HISTOCHEMICAL STUDY OF MITOCHONDRIA IN THE FEMUR DEVELOPMENT OF PIGEON EMBRYO (*Columba livia*)

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ABSTRACT

Twenty fertilized eggs of pigeon (*Columba livia*) embryo were collected from local markets and pigeon breeders in Basrah governorate.They incubated in eggs incubator .The study comprised histochemical description of mitochondria decreasing and increasing in it density in femur bone development of pigeon embryo (*Columba livia*) which included appearance of the mitochondria during the embryological stages. The study showed the mitochondria is spindle in shape and few in resting chondrocyte stage , while it was more in hypertrophy chondrocyte and more density in ossification in osteoblast stage .The aim of this study is know the density of mitochondria during the development of the femur bone of pigeon embryo.

INTRODUCTION

The bones of the axial and appendicular skeleton are formed by one of two processes, intramembranous or endochondral bone formation. In intramembranous bone formation is formed in the absence of a cartilage model while in endochondral bone formation, a cartilage model is first formed and then replaced by bone tissue(1,2).The flat bones of the skull and face are formed by intramembranous ossification(3).Osteoprogenitor cells,which gives rise to osteoblasts and osteocytes are present within the mesenchyme (1, 4) . These cells aggregate at the sites where new bone is to be formed and differentiate into osteoblasts that actively

synthesize new bone matrix(5). Endochondral ossification involves the formation of cartilage tissue from aggregated mesenchymal cells and the subsequent replacement of this cartilage tissue by bone tissue (6) . All the skeletal components of the vertebral column, pelvis, and appendicular skeleton (limbs)was developed via endochondoral ossification(7).The process of endochondral ossification is divided into five stages(8,9). First, the mesenchymal cells are committed to become cartilage cells.During the second phase of endochondral ossification, the committed mesenchymal stem cells condense into compact nodules and differentiate into chondrocytes.During the third phase of endochondral ossification, chondrocytes proliferate rapidly to form the cartilage model that will eventually be replaced by bone tissue(10).As they divide, chondrocytes secrete a cartilage- specific extracellular matrix. In the fourth phase, the chondrocytes stop dividing and become hypertrophic. Hypertrophic chondrocytes have increased production of collagen type X and fibronectin, thus altering the remaining cartilage matrix so that it can be mineralized by calcium carbonate. Finally, in the fifth phase, blood vessels begin the invasion of the cartilage model. The hypertrophic chondrocytes undergo apoptosis and the spaces are invaded by ingrowing blood vessels(11). As the cartilage cells die, osteoprogenitor cells differentiate into osteoblasts and begin to lay down bone matrix on the partially-degraded, mineralized cartilage remnants (12,13). The mitochondria were most numerous in large osteoblasts, in which they occupied every part of the cytoplasm except the juxta-nuclear vacuole, and generally tended to be orientated in the long axis of the cell (14) .In the development of cartilage , the mitochondria were most numerous in the perichondrial cells(15). In both the osteoblastic and chondroblastic series of cells, the mitochondria were most numerous where the rate of deposition of intercellular matrix was greatest.The mitochondrial content of the mesenchymal cells, reticular cells and of the osteoblasts was increased as these cells became osteoclasts(16,17,18).Chang stated that the osteoclasts contained more mitochondria than any

other cell in the area of bone formation(14) .The aim of this study is detect the mitochondria density, while the femur bone development of pigeon embryo have been occurred .

MATERIALS AND METHOD

Twenty fertilized eggs of pigeon embryo (*C. livia*) were collected after laying ,then incubated in an automatic incubator at 37 ◦C with humidity (50%-70%) for different stages, in order to obtain embryo at different ages. The incubated eggs with different ages (8,10,12,14,16, 18) days were tested by candling to know the presence of whole embryo to start histochemical study, then all embryonated egg shell was broken at wide side by small scissors ,then washing with normal saline to remove the yolk. The embryo were carefully taken out from the egg shell by forceps and put in a petri dish containing formalin. The skin was removed and then the viscera were taken out from the body by forceps carefully. Skeleton was cut to parts , the femur was taken,because of bone hardness , it must be undergo decalcification by put it in diluted nitric acid solution 5% for 3 day . This solution dissolve all the calcareous salts , but in the same time , the bone retains with shape and structure . It just becomes simple to cutting and banding.`Histological sections of femur of embryo with different ages were prepared by steps according to Luna(19) . Staining the sections with acid fuchsin , methyl blue and HCl according to (20).

RESULT

Mitochondria are present as spindle elongated bodies red in color in the resting chondrocyte stage , (figure 1,2), and increase in density in hypertrophy chondrocyte stage ,(figure 3,4), but during the late stages of embryonic bone development , the mitochondria was more increasing in density in osteoblast in ossification stage, (figure 5,6)

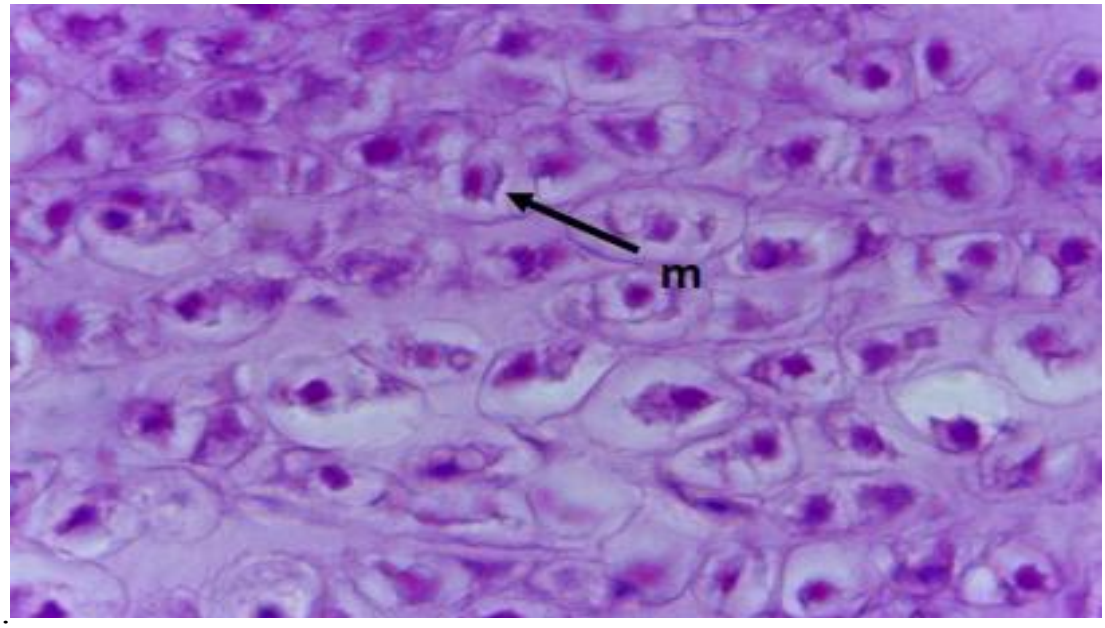

Figure (1):Longitudinal section in femur in pigeon embryo at 8 days of incubation showed mitochondria (m) in resting chondrocyte stage stained with acid fuchsin stain.1000x

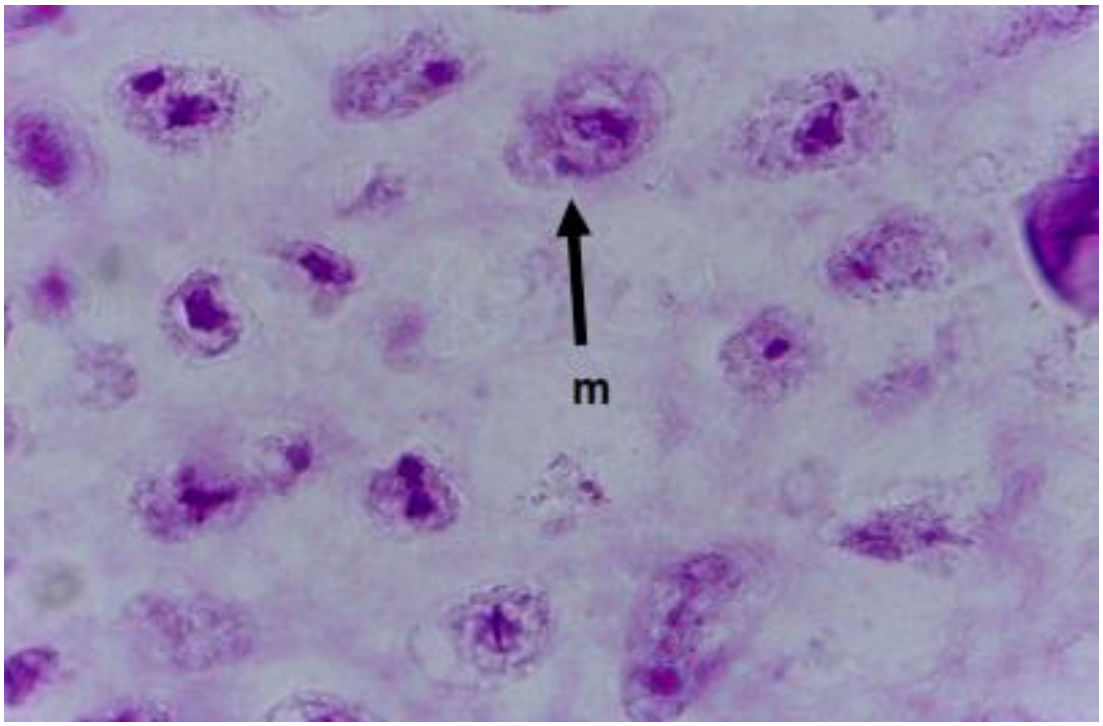

Figure (2):Longitudinal section in femur in pigeon embryo at 10 days of incubation showed mitochondria (m) in resting chondrocyte stage stained with acid fuchsine stain.1000x

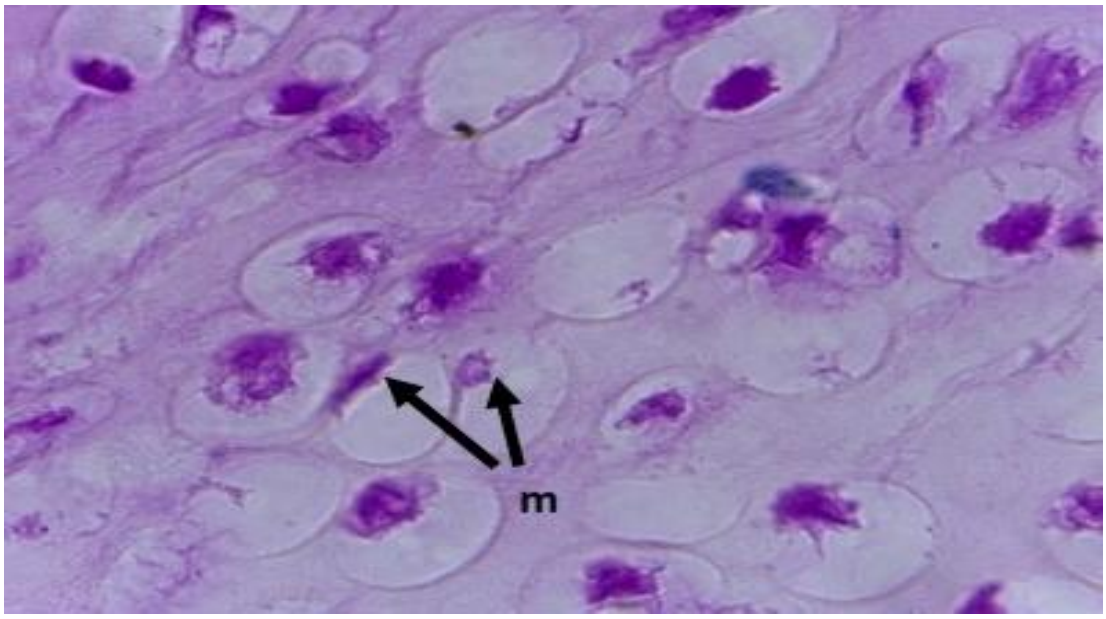

Figure (3):Longitudinal section in femur in pigeon embryo at 12 days of incubation showed mitochondria (m) in hypertrophy chondrocyte stage stained with acid fuchsine stain.1000x

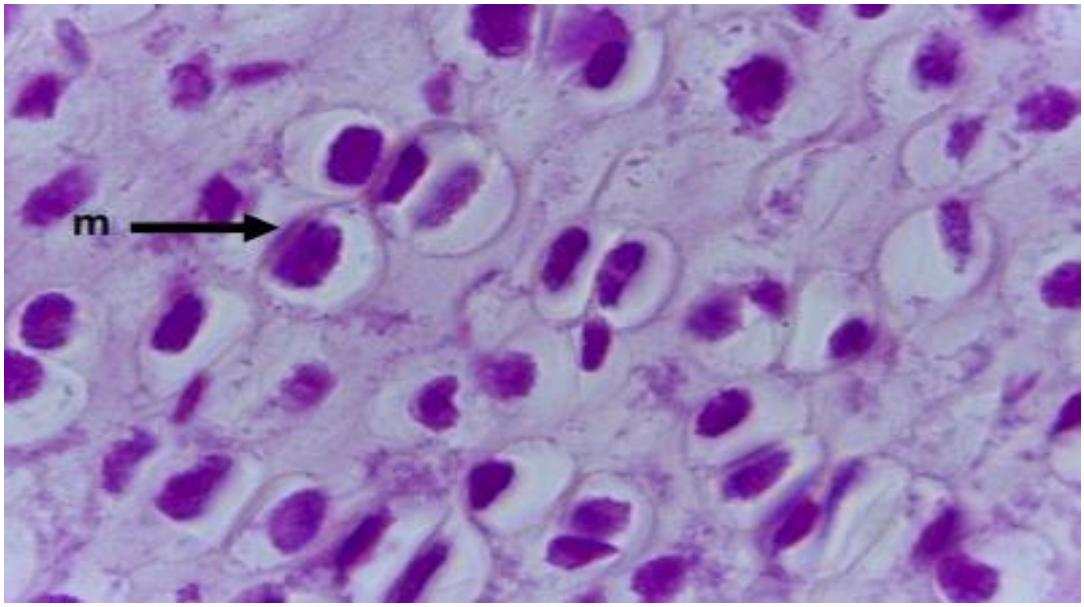

Figure (4):Longitudinal section in femur in pigeon embryo at 14 days of incubation showed mitochondria (m) in hypertrophy chondrocyte stage stained with acid fuchsin stain.1000x

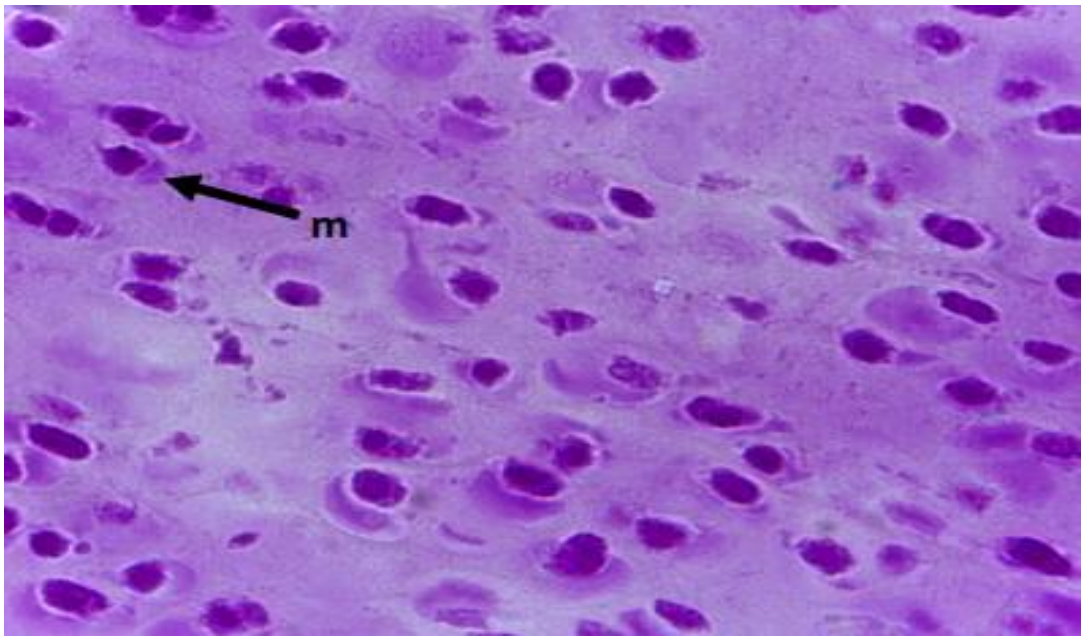

Figure (5):Longitudinal section in femur in pigeon embryo at 16 days of incubation showed mitochondria (m) in ossification stage stained with acid fuchsin stain.1000x

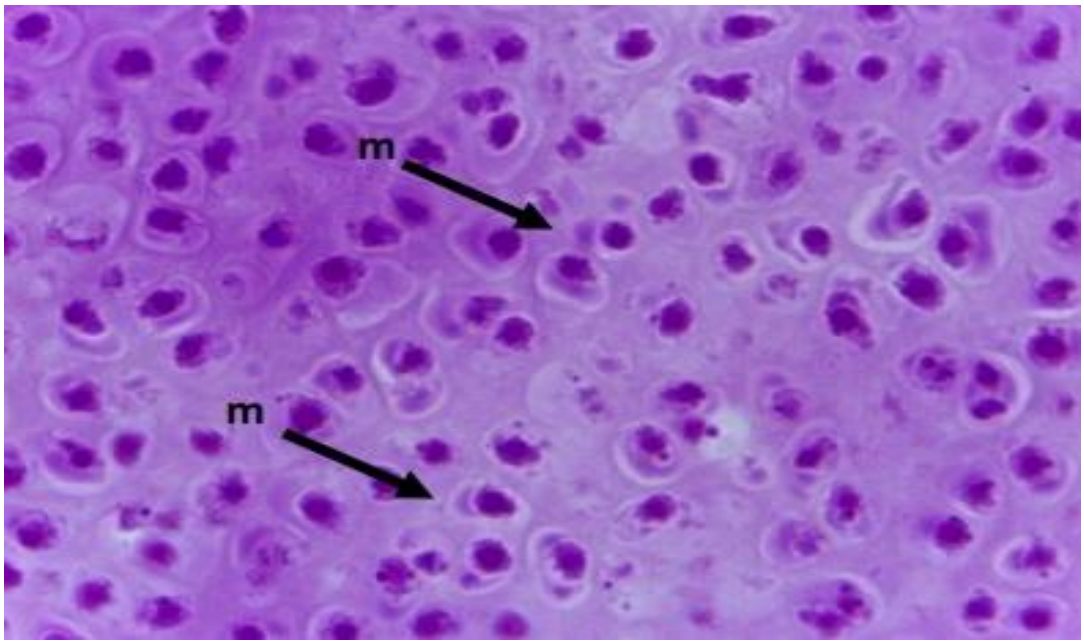

Figure (6):Longitudinal section in femur in pigeon embryo at 18 days of incubation showed mitochondria (m) in ossification stage stained with acid fuchsine stain.1000x

DISCUSSION

Mitochondria has active role in the cytoplasmic transport of calcium ions which may accumulate also inorganic deposits during ossification process, so mitochondria participates in calcification of bone, also mitochondria provides the bone cells with energy that it produce in form as adenosine triphosphate (ATP) during bone formation(21). The results of primary stages of bone development are in agreement with the findings of Pritchard, (1952) (22).The formation of the matrix, mitochondrial content increased rapidly in the osteoblasts. In the later stages of development the density of mitochondria increase in the cells area of rapid bone formation. In either areas, at the late stages of development, the density of mitochondria is parallel the rate of deposition of the bone matrix. The cells with a large density of mitochondria is metabolically very active cells(23). The osteoblasts are active cells in this observation. when considered in view of the other histochemical findings reported in this investigation, strongly suggests that the osteoblast is actively engaged in the synthesis and secretion of certain components of the bone matrix. After the formation of the matrix on the 12 day of incubation, the density of mitochondria in the osteocytes indicate that these cells continue to produce the bone matrix, not as rapidly as osteoblasts.

CONCLUSIONS

The observations in this study confirmed that the mitochondria are increased in density with development of bone. In the primary stages of development bone (8 days of incubation) of the femur bone , the chondrocyte contains slight density of mitochondria. During the 14 day of incubation , the chondrocyte matured and contained increasing in the density of mitochondria.The increasing of mitochondria density in ossification stage continue to 16 days of incubation.

دراسة كيمونسيجية لتطور المايتوكوندريا في عظم الفخذ في اجنة الحمام(*Columba livia*)

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الخلاصة

جمعت عشرون بيضة مخصبة لاجنة الحمام (*Columba livia*) من الاسواق المحلية ومربي الحمام في محافظة البصرة. حضنت في حاضنة للبيض. تضمنت الدراسة الوصف الكيمونسيجي للمايتوكوندريا من حيث زيادة او نقصان كثافتها في تطور عظم الفخذ لأجنة الحمام والتي تضمنت ظهور المايتوكوندريا خلال المراحل الجنينية (8، 10، 12، 14، 16، 18)يوم . حيث بينت الدراسة ان المايتوكوندريا تكون مغزلية الشكل قليلة العدد في المراحل المبكرة من التطور و ،من ثم تزداد في الكثافة في مرحلة تضخم الغضروف. و تزداد كثافتها في مرحلة التعظم. هدف الدراسة هو معرفة كثافة المايتوكوندريا من خلال تطور عظم الفخذ في جنين الحمام.

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