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Histomorphometric characterization of the interventricular septum in human specimens from Carnegie stage 14 to the early fetal period

Caracterización histomorfométrica del tabique interventricular en especímenes humanos del estadio 14 de Carnegie al periodo fetal precoz

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ABSTRACT

Introduction: The interventricular septum, whose hypertrophy is part of some heart diseases and results from maternal factors such as diabetes mellitus. **Objective:** To morphometrically describe the development of the interventricular septum in human embryos and fetuses. **Method:** A quantitative, descriptive, and cross-sectional study was conducted from January 2024 to June 2025 in the morphometry laboratory of the Faculty of Medicine at the *Universidad de Ciencias Médicas de Villa Clara*. The sample, performed by intentional non-probability sampling, consisted of 23 embryos and 4 fetuses from the early period. Morphometric data were obtained using ImageJ software. **Results:** The mean thickness at the basal region of the septum was 209.79 μm, increasing from 73.67 μm to 307.70 μm. In the apical region, the mean thickness was 184.20 μm, increasing from 89.93 μm to 345.51 μm. The mean length was 942.37 μm, ranging from 253.50 μm at Carnegie stage 14 to 1357.61 μm during the fetal period studied. Area values were converted to mm^2. The mean septal area was 0.26 mm^2, ranging from 0.01 mm^2 at Carnegie stage 14 to 0.27 mm^2 during the fetal period studied. **Conclusions:** Measurements obtained from the interventricular septum reveal the anatomical irregularity of this structure. The interventricular area shows continuous growth as the specimens develop.

Keywords: embryonic heart, interventricular septum, morphometry

RESUMEN

Introducción: La hipertrofia del tabique interventricular forma parte de algunas cardiopatías y resulta de algunos determinantes maternos como la diabetes mellitus. **Objetivo:** Describir morfométricamente el desarrollo del tabique interventricular en embriones y fetos humanos. **Método:** Se realizó una investigación cuantitativa, descriptiva y transversal, desde enero 2024 a junio de 2025, en el laboratorio de morfometría de la Facultad de Medicina de la Universidad de Ciencias Médicas de Villa Clara. La muestra, por muestreo no probabilístico intencional, quedó constituida por

23 embriones y 4 fetos del periodo precoz. Los datos morfométricos se obtuvieron por el programa ImageJ. **Resultados:** La media del grosor en la región basal del tabique es 209,79 µm, con un incremento que va de 73,67 µm a 307,70 µm. En la región apical, la media del grosor fue de 184,20 µm, que creció de 89,93 µm a 345,51 µm. La media de la longitud fue de 942,37 µm; esta varió desde 253,50 µm en el estadio 14 de Carnegie hasta 1357,61 µm en el periodo fetal analizado. Los valores del área fueron llevados a mm^2. El valor de la media del área septal fue de 0,26 mm^2 y varió de 0,01 mm^2 en el estadio 14 de Carnegie a 0,27 mm^2 en el periodo fetal estudiado. **Conclusiones:** Las mensuraciones obtenidas del tabique interventricular evidencian la irregularidad anatómica de esta estructura. El área del tabique interventricular presenta un crecimiento continuo según el desarrollo de los especímenes.

Palabras clave: Corazón embrionario, Tabique interventricular, Morfometría

INTRODUCTION

The definitive interventricular septum is formed, in a mosaic-like manner, from the primitive interventricular septum, the ventrosuperior and dorsoinferior endocardial cushions of the atrioventricular canal, and the conotruncal ridges.[1]

Despite the widespread use of conventional ultrasonography in screening for congenital heart diseases, prenatal detection still presents diagnostic gaps, which drives the development of new imaging acquisition techniques aimed at improving cardiac screening during routine prenatal care. From this perspective, current research delves deeper into the state of development of the interventricular septum, whose hypertrophy is associated with certain cardiopathies and influenced by maternal determinants such as diabetes mellitus.[2,3]

Using ultrasonography to measure the area of the interventricular septum is a direct way to assess septal defects – the most commonly found congenital heart defects (about 30% of heart diseases) – particularly membranous defects. Additionally, it allows for indirect analysis of other cardiac malformations, such as valvular stenosis, which lead to ventricular hypertrophy. Another advantage is the early detection of septal hypertrophy in fetuses of diabetic mothers, as it can identify those fetuses at higher risk of developing macrosomia and cardiomyopathy.[3]

It is possible to obtain measurements of the interventricular septum area in histoembryological series of human hearts in embryos and fetuses, with proper image selection and cutting plane, a factor that has not been extensively studied in the morphometry of the heart during such early stages of development[4]. This parameter would be an important reference for fetal ultrasonographic studies.

The development of embryonic medicine requires precise knowledge of its morphology and morphometry, which serve as a reference for prenatal histopathological and ultrasonographic diagnosis; these are reasons that justify efforts to progressively expand the field of quantitative knowledge of the human organism at this early stage of life.[5]

One of the methods introduced to achieve a greater understanding of embryonic development is the morphometric method, which allows for various quantitative studies with a bidimensional projection in the space of the object being analyzed, contributing to corroborating the findings of studies carried out from a qualitative approach.[5,6]

The application of morphometric techniques in studies of the embryonic heart has allowed for the exploration of linear, bidimensional, and tridimensional variables, providing more information on the complex processes involved in the development of this organ, whose malformations have a significant impact on perinatal morbidity and mortality.[5]

For this reason, we propose as the objective of this study to morphometrically describe the development of the interventricular septum in human embryos and fetuses.

METHODS

A quantitative, descriptive, and cross-sectional study was conducted from January 2024 to June 2025 in the morphometry laboratory of the Faculty of Medicine at the *Universidad de Ciencias Médicas de Villa Clara*. The sample consisted of all human embryos (up to eight weeks) and fetuses (up to ten weeks) from the embryo bank of this faculty. The specimens were grouped according to crown–rump length and classified following the Carnegie staging system.[7,8]

The sample, selected through intentional non-probability sampling[9,10], included 23 embryos and 4 early-period fetuses, sectioned in different anatomical planes (longitudinal, transverse, and frontal), all meeting the following four inclusion criteria:

1. known crown–rump length of the embryo/fetus;
2. technical and image quality allowing adequate visualization of cardiac structures;
3. cutting plane permitting proper observation of the interventricular septum (transverse and frontal);
4. and absence of documented malformations in the embryo/fetus.

All embryos and fetuses were processed using the paraffin histological technique. The observation, in turn, was carried out using optical microscopy to assess cardiac morphology and the interventricular septum in the complete series of organs of the specimens. The sequential histological study comprised 1004 slides, from which those showing cardiac structures were reviewed. For morphometric analysis, slides of the interventricular septum with the best quality and structural integrity were selected and photographed.

Measurements were obtained using the ImageJ software[11], previously calibrated to express results in micrometers (μm). For each variable, seven readings were taken, and the mean, extreme values, and standard deviation were recorded. Final data for each developmental stage were calculated as the mean of all specimens, or, in cases of a single individual, the maximum value was used. Using an optical microscope, a qualitative technique was employed, consisting of the evaluation of general cardiac morphology and, in particular, the interventricular septum in the embryos and fetuses under study, after examining the complete series of the organ of interest.

Statistical Methods

Data were organized in a Microsoft Excel file and processed using RStudio (**R 4.4.2**). Due to the exploratory nature and small sample size, a descriptive approach was prioritized, complemented by cautious inferential analyses.

For quantitative variables, medians and interquartile ranges (IQR) were calculated due to non-normal distribution (Shapiro–Wilk test, $p < 0.05$ in most cases). Variables following a normal distribution were summarized using means and standard deviations (SD). Qualitative variables were presented as absolute frequencies and percentages.

Operationalization of the Variables

- Basal interventricular septum thickness (BIVST). Conceptual definition: Mean distance of the interventricular septum in the inflow–outflow segment, measured from right to left.
- Apical interventricular septum thickness (AIVST). Conceptual definition: Mean distance of the interventricular septum in the apical segment, measured from right to left.
- Interventricular septum length (IVSL). Conceptual definition: the mean of the distances of the interventricular septum length, from the observed muscular portion in the image, measured from the apical to the basal segment.
- Interventricular septum area (IVSA): area corresponding to the interventricular septum in each section. Conceptual definition: it was obtained by delineating the external boundaries of the interventricular septum from the apical segment to the inflow–outflow segment.
- Developmental stages: Operational definition: a) Embryonic: According to Carnegie stages (CS) from CS14 to CS23. b) Fetal: Fetuses at the 9th and 10th weeks, corresponding to the early fetal period.

Study Limitations:

The specimens had been previously classified by developmental stage or week, which was verified through expert consultation when necessary. A technical limitation identified was the variability in the cutting planes of measurable images. Although previous studies have not reported significant differences attributable to this factor, the frontal plane posed greater measurement challenges.

Ethical Aspects

The specimens belong to the embryo bank collection of the Faculty of Medicine at the *Universidad de Ciencias Médicas de Villa Clara*. Their original acquisition included informed consent from donors and approval of the ethics committees of the participating hospitals: *Hospital Gineco-Obstétrico "Mariana Grajales"* of Santa Clara and *Hospital Provincial Clínico-Quirúrgico Universitario "Arnaldo Milián Castro"* of Villa Clara. For this research, authorization was obtained from the Faculty of Medicine Ethics Committee, which adheres to the principles of the Declaration of Helsinki.[12]

RESULTADOS

Morphometric variables of the interventricular septum were analyzed in 27 embryos/fetuses, some sectioned transversely and others frontally. This allowed the research team to measure the thickness, length, and area of the interventricular septum; results are shown in **table 1**. The mean basal septal thickness was 209.79

µm, with an increase observed from Carnegie stage 14 to the analyzed fetal period, ranging from 73.67 µm to 307.70 µm. In the apical region, the mean thickness was 184.20 µm, increasing from 89.93 µm to 345.51 µm.

The mean length of the muscular interventricular septum was 942.37 µm, ranging from 253.50 µm at Carnegie stage 14 to 1357.61 µm in the analyzed fetal period.

Table 1. Morphometric variables of the interventricular septum during developmental stages

		Developmental stages								
	Global (N = 27)	CS-14 (n=1)	CS-15 (n=2)	CS-18 (n=3)	CS-19 (n=1)	CS-20 (n=6)	CS-21 (n=1)	CS-22 (n=5)	CS-23 (n=2)	Fetal (9a-10a sem.) (n=2)
Interventricular septum										
BIVST (µm)	209,79 (237,95)*	73,67	81,57 (20,70)	78,20 (8,30)	132,79	165,74 (69,50)	270,53	349,46 (145,13)	314,87 (51,56)	307,70 (25,65)
AIVST (µm)	184,2 (247,04)*	89,93	130,50 (51,95)	95,08 (7,47)	143,67	174,90 (52,52)	380,83	377,01 (167,53)	366,19 (16,87)	345,51 (2,29)
IVSL (µm)	942,37 (721,42)†	253,5	194,15 (41,64)	775,24 (90,49)	436,51	790,01 (222,62)	769,89	1430,84 (368,96)	1043,31 (165,83)	1357,61 (55,32)
IVSA (mm^2)	0,26 (0,18)*	0,01	0,01 (0,00)	0,08 (0,02)	0,06	0,13 (0,04)	0,21	0,24 (0,09)	0,27 (0,00)	0,27 (0,01)

Source: Embryo bank at the *Universidad de Ciencias Médicas*, Villa Clara.

* Median (interquartile range); Shapiro p<0.001

† Mean (standard deviation); Shapiro p>0.05

AIVST: Apical Interventricular Septum Thickness; BIVST: Basal Interventricular Septum Thickness; CS: Carnegie Stage; IVSA: Interventricular Septum Area; IVSL: Interventricular Septum Length; mm^2: square millimeters; µm: micrometers

DISCUSIÓN:

Evidence indicates that there is a single muscular septum that forms as the apical components of the ventricles grow outward from the primary cardiac tube. Each ventricle initially develops with an apical component arising from the outer curvature of the cardiac tube. The muscular interventricular septum is interposed mainly between the apical components of the ventricles. The first signs of interventricular septum development have been described at Carnegie stage 11.[13]

The measurement of fetal interventricular septum thickness performed by two-dimensional echocardiography using M-mode is limited in accuracy, as the septum is not a linear, perfectly horizontal, or symmetrical structure.[3,14,15]

In early stages of septation, the ventricle exhibits a bilobed structure: as the septum extends superiorly, the separation becomes nearly complete, as shown in **figures 1** and **2**. The mechanisms underlying the rapid extension of the septum to form a complete wall between the left and right ventricles remain debated (**Figure 3**). A plausible explanation involves the rapid proliferation of these myocytes, which retain the ability to divide even within the functional myocardium.[15,16,17,18]

This proliferative capacity, however, is lost shortly after birth, when mature myocardium ceases to undergo significant mitotic activity. Small muscular ventricular septal defects are very common in newborns. A monitoring study of asymptomatic neonates reported an incidence of 53 per 1000 live births. Only one in ten of these patients showed clinical signs of the defect. However, nearly 90% of these small defects had closed spontaneously by 10 months of age. These findings suggest that, although diminished, myocardial proliferation continues postnatally in humans[3,15,16].

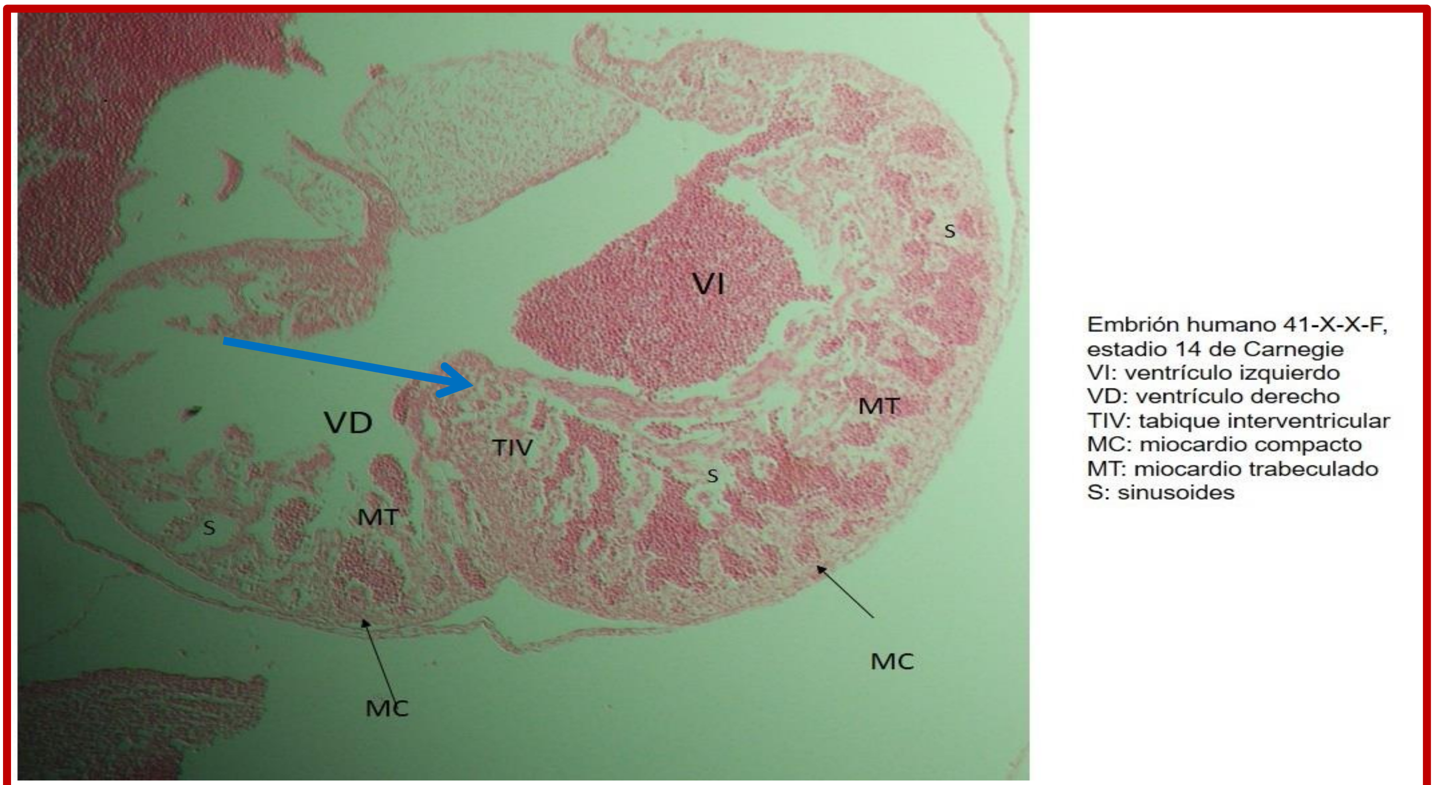

Figure 1. Human embryo at Carnegie stage 14. The lobulated appearance of both ventricles is observed, seen in a cross-sectional view, with the left ventricle being slightly larger. At this stage, the beginning of ventricular septation is observed, the muscular interventricular septum (arrow) extends between the two ventricles, and the primary interventricular foramen is wide. VI (left ventricle), VD (right ventricle), TIV(internventricular septum), MC (compact myocardium), MT (trabeculated myocardium, S (sinusoids)

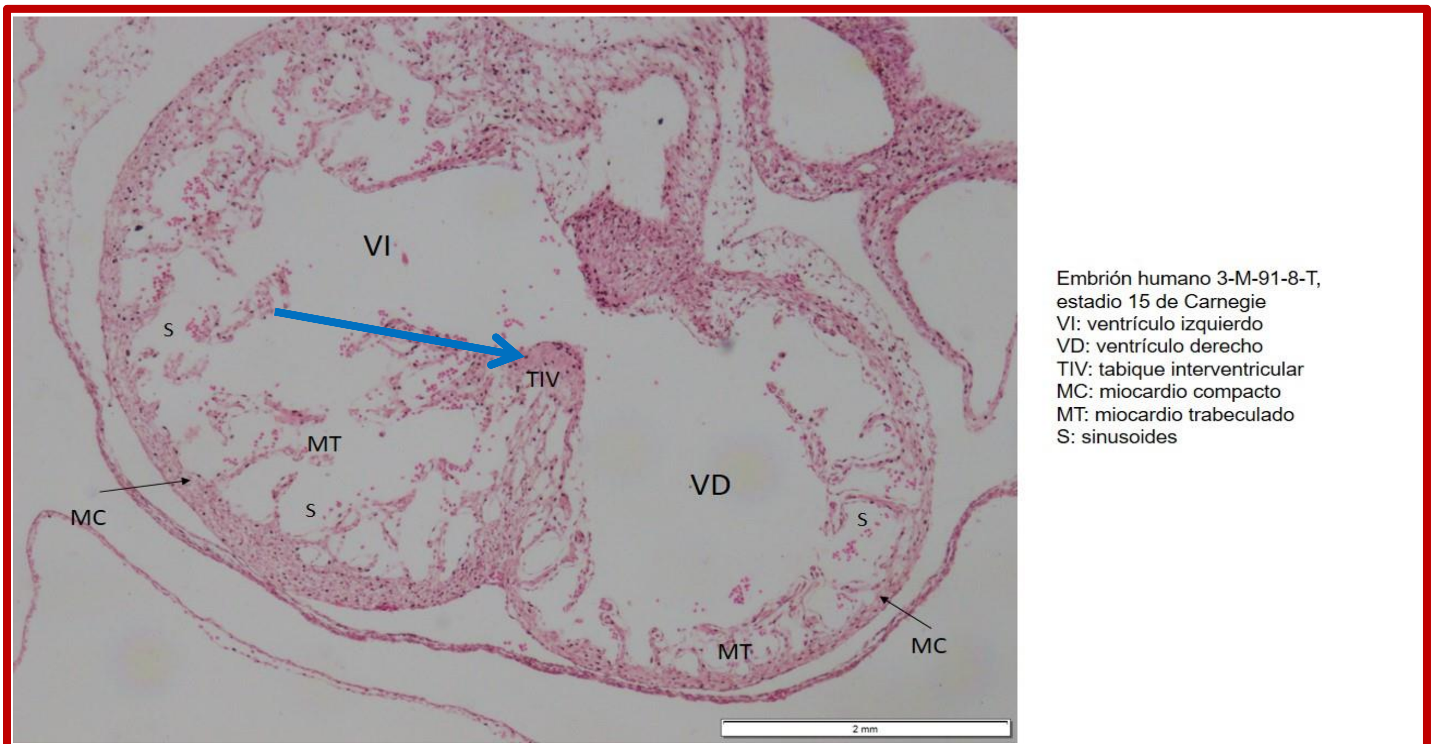

Figura 2. Human embryo at Carnegie stage 15. Both ventricles maintain a rounded appearance, with the left ventricle being larger than the right. The muscular interventricular septum (arrow) grows apically between the two ventricles, and the primary interventricular foramen remains wide. VI (left ventricle), VD (right ventricle), TIV(internventricular septum), MC (compact myocardium), MT (trabeculated myocardium, S (sinusoids)

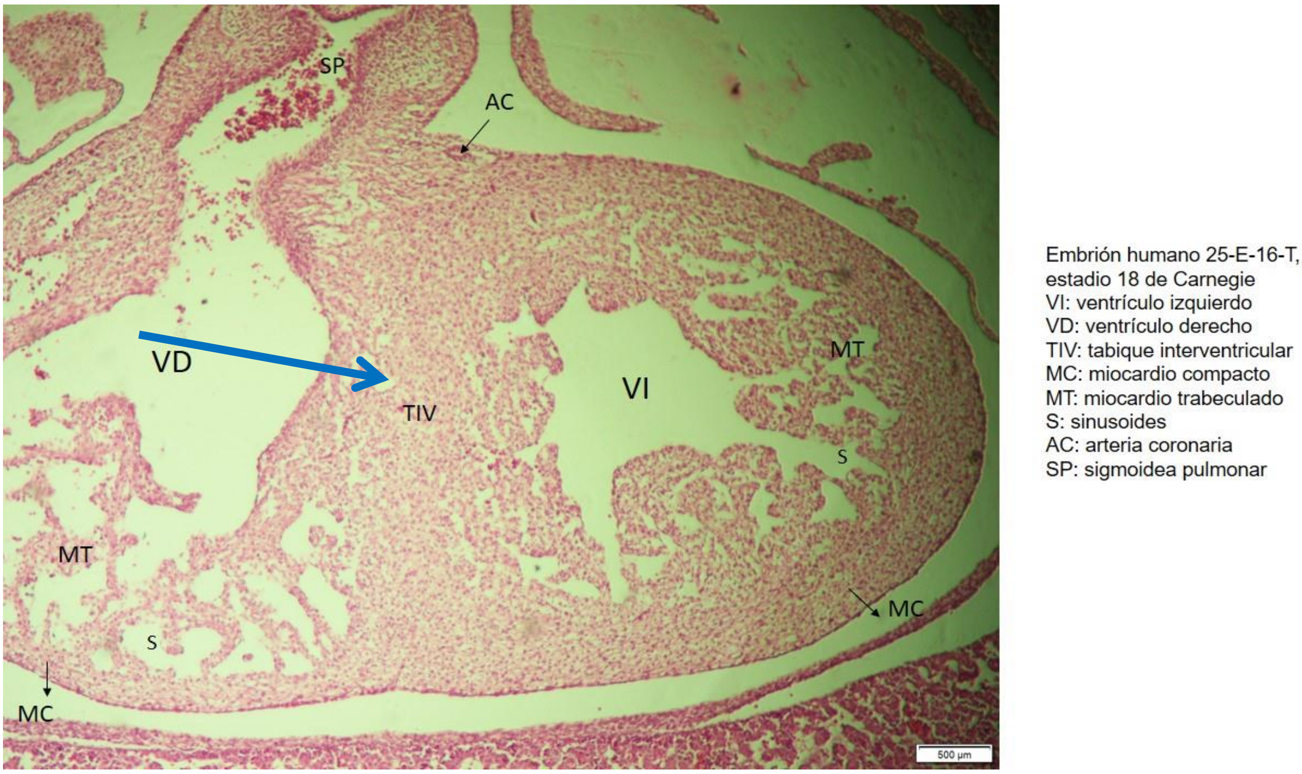

Figura 3 Human embryo at Carnegie stage 18. The interventricular septum almost completely separates both ventricles. VI (left ventricle), VD (right ventricle), TIV(internventricular septum), MC (compact myocardium), MT (trabeculated myocardium, S (sinusoids)

A study employing Spatio-Temporal Image Correlation demonstrated that all cardiac dimensions, including interventricular septum thickness, increase steadily with gestational age (14 to 40 weeks). This finding is consistent with prior two-dimensional M-mode ultrasound studies, in which septal area was not reported. The measurements obtained in the present study align with those previously reported by the authors in their series.[15]

The values of the interventricular septum area obtained from the analyzed specimens were converted to mm^2. The mean septum area was 0.26 mm^2, ranging from 0.01 mm^2 at Carnegie stage 14 to 0.27 mm^2 in the analyzed fetal period.

In a study involving fetuses from 18 to 33 weeks of gestation, the mean septum area was 0.47 ± 0.10 cm^2 (ranging from 0.36–0.59 cm^2) at week 18 and increased to 2.42 ± 1.13 cm^2 (ranging from 1.26–3.92 cm^2) at week 33, showing a strong correlation with gestational age ($r = 0.81$).

Measurement of the interventricular septum area is is particularly relevant in fetuses at risk of developing hypertrophic cardiomyopathy, such as those of diabetic mothers, who often exhibit marked thickening of the interventricular septum, potentially leading to left ventricular outflow tract obstruction.[3]

Severe complications in both the mother and the newborn resulting from fetal macrosomia underscore the need for early diagnosis and prevention. This common condition affects approximately 10% of fetuses. Unfortunately, current predictors, such as fetal biometry, fundal height, and amniotic fluid index, appear insufficient. Interventricular septum thickness is a potential diagnostic marker of fetal macrosomia, as its measurement enables detection in up to 71.43% of cases.[19,20]

In one study, septal thickness showed a weak correlation with gestational age, following an almost linear trend between 18 and 33 weeks, without statistically significant correlation with the area obtained by employing Spatio-Temporal Image Correlation. This result suggests that septal area may serve as a more reliable ultrasonographic parameter than septal thickness in diagnosing septal hypertrophy[3].

To objectively identify cardiac anomalies in early gestation, it is essential to establish normal reference ranges for cardiac dimensions at each gestational week. Although several studies have published cardiac nomograms using two-dimensional ultrasound in M- or B-mode, most are based on small samples and do not include fetuses younger than 19 gestational weeks.[19,20]

The reference values determined in the present study for fetal interventricular septum area may therefore provide a useful benchmark for the assessment of fetuses at risk of septal hypertrophy, particularly offspring of diabetic mothers, facilitating earlier detection of functional and structural cardiac abnormalities. These results are consistent with those reported by Rolo L et al.[3]

CONCLUSIONS:

The measurements obtained from the interventricular septum demonstrate the inherent anatomical irregularity of this structure across its different regions. The area of the interventricular septum shows accelerated growth up to Carnegie stage 23, according to the developmental progression of the specimens.

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