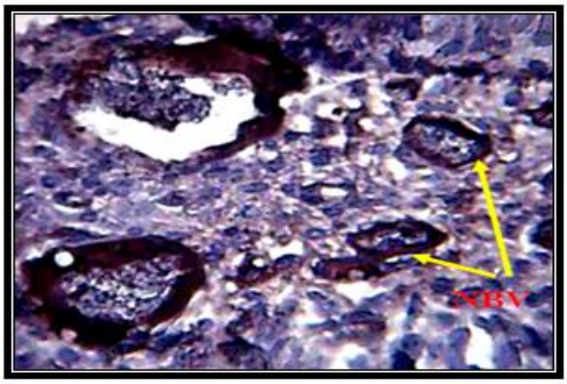

Figure 17

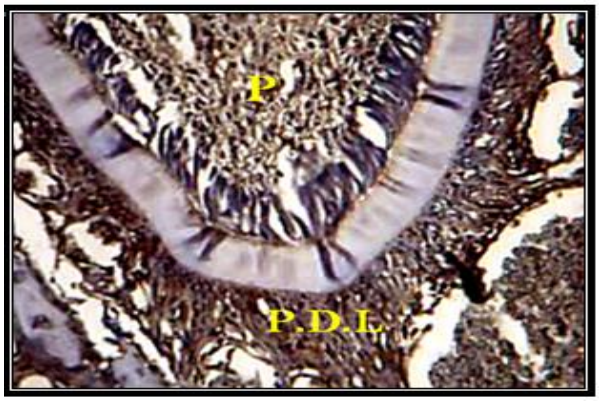

Figure 18

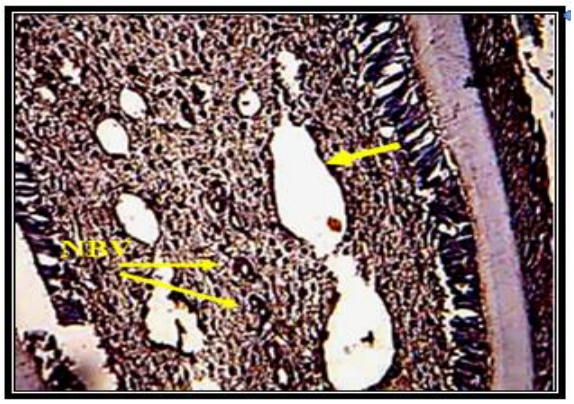

Figure 19

DISCUSSION

Positive expression of CD34 in pulp expressed by vascular endothelial cells and paravascular cells which may be endothelial cell or progenitor cells. Also positive expression of CD34 was detected in pulp and dental sac of developing tooth at 4,7,10 and 13 day of eruptive periods of mouse and in different studied groups (Amnion, Chorion, Amniotic fluid and Control) but in different level and scoring. The present results reported an early eruption of mouse tooth at day 13 in Amniotic fluid showing well arranged P.D.L groups, well developed surrounding bone and root formation, with angiogenesis illustrates in bone and P.D.L sites. For angiogenesis it creates change in blood volume and in turn increases vascular pressure. All these histological features aids in lifting up the tooth for eruption in supraosseous level. And all these features need for high cell proliferation that could be attributed to administration of stem

cell from amniotic fluid. Presentation of CD34 marker which is specifically expressed by the progenitors of the endothelial and mesenchyme was studied [6,7].

Abedini' et al [8] studied the CD34 and alkaline phosphatase activity of the stem cells in pulp of deciduous tooth and they propose the probable role of endothelial progenitor cells as well as the neural crest in the derivation of pulp stem cells.

Maltby et al [9] reported that various endothelial markers have been used in order to identify the antigen reactivity of vessels in a variety of tissues. And they illustrate CD34 marker in most of endothelial cells, and it has been suggest that CD34 regulate early events in blood cell migration and differentiation, and it may help in cell adhesion molecules.

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