PRE AND POSTNATAL VASCULARIZATION OF LONG BONES IN GUINEA PIG (CAVIACUTLERI)

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ABSTRACT

The present study was carried out to determine the vascularization of long bone in laboratory animal. Fourty healthy guinea pigs have been used and the result showed that, at 10 days old embryo showed newly differentiated blood cells at the future cartilaginous template. At age of 25-35 days only the osteoprogenitor cells, osteoblasts and osteocytes were seeing. At 43 old embryos the hematopoiesis and vascularization were clearly by formation free blood cells and newly formed blood vessels in the marrow cavity. At 54 old day embryo the hematopoiesis was increased and the differentiation of immature white blood cells was noticed at age of 65 day. At the postnatal periods showed a nutrient artery within marrow cavity.

INTRODUCTION

The vasculature in bone is important for skeletal development and growth by affecting both bone modeling and remodeling processes. The majority of bones in the vertebrate skeleton are formed by endochondral ossification, a process initiated in the embryo by mesenchymal condensation، that gives rise to cartilage elements which, in turn, are invaded by blood vessels that bring along perivascular osteoprogenitor cells and recruit osteoclasts leading to these elements being resorbed and gradually replaced by bone tissue (1,2).The developmental pattern of the vascular networks an important component coordinating or directinglimb morphogenesis(3).

MATERIALS AND METHODS

Forty healthy guinea pigs that included both sexes (10 males and 30 females) have been used in this study. Males and female were put together in mating cages for a half day and the pregnancy test was done by vaginal smear test. Ten embryos have been collected at 25 and 35 days of pregnancy periods by cesarean section. Age detection, was done by dependent the crown-rump length (CRL) for corrections.

CRL is the measurement from the vertex of the skull to the midpoint between the apices of the buttocks for prenatal only (4). The embryos and newly born animals were fixed in 10% formalin for 7 days. For well decalcification, the specimens were placed in formic acid-sodium citrate solution 12-36 hours (5). Then routine paraffin technique was dependent and the tissue samples were sectioned at 5-6 µm. The tissue sections were stained with Hematoxyline and Eosin stains (5).

RESULT AND DISCUSSION

Prenatal periods

At 10 days old embryos, the vascularization was started as newly differentiated blood cells at the stage which the mesenchymal cells of the future cartilaginous template condense and differentiate into chondrocytes to form the cartilaginous template of the bone (Fig.1). This result was not compatible with author whom record that the bone did not formed till now, while most studies showed that the endothelium is one of the most important vasculature components on the long bone, because it's functions as an essential barrier that limits the movement of cells and molecules between the circulation and tissues and it is a dynamic organ actively capable of directly communicating with adjacent tissue and circulating blood cells (6, 7).

At age of 25 days old embryo the vascular invasion of the diaphysis of the cartilage template has occurred via the periosteal buds (Fig.2, 3), this results was confirmed by author like (8, 9) whom revealed that the invasion of blood vessels of the mid-diaphysis represents the first hint of bone formation in a continuing process of the formation of primary center of ossification. Localized vascular invasion accompanied by osteoprogenitor cells differentiation into osteoblasts which secrete the matrix. Floyd(10) demonstrated that the development of central hypertrophic cartilage cells preceded and was linked to the rapid vascular invasion of the epiphysis in mice. The vascular bud starts toproject into the bone cartilage interface through the bone plate from the bone marrow of rabbits about 4 weeks after its birth (11).It is generally held that periosteal arteries play some part in the vascularization of tubular bone cortex, because of a free anastomosis between nutrient and periosteal arterial systems within Haversian canals (12).

In advanced age (35 days) the periosteal buds have carried groups of cells from periosteum which represented the osteoprogenitor cells that attached to bone spicules in order to form osteocytes within lacunae and this stage showed no vascularization at the primary center of ossification (Fig.4, 5).These result were parallel with results of (10, 13, 14) who showed the invasion by a vascular budat the diaphysis is closely associated biologically and temporally with the onset of osteogenesis. (8) investigated the invasion of capillaries into the mesenchymal zone, and the emergence and differentiation of mesenchymal cells into mature osteoblasts, these osteoblasts constitutively deposit bone matrix leading to the formation of bone spicules. These spicules grow and develop eventually fusing with other spicules to form trabeculae.

At age of the 43 old embryo the hematopoiesis and vascularization were seeing by formation of free blood cells within marrows cavity (Fig.6,7) in addition to that some of these blood cells were inside newly formed blood vessels (Fig.8), at this stage the population of blood cells was relatively fewer than that of osteoprogenitor cells. These results were compatible with results of (15) who noticed that the endothelial cells were developed into immature vascular networks that enter the bone via cartilage canals already established in the expanding cortical bone in human and mice embryos. Several investigators have suggested that the formation of hypertrophic chondrocytes plays a key role in the vascularization of cartilage and in the formation of bone (16, 10).The significance of vascular canals entering cartilage from its perichondrium is taken to be twofold, to improve the nutrition of the cartilage mass in it deeper parts and to provide blood and possibly osteoblasts for the formation of a new ossify center (17).

At age of 54 day embryo the blood cells population was increased and beat than that of osteoprogenitor cells (Fig.9).These results were parallel with (18) who referred to the great increase of blood cells in the bone marrow. Suleyman (19) observed that the formation of colonies containing multiple blood cell lineages including granulocytes, erythrocytes, monocytes, and megakaryocytes indicates the presence of multilineage hematopoietic stem.

At the 65 day embryo showed differentiation of band (immature) white blood cells within hemopoitic tissue (Fig.10). These result also recorded by (20) who found during or after vascular invasion, the hypertrophied cartilage core was degenerated by chondroblasts, osteoclasts then replaced by bone marrow and later by bone that

initially deposited on calcified cartilage spicules. Roodman (21) reported that the Bone resorbing osteoclasts derive from hematopoietic precursors present in both the bone marrow and the peripheral circulation.In both compartments, endothelial cells separate osteoclast precursors from the bone surface, to reach future sites of bone resorption, osteoclast precursors therefore need to adhere to and migrate through endothelium. This process is likely to be tightly regulated, similar tomechanisms governing the trans endothelial migration of leukocytes and monocytes (22.).

At postnatal periods

The post natal periods showed that the vascularization of bony tissue characterized by marked formation of nutrient artery within marrow spaces which extended into the long bone ends (Fig. 11).These results also showed by (1) that found during postnatal bone growth, canal formation for blood vessels develop in unison with vessel formation at the growth plate region. This result disagrees with (10) who notice that the distal femoral epiphysis was avascular at approximately five days postnatal.

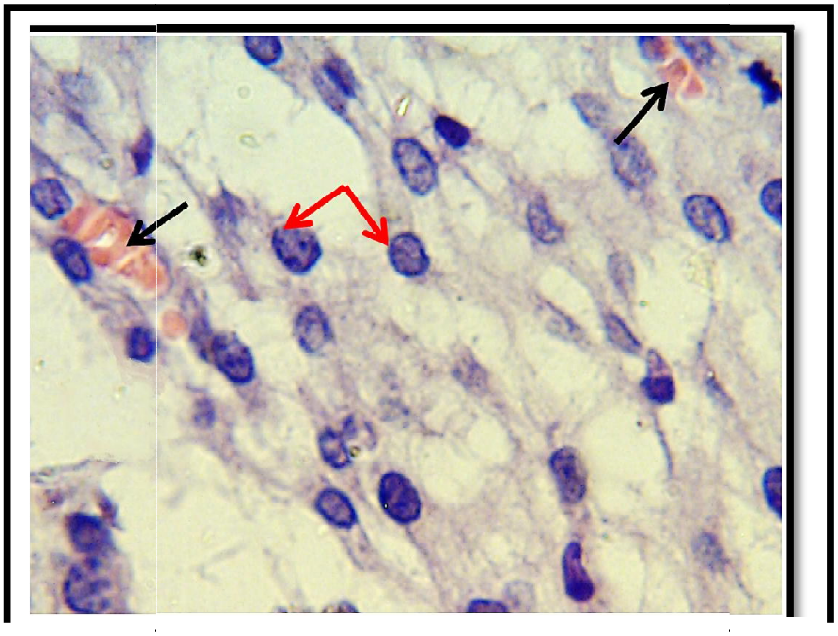

Fig.1: section of mesenchymal tissue in early embryo (10 days old) shows: Mesenchymal cells (Red arrows) and blood cells (Black arrows). H&E stain 400X.

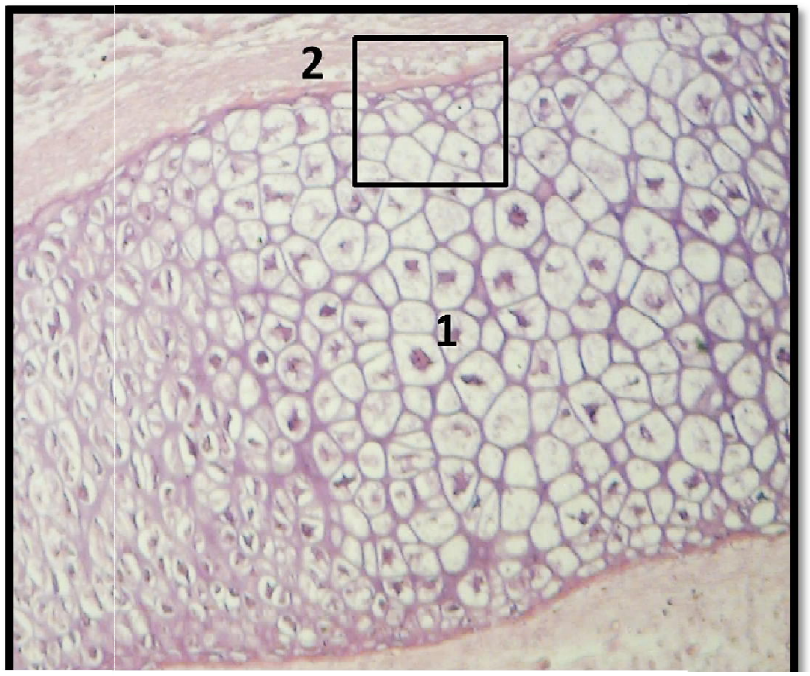

Fig.2: section of fore lime cartilaginous template in early embryo (25 days old) shows: Diaphysis with hypertrophied chondrocytes (1) and perichondrium (2). H&E stain 100X.

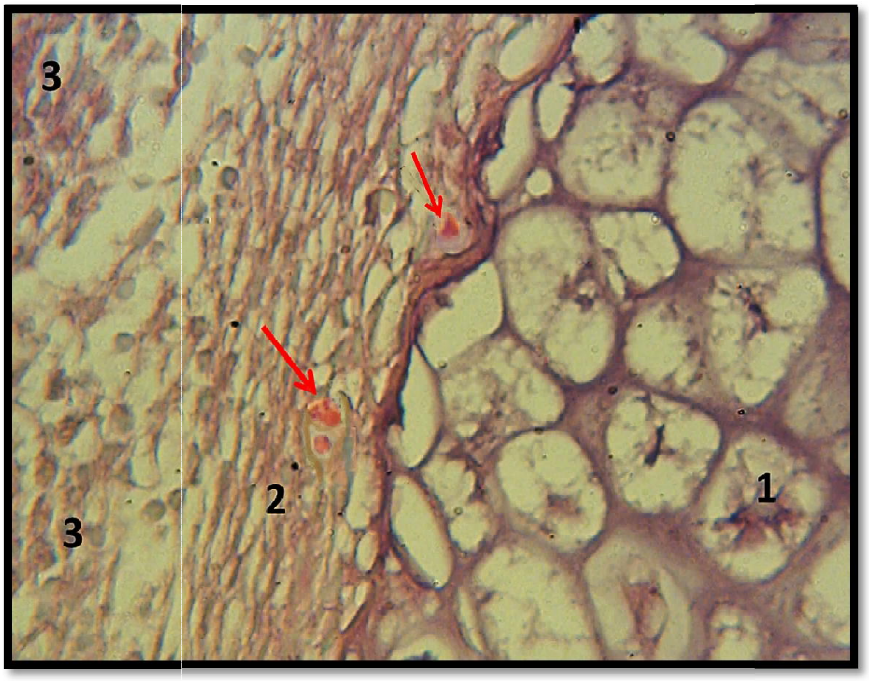

Fig.3: magnified section of black box area in fig (2) shows: hypertrophied chondrocytes (1), perichondrium (2), mesenchymal tissue (3) and blood cells within buds (arrows). H&E stain 400X.

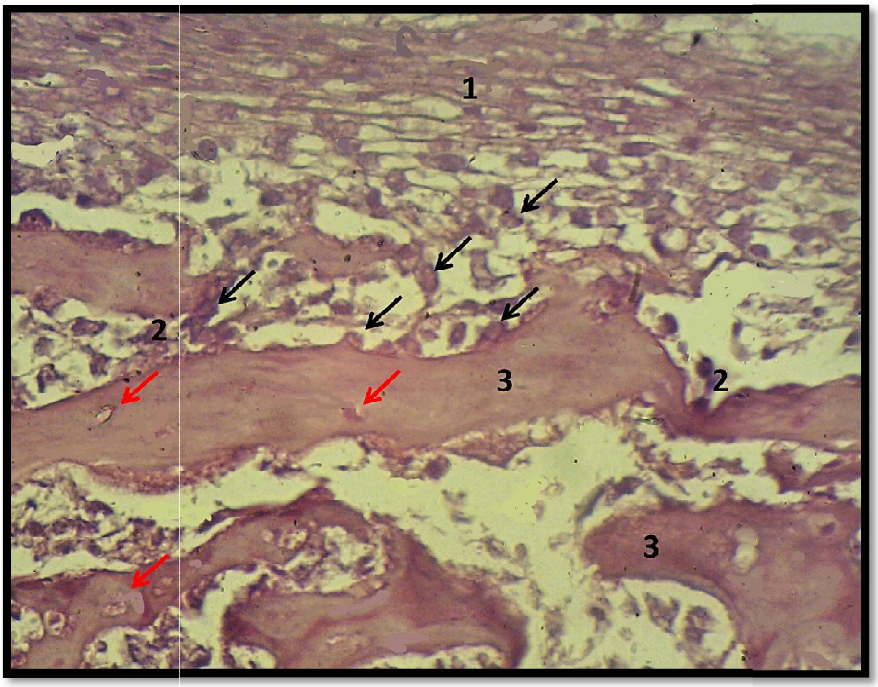

Fig.4: section of fore lime cartilaginous template in early embryo (35 days old) shows: perichondrium (1), buds (2), boney spicules (3), osteoprogenitor cells (Black arrows) and osteocytes(Red arrows). H&E stain 400X.

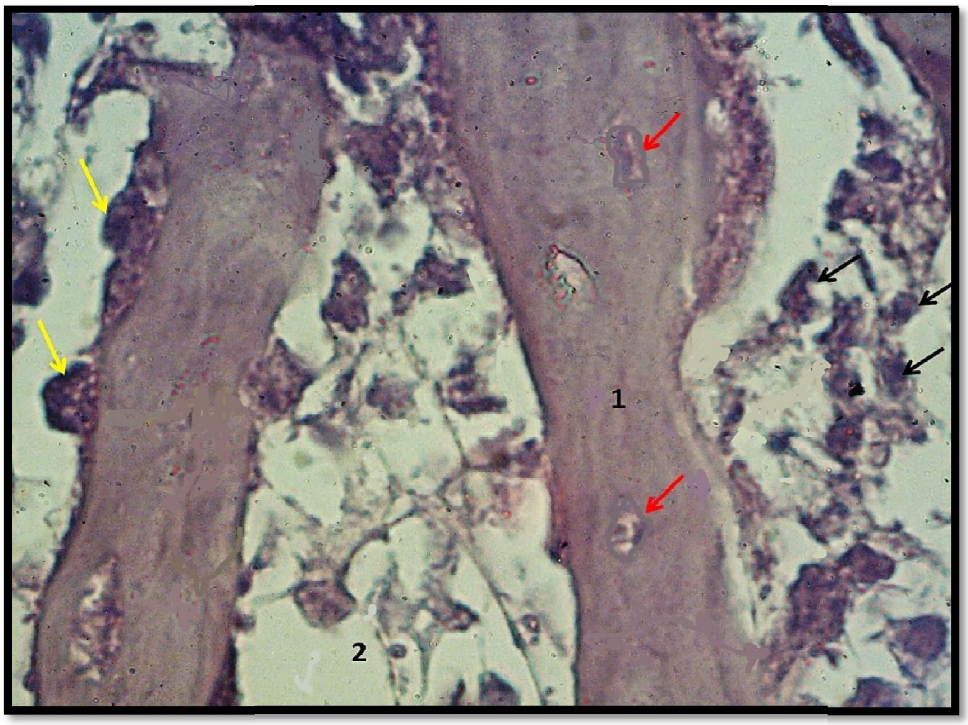

Fig.5: section of fore lime template in early embryo (35 days old) shows: bone spicules (1), marrow cavity (2), osteoprogenitor cells (Black arrows) and osteocytes (Red arrows) and osteoblasts (yellow arrows). H&E stain 1000X.

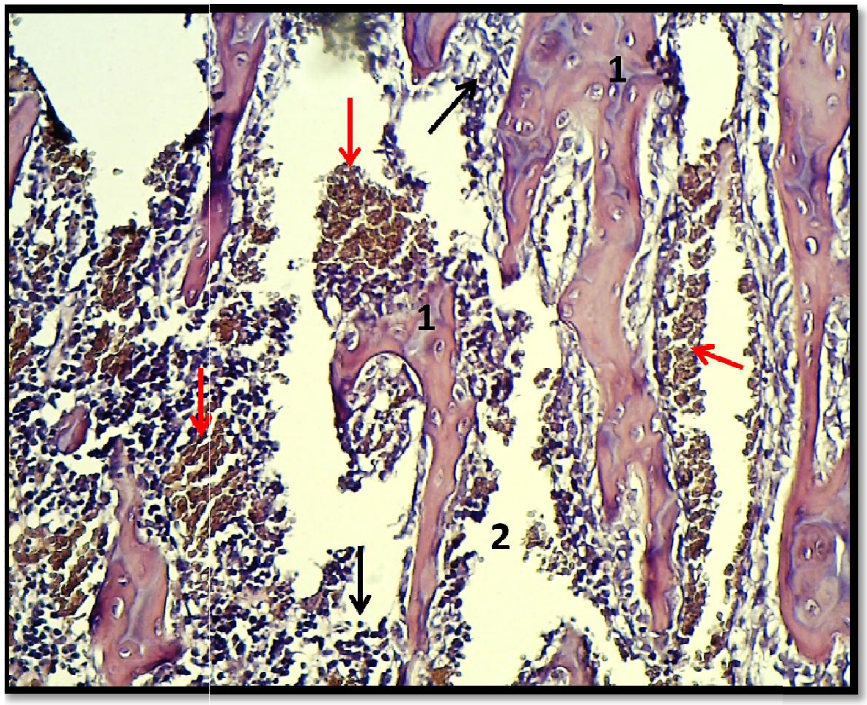

Fig.6: section of femur early embryo (43 days old) shows: bone trabeculae (1), marrow (2), osteoprogenitor cells (Black arrows) and RBCs (Red arrows) . H&E stain 100X.

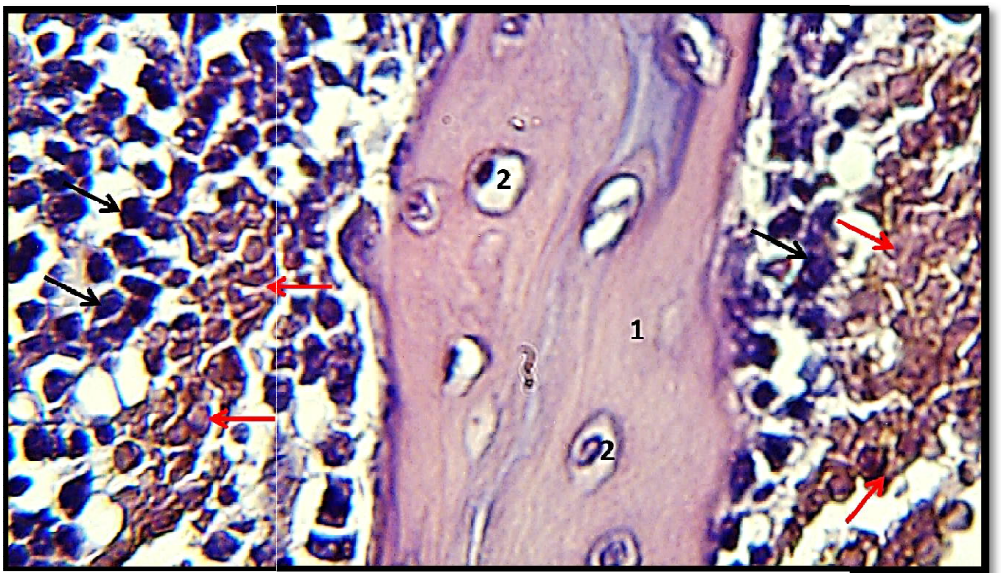

Fig.7: magnified section of femur early embryo (43 days old) shows: bone trabeculae (1), osteocytes (2), osteoprogenitor3 cells (Black arrows) and RBCs (Red arrows) . H&E stain 400X.

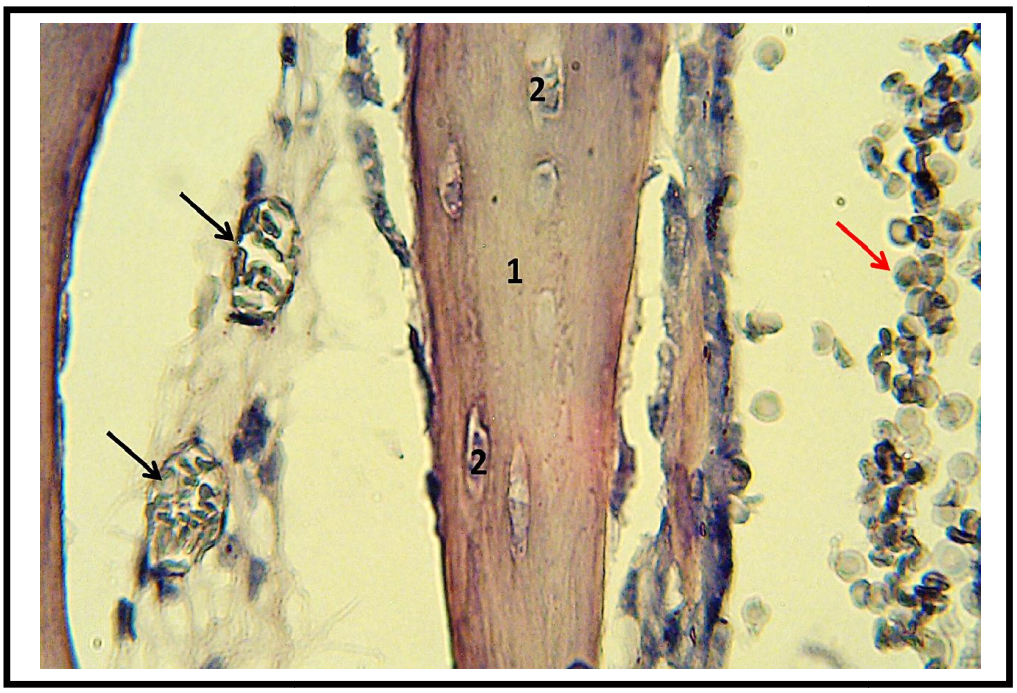

Fig.8: section of femur early embryo (43 days old) shows: bonetrabicula(1), osteocytes (2), free RBCs (Red arrows) and blood vessles (Black arrows). H&E stain 400X.

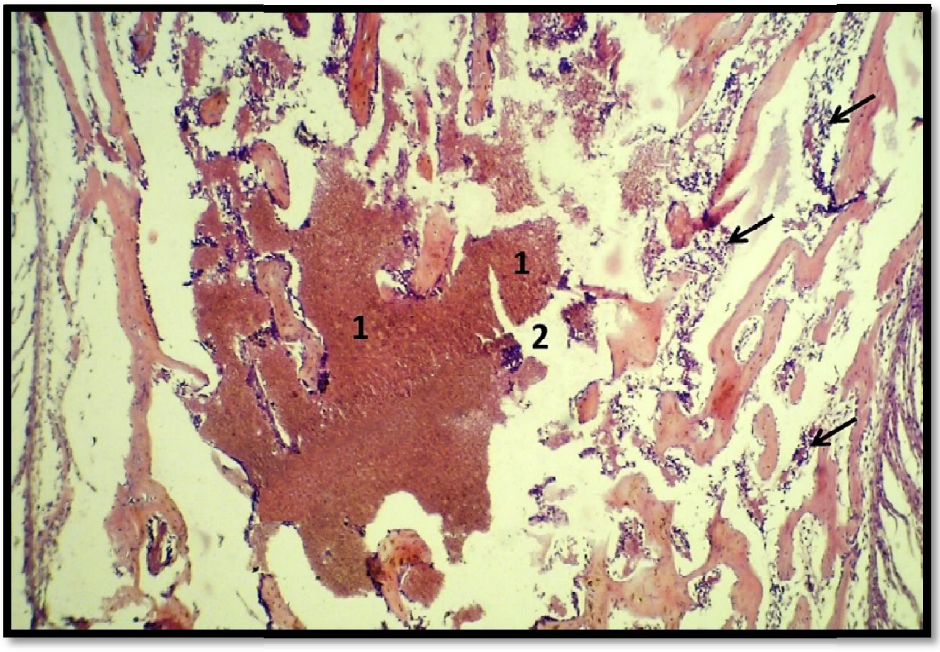

Fig.9: section of femur (54 days embryo) shows: RBCs (1) marrow cavity (2) and osteoprogenitor cells (Black arrows). H&E stain 100X.

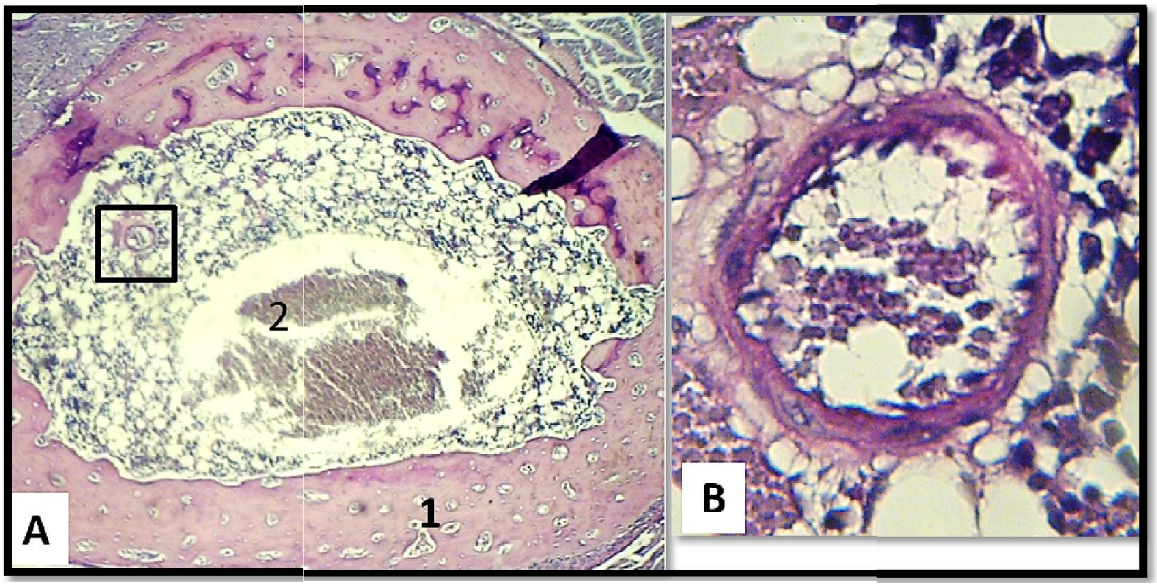

Fig.10: transvers section of ulna (1 day old) Micrograph (A) show: bone wall (1) and red marrow (2). Micrograph (B) shows magnification of black box area in (A) show nutrient artery. H&E stain 400X.

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تطورات ما قبل وبعد الولادة للأوعية الدموية في العظام الطويلة في خنزيرغينيا

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الخلاصة

أجريت هذه الدراسة لتحديد تطور الأوعية الدموية في العظام الطويلة في الحيوانات المختبرية. وقد استخدمت أربعين من خنازير غينيا و أظهرت النتيجة أنه في 10 يوم من عمر الجنين أظهرت تطور خلايا الدم حديثا في قالب الغضروف العظم. في عمر 25-35 يوما من اظهرت تطور خلايا المولدة للخلايا العظمية وخلايا الارومات العظمية والخلايا العظمية ولم تسجل تكون للاوعية. في عمر 43 يوم اظهرت النتائج تكون الدم و الأوعية الدموية بوضوح من خلال تكوين خلايا الدم وتكون الأوعية الدموية في تجويف نخاع . في 54 عمر يوم سجلت زيادة تكون الدم ، ولوحظ تمايز خلايا الدم البيضاء غير الناضجة في سن 65 يوم. في اعمار ما بعد الولادة أظهرت تكوين الشريان المغذي داخل تجويف نخاع .

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