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Brief Article

Cardiocentro ERNESTO CHE GUEVARA SANTA CLARA CUBA

An approximation of cardiac dimensions in the human embryo at Carnegie stage 22

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Acronyms
3DUS: three-dimensional ultrasound

ABSTRACT

Introduction: Cardiac prenatal growth has been a topic of research, and it has allowed establishing the normal curve of fetal heart volume.

Objective: To obtain, in a novel way in our country, the volume of the embryonic heart at Carnegie stage 22, at week 8 of development.

Method: Two human embryos from this stage were studied at the embryo gallery of the Faculty of Medicine of Villa Clara. The two specimens were processed by paraffin technique, their cuts were digitized and the heart areas were measured in all serial sections of the heart. To calculate the volume, the thickness of the cut was multiplied by the sum of partial areas.

Results: Volumes of 6.137 mm3 and 6.004 mm^3 were obtained in both specimens.

Conclusions: The results provide a scientific approximation of the actual dimensions of the heart at this stage of development.

Key words: Heart, Human embryo, Morphometry

Una aproximación a las dimensiones cardíacas en el embrión humano del estadio 22 de Carnegie

RESUMEN

Introducción: El crecimiento cardíaco en la etapa prenatal ha sido motivo de investigación, y ha permitido establecer la curva de normalidad del volumen del corazón fetal.

Objetivo: Obtener, de forma novedosa en nuestro medio, el volumen del corazón embrionario en el estadio 22 de Carnegie en la semana 8 del desarrollo.

Método: Se estudiaron dos embriones humanos de este período, pertenecientes a la Embrioteca de la facultad de Medicina de Villa Clara. Ambos especímenes fueron procesados por la técnica de parafina, digitalizados sus cortes y medidas las áreas cardíacas en la totalidad de las secciones seriadas del corazón. Para el cálculo del volumen se empleó el espesor del corte multiplicado por la sumatoria de áreas parciales.

Resultados: Se obtuvieron volúmenes de 6,137 mm^3 y 6,004 mm^3 en ambos especímenes.

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Conclusiones: Los resultados obtenidos brindan una aproximación científica a las dimensiones reales del órgano en esta etapa de su desarrollo.
Palabras clave: Corazón, Embrión humano, Morfometría

INTRODUCTION

Human development, in the first 8 weeks, includes the pre-embryonic and the embryonic periods. In them, there are typical processes such as segmentation, blastulation, implantation, gastrulation and organogenesis[1]. For this space of time, 23 stages have been identified in relation to the size of the embryo and its degree of development[1,2].

The formation of the human heart begins in the middle of the third week when the cardiogenic field is defined; which subsequently rotates due to the folding of the embryo and its lateral portions merge to form a single tubular heart that starts functioning in the fourth week[3,4]. Shortly after, there are complex processes of folding, intracardiac changes and septations that transform the internal and external morphology of the organ, to clearly anticipate, in the eighth week, what will be its final anatomy.

Currently, the genes, transcription factors and proteins involved in cardiogenesis are well known. It includes the gene NKX 2.5, the combination of BMP activity and inhibition of WNT proteins, expression of FGF-8, TBX-5; laterality genes such as nodal and lefty-2 genes; and transcription factors such as PITX 2, HAND 1 and HAND 2[4].

Cardiac prenatal growth has also been investigated, both, through postmortem studies[5,6] and *in vivo* studies by 3D and 4D ultrasonography including the STIC technology (Spatio-Temporal Image Correlation), which has allowed establishing the normal curve of fetal heart volume[7,8]. In the embryonic period, these investigations become more complex due to the smallness of the embryo, particularly the heart, and due to the dynamic nature of cardiac morphology between the fourth and the eighth week, when the main congenital heart defects are generated. For this reason, the embryonic stage of the heart is now a topic of interest for the scientific community. This study was conducted in order to obtain, in a novel way in our country, the cardiac volume from two human embryos at Carnegie stage 22.

METHOD

Two specimens from the embryo gallery of the Faculty of Medicine of Villa Clara were studied. They were previously classified according to the Carnegie criteria[2]. Both came from medicated abortions (misoprostol), with traces of normalcy in their external appearance, and had been labeled as M-75 and M-88. Their maximum skull-spine lengths were 25 and 27 mm, respectively, which, in conjunction with the external appearance, allowed their classification at Carnegie stage 22, week 8.

Tissue processing was carried out through paraffin technique, staining with hematoxylin and eosin, and serial cuts of 10 micron thickness, in the sagittal plane in the case M-75, and transverse plane, in M-88.

For a morphometric study of the organ, 278 cuts were digitized in M-75 and 157 in M-88, with the use of a DCM 500 digital camera coupled with a stereo microscope MBC-10 (1x objective). The Scope Photo 3.0 software and its polygon option was used to obtain the cardiac area variable (**Figure**), which was measured 7 times in each cut and then the arithmetic mean was calculated per cut. The formula suggested by Marantos Gamarra[5] in his doctoral thesis on cardiac morphometry of the human embryo at O'Rahilly stage 16 was used to calculate the volume:

$$V = e\sum_{i=1}^{i=n} na_i$$

where:
e = thickness of the cut
ai = area of the organ in each cut
n = number of cuts

RESULTS

In the 278 means of cardiac area that were obtained in M-75, the minimum value was 0.01 mm^2, the

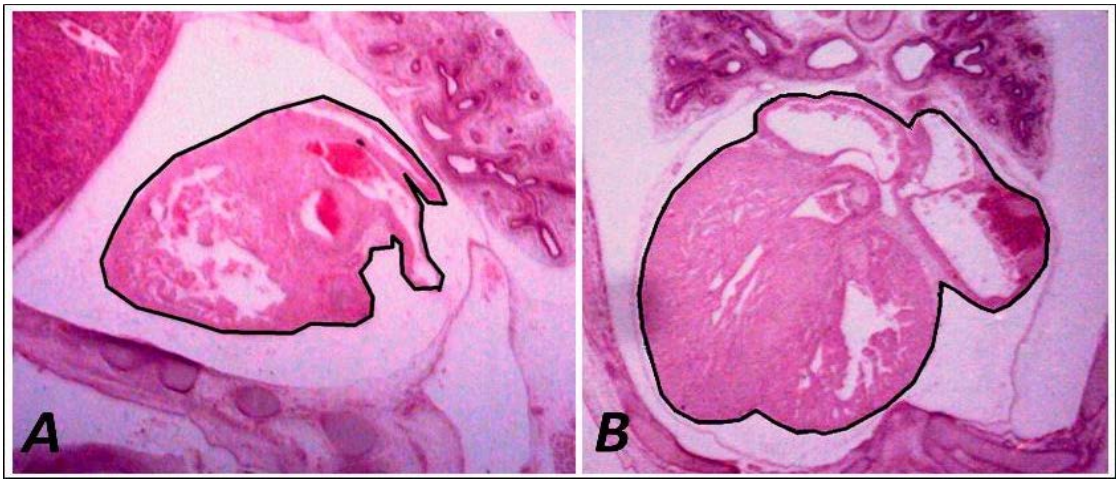

Figure. Microphotographs of human embryos (hematoxylin and eosin). **A.** M-75 sagittal plane cut. **B.** M-88, transverse plane.

Table. Descriptive statistics of cardiac area in both embryos.

Embryo	Nº of cuts	Cardiac area			
		Minimum	Maximun	Mean	Std. deviation
M-75	278	0,01	3,72	2,2076	1,07143
M-88	156	0,17	7,56	3,8493	2,60540

maximum was 3.72 mm^2 and the average was 2.20 mm^2; on the other hand, in the 156 cardiac area means from M-88, the minimum value was 0.17 mm^2, the maximum value was 7.56 mm^2 and the average was 3.84 mm^2 (**Table**).

As it was explained in the methodology of this study, the average of the calculated areas was used for implementing the formula of volume, obtaining a volume of 6.137 mm^3 in M-75, and 6.004 mm^3 in M-88.

DISCUSSION

The importance of early morphometric studies was stated early in the literature in the words of Thompson (1948): "... the numerical precision is the very soul of science, and its attainment affords the best, perhaps the only criterion of truth of theories and the correctness of the experiments." This phrase was quoted by Marantos Gamarra[5], who said that the quantitative study of heart development in the post-somite period is based on the measurement of the maximum lateral and anteroposterior heart diameters, and the calculation of the total volume of the organ; the latter, being a three-dimensional measurement, reflects more accurately the size than other variables, such as diameter and area, which are one-dimensional and two-dimensional, respectively. It is important to point out that, in these studies, volume refers to the three-dimensional extension of the heart, not its blood volume.

Volumetric analyses are reported by the technique of three-dimensional ultrasound (3DUS), as it is possible to get the area in successive cuts of the structure, at intervals that are defined by a scale that is the basis for its reconstruction. The volume that is estimated this way avoids the bias of assuming a particular morphology, a limitation of two-dimensional ultrasonography. With the use of 3DUS technique, different structures of obstetric interest have been studied from a volumetric point of view, for example, the lungs, kidneys, heart and liver; being the liver a possible marker of intrauterine growth delay[9].

Leaving out the differences between the procedures, the methodology by which 3DUS obtains the volume of organs is similar to the one used for obtaining cardiac volume in these embryos.

Fetal growth, and organ growth in particular, has been the subject of many investigations in order to quantitatively characterize it and identify its irregularities early. For this purpose, various general ultrasound biometric parameters have been used, as well as those from specific organs[7-10]. Some biometric indicators are closely related to intrauterine growth and gestational age, while others do not undergo changes when the fetus has an impaired growth, at least until the process becomes irreversible. An example of the above is the transverse diameter of the cerebellum in fetuses with intrauterine growth restriction; therefore, it is a useful tool in predicting gestational age[11].

Previous studies with specimens from the same embryo gallery have reported results of morphometric studies which have been based on cardiac diameters and areas, without actually making a calculation of volume[12,13]. According to Gonzalez Lorrio[6], the assessment of the human embryonic heart growth is best made taking into account the volumetric variation of the organ instead of the variation of linear measurements, an issue with which we fully agree. References to the volume of embryonic organs have only been found in the doctoral theses of the above-mentioned autor[6] and Marantos Gamarra[5], referring to the heart, and in the work of Martinez Lima *et al*[14]**,** on liver volume.

Marantos Gamarra[5], in a sample consisting of 11 embryos at O'Rahilly stage 16, reported volumes between 3.05 and 5.16 mm^3. Ours were higher (6.137 and 6.004 mm^3 in the two cases that were studied), a difference that is logical since that author's specimens[5] belonged to an earlier stage of embryonic development.

CONCLUSIONS

To affirm more consistently that cardiac volume in the human embryo at stage 22 is at the calculated values may require a larger study sample; however, this does not diminish the worth of the results of the scientific approximation to the actual cardiac dimensions at this stage of development. Moreover, the similarity in cardiac volume, despite the differences in the lengths of the embryos, may be a quantitative reaffirmation of the criteria that support their inclusion in the same period.

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