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THE STUDY OF THEHISTOGENESIS OF SKELETAL MUSCLESTISSUE TO THE AORTIC SMOOTH MUSCLES TISSUE DURING EMBRYONIC OF RAT

VAHID ROSTAMI ZADEH[1*], IRAJ POUSTY[2]

M.A Student, Department of Basic Sciences, Science and Research branch, Islamic Azad University, Tehran, Iran (vahidrostamizadeh92@gmail.com)

Professor, Department of Basic Sciences, Science and Research branch, Islamic Azad University, Tehran, Iran

ABSTRACT

In this study, Wistar rats were used. Two male and female rats were placed in a cage in order to intercourse. After 14 hours, vaginal sperm test was taken from the female rats. After determining the sample containing the sperm, the rats were marked and that day was considered the zero day of rat pregnancy. On the seventh day of pregnancy, the fetus sample was taken from the rats; the pregnant female rats were anesthetized by a dose of anesthesia of tiopentan or a combination of ketamine and xylazine intraperitoneally. After anesthesia, the fetus was separated and then it was fixed by Bouin solution. Samples from pregnant rats were taken on the seventh day to the twenty-first of the pregnancy. The results showed that changes in skeletal muscles tissue to the aortic smooth muscles tissue were observed intangibly during the embryonic. On the one hand, the heart muscle cells were reduced by going away from the intersection of the heart to the aortic heart skeletal muscle, and the smooth muscle cells of aorta were seen at the end. Changes in the fetal period were the same. Of course, the number of skeletal muscles in aorta was gradually declined compared to the past days and different periods approaching from the fifteenth day of the embryo to the birth so that on the twenty-first day, the aorta was formed entirely from smooth muscle cells.

Key words:histogenesis, skeletal muscles tissue, smooth muscles tissue, Aortic, Heart, Rat

INTRODUCTION

The heart is the first organ to undergo practical differentiation. In the early stages of fetal life, the spontaneous contractions of the heart muscle cells appear and this spontaneous beat is the main basis for life. In the rodents' heart, the blood is supplied to the heart by branches of two arteries internal thoracic artery and subclavian artery in addition to coronary arteries (2). The unique characteristic of cardiac muscle cells, by which can be distinguished from other muscle cells is the cross dark band that cut the chain of heart cells at irregular distances (1). There are tissue changes in skeletal muscle in the heart to the aortic smooth muscles, and these changes vary in different times of histogenesis. Moreover, it is histologically important when the changes happen. The results of this study can serve as a base knowledge on the issues of comparative histology of heart and aorta, heart anatomy, heart physiology, heart pathology and cardiology for the scholars and experts.

In a study of the embryonic development in a chicken's heart using light and electron microscope, Monask (1968) found that fetal heart muscle cells contain large amounts of free ribosomes and glycogen particles associated with granular endoplasmic reticulum. Unlike the developing skeletal muscle, some granular endoplasmic reticulum in the cytoplasm of heart muscle cells belongs to the Golgi apparatus of hypertrophy (6).

Hirooma and Hirroka (1992) examined the embryology of layers of the tunica media of the chicken's aorta and said that the first distinction of the smooth muscle cells in the tunica media was accompanied by increasing the number of cell layers that reached about 20 layers during 8 days. Moreover, aortic tissue is similar to the maturation stage in adult hens one day after hatching chicks(4).

Heterogeneous virus of Avian Leukemia and vectors causing liver necrosis in different types of are preferably expressed in the heart ofAvin embryos.Using a computer method, the clones tagged by two vectors are analyzed in terms of frequency, anatomical and sub-anatomic position, the number of nuclear chain Hoecht and average cell division time during the formation of the heart (heart morphogenesis: from 17-19 to 37 stages). The analysis shows that clones tagged by these vectors have the same features and lead to the knowledge of the relationships between meiosis and immigration properties of myocardial cells and histogenesis of the heart. Since only external cells were incubated with our method, the analysis shows that (1) in the stages 17-19, myocardium is made up of cells

that have different capabilities. Some cells retain the capacity to divide and become heart muscle cells, whereas most cells have a limited proliferation and make up discrete layers of muscle cells (3).

With the increased aging of human, the changes that occur in the aorta are very significant and recognition of the changes is important to interpret the form of aortic tissue. From the beginning of the birth, aorta has a complex structure, so we must understand the process of formation of this tissue in order to understand the origin and relationships of different parts of the aorta (5). Chicken embryonic myocardium (the stages +8 to -12) were studied by light and electron microscope. Myocardium, which initially has concentric cells with large intercellular spaces, gradually becomes more concentrated. Intercellular spaces are reduced and the cells are in asymptotic position (7).

MATERIALS AND METHODS

mating: is the cycle of rat activity during the dark. Therefore, two female and male rats were placed in a cage so that they could intercourse.

Pregnancy test: After 14 hours, vaginal sperm test was taken from the female rats. After determining the samples containing the sperm, the rats were marked and that day was considered the zero day of rat pregnancy.

Sampling: the pregnant female rat was anesthetized by a dose of anesthesia of tiopentan or a combination of ketamine and xylazine intraperitoneally. Samples from all the pregnant rats were taken on the seventh day to the twenty-first of the pregnancy and were fixed by Bouin solution. One female rat per day and five samples per female rat were tested as iteration. The difference is that since the seventh to eleventh days old embryos were small and could not be removed from the uterus, fixing was carried out in the uterus. However, the embryos of the twelfth to the twenty-first days old were removed from the uterus and amniotic sac and then fixing was carried out.As the intended organ in this study is the heart and the aorta, the heart of fifteen to twenty-one-day-old embryos was removed by forceps and scalpel beneath the loop. Thefifteenth to the twenty-first day embryos were first fixed and the fixed embryo was removed beneath the heart loop after 2 days.The early fixing of embryos is why that its heart cannot be removed and as the fetal heart is not fixed, it even seems impossible. Moreover, embryos under fifteen days were very young and it is very difficult to remove their heart.

Cell count: after preparation of slides for further study, the heart skeletal cells and smooth cells of aorta were counted. After

checking the slides under the microscope, their hearts were observed from the fifteenth day of the twelfth day and the aorta was visible from the fifteen. The fifteenth to twenty-one-day samples were evaluated that were originated from the heart. Thus, five iterations were counted per sample. Two areas in the heart and five areas in theconfluence of the heart to the aorta to the aorta with area 0.3 mm× 0.3 mm with a lens magnification of 40 were consecutively counted from each of the slides. Then, the number of muscle cells and aortic smooth muscle cells of the heart were counted. Similarly, all the samples were counted from the fifteenth to the twenty-first day.

RESULTS AND DISCUSSION

The effect of different areas of sampling and different embryonic days on the number of cells: according to the analysis of variance table, different levels of sampling and different embryonic days of the cells are significant at 0.5%. As shown in the table, the number of skeletal cells reduced in all areas from three to seven approaching the twenty-first day and the number of aortic smooth cells has been increased. So that, the number of skeletal cells reached zero on the twenty-first day in all the areas and the greatest number of aortic smooth cells were observed at the same day. However, all the muscles are skeletal in the first and second areas and there was no aortic smooth cell. As seen in the table, the number of cells varies in different days so that the number of skeletal cells is greater than the aortic smooth cells in all the areas on the fifteenth, sixteenth and seventeenth days. The number of skeletal cells, except for the seventeenth day in the third, fourth and fifth areas and for the sixteenth day in the fifth area that had no significant difference from the number of aortic smooth cells, were statistically significant in all other areas on the fifteenth to seventeenth day ($p<0.05$) and the number of number of skeletal cells was significantly greater than the aortic smooth cells. However, it reached a relative balance on the eighteenth day. The number of skeletal cells was significantly greater than the aortic smooth cells in the third area on the eighteenth day. The number of skeletal cells was greater than the number aortic smooth cells in the fourth area on the eighteenth day but there was no significant difference in terms of the number of cells. The number of the cells was reversed in the fifth day on the eighteenth day and the number of aortic smooth cells was greater than the number of skeletal cells but the two cells did not statistically have significant difference. However, the number of aortic smooth cells was significantly greater than the

number of skeletal cells in the sixth and seventh day. The condition was the same during the nineteenth to the twenty-first days and in the number of aortic smooth muscle cells are greater than the number of skeletal muscle cells, and this difference was statistically significant.

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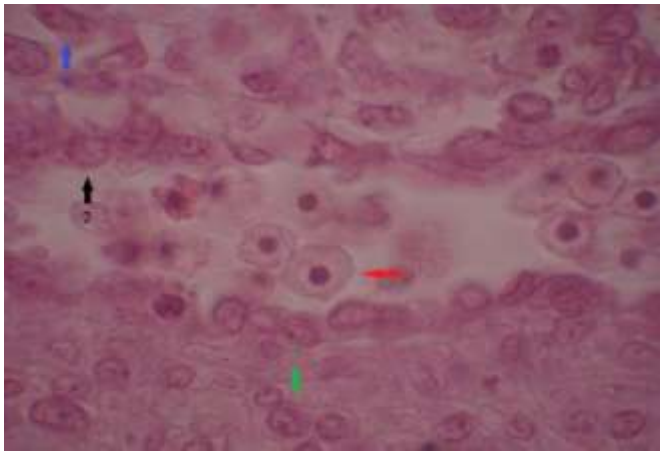
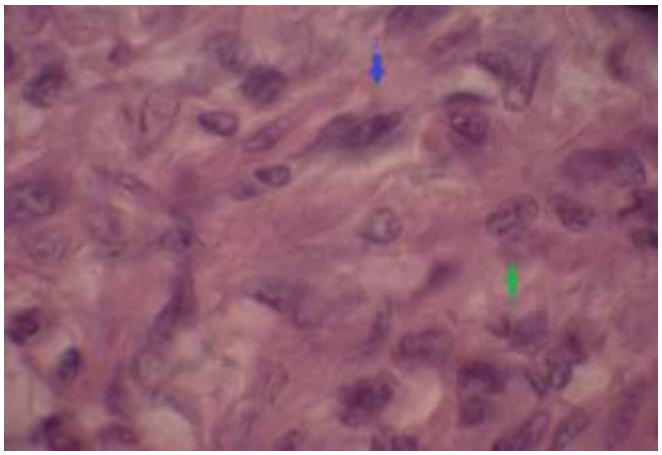


H&E ,X1600 A slice of the heart to the aorta in the fetus 15 days
1.Erythrocytes cell 2.Endothelium cell 3.Skeletal muscle cell 4. Smooth muscle cell



H&E , X2000 A slice of the heart to the aorta in the fetus 21 days
1. Skeletal muscle cell 2. Smooth muscle cell

	Cell type	Day 15	Day 16	Day 17	Day 18	Day 19	Day 20	Day 21
District 1	Skeletal muscle cell	6/73±0/32	8/23±0/42	6/72±0/38	7/38±0/45	6/84±0/36	6/37±0/27	5/43±0/78
		B	**A**	**C**	**c**	**de**	**e**	**e**
	Smooth muscle cell	0±0	0±0	0±0	0±0	0±0	0±0	0±0
		e	**e**	**De**	**d**	**c**	**c**	**bc**
District 2	Skeletal muscle cell	7/26±0/51	8/78±0/32	7/16±0/87	6/94±0/43	6/68±0/34	5/92±0/64	6/17±0/25
		bc	**b**	**c**	**cd**	**e**	**e**	**e**
	Smooth muscle cell	0±0	0±0	0±0	0±0	0±0	0±0	0±0
		e	**d**	**d**	**cd**	**bc**	**c**	**bc**
District 3	Skeletal muscle cell	6/27±0/38	6/95±0/52	4/83±0/83	4/71±0/68	2/18±0/83	1/53±0/54	0/95±0/61
	Smooth muscle cell	**c**	**bc**	**c**	**cd**	**e**	**e**	**e**
		1/21±0/13	1/75±0/31	2/11±0/68	2/83±0/69	4/18±0/54	5/01±0/68	5/71±0/51
		de	**d**	**cd**	**c**	**c**	**bc**	**bc**
District 4	Skeletal muscle cell	5/75±0/26	6/39±0/48	4/52±1/22	3/42±0/46	2/01±1/16	0/95±0/69	0±0
	Smooth muscle cell	**c**	**bc**	**c**	**d**	**e**	**e**	**e**
		1/63±0/19	2/25±0/58	2/7±0/68	3/14±0/49	4/95±0/32	5/11±0/78	6/08±0/41
		de	**d**	**cd**	**c**	**bc**	**bc**	**bc**

District 5	**Skeletal muscle cell**	5/27±0/27	5/71±1/03	4/48±0/75	3/52±0/71	2/01±1/21	0/32±0/11	0±0
		c	**bc**	**c**	**de**	**e**	**e**	**e**
	Smooth muscle cell	2/25±0/28	3/02±0/38	3/31±0/28	4/16±0/35	5/12±0/75	5/83±0/61	6/22±0/46
		d	**cd**	**cd**	**c**	**bc**	**bc**	**bc**
District 6	**Skeletal muscle cell**	4/95±0/51 **c**	5/42±0/78 **c**	3/94±0/45 **c**	2/82±0/28 **c**	1/17±0/73 **c**	0/62±0/83 **c**	0±0
	Smooth muscle cell	2/51±0/12 **c**	3/12±0/46 **c**	3/75±0/45 **c**	4/32±0/84 **c**	5/78±0/71 **c**	5/62±0/53 **c**	6/21±0/65
District 7	**Skeletal muscle cell**	4/65±0/66 **c**	5/87±0/38 **c**	4/21±0/52 **c**	2/43±0/77 **c**	1/32±0/44 **c**	0±0 **c**	0±0
	Smooth muscle cell	2/85±0/36 **c**	3/21±0/43 **c**	3/78±0/55 **c**	4/65±0/26 **c**	5/46±1/15 **c**	5/85±0/34 **c**	**c**6/15±0/63

The interaction between different areas and different days of embryonic sampling on the number of cells