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Histomorphometric and Qualitative Analysis of Umbilical Cord Vessels in Normal and Malformed Human Fetuses Between 13 and 28 Post-Conception Weeks

Análisis Histomorfométrico y Cualitativo de los Vasos del Cordón Umbilical en Fetos Humanos Normales y Malformados Entre 13 y 28 Semanas Post-Concepción

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SUMMARY: The umbilical cord is essential for fetal development, with its vessels undergoing continuous remodeling to sustain growth. Disturbances in vascular wall structure and extracellular matrix organization have been linked to impaired development and congenital malformations. However, systematic analyses of umbilical cord vessel histomorphometry and collagen maturation across gestational stages remain scarce. Ninety-seven human fetuses (51 normal, 46 malformed) were stratified into post-conceptional week subgroups (13–16, 17–20, 21–24, 25–28). Proximal cord segments were processed with Weigert's resorcin-fuchsin and Picrosirius Red. Vessel and cord areas and wall thicknesses were measured with ImageJ. Collagen distribution was qualitatively assessed under polarized light. Data normality was tested by Kolmogorov-Smirnov; intragroup comparisons used Kruskal-Wallis with Dunn's post hoc; intergroup comparisons used Student's t or Mann-Whitney U tests; correlations were analyzed with Spearman's rho. Significance was set at $p < 0.05$. In normal fetuses, all morphometric parameters positively correlated with gestational age ($p < 0.001$), including cord area ($r = 0.502$), vein area ($r = 0.735$), artery area ($r = 0.725$), and wall thicknesses. Both vein and artery cross-sectional areas and wall thicknesses increased significantly across intervals. For example, arterial wall thickness rose from 0.17 ± 0.07 mm at 13–16 PCW to 0.35 ± 0.09 mm at 25–28 PCW ($p < 0.05$). In malformed fetuses, only artery area ($r = 0.392$, $p = 0.007$) and arterial thickness ($r = 0.570$, $p < 0.001$) correlated with gestational age, while vein wall thickness and cord area showed no significant progression. Between groups, vein area was greater in malformed cords at 13–16 PCW ($p = 0.0041$), while at 17–20 PCW both vein and artery wall thicknesses were reduced ($p = 0.0027$ and $p = 0.0003$). No differences were observed at 21–28 PCW. Picrosirius Red revealed progressive collagen maturation in normal cords, contrasting with persistent disorganization and delayed maturation in malformed cords. Normal umbilical vessels exhibit predictable morphometric and collagen maturation patterns, while malformed fetuses demonstrate impaired remodeling, supporting cord histology as a potential marker of fetal pathology.

KEY WORDS: Umbilical cord; Fetal malformation; Vessel wall thickness; Elastic fibers; Collagen; Histomorphometry.

INTRODUCTION

The human umbilical cord is a transient yet essential structure that sustains fetal development by allowing the exchange of nutrients, and waste between the placenta and fetus. It normally contains two arteries and one vein, supported by Wharton's jelly, a mucoid connective tissue that protects the vessels from compression and torsion (Stehbens *et al*., 2005; Kozadinos *et al*., 2025). The umbilical vessels, lacking vasa vasorum, are highly sensitive to systemic changes in perfusion and oxygenation (Stehbens *et al*., 2005).

As gestation progresses, the cord undergoes structural modifications reflecting vascular maturation. These changes include gradual thickening of vessel walls, increased organization of the elastic fiber system, and remodeling of the extracellular matrix (Sexton *et al*., 1996; Junek *et al*., 2000; Yousif *et al*., 2023). In pathological pregnancies, particularly those affected by pre-eclampsia or growth restriction, this progression may be disrupted. Studies have reported reduced vessel caliber, thinner walls, and alterations in collagen content and fiber architecture

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in such cases (Blanco *et al*., 2011; Lan *et al*., 2018; Chillakuru *et al*., 2020).

Less explored are the histological features of umbilical cords in fetuses with congenital malformations. Structural anomalies, such as single umbilical artery (SUA), have been associated with higher rates of chromosomal defects and perinatal complications (Kim *et al*., 2017; Yu *et al*., 2024). However, most of the literature focuses on clinical associations (e. g. hypertension, diabetes) rather than on microscopic or morphometric details. Few studies examine how elastic or collagen components differ in malformed fetuses, or how these changes evolve with fetal age (Stehbens *et al*., 2005; Mailath-Pokorny *et al*., 2015; Lan *et al*., 2018; Salem *et al*., 2020).

The umbilical vasculature, as an extension of the fetal cardiovascular system, can offer valuable clues about intrauterine conditions. Elastic fibers and collagen Types I and III contribute to vascular integrity, tone, and resistance to stress. Deviations in their distribution may indicate altered hemodynamic load or intrinsic developmental errors (Altunkaynak & Yahyazadeh, 2021; Dubetskyi *et al*., 2023). Yet, systematic evaluations of these features remain scarce, especially in malformed fetuses outside the context of maternal comorbidities.

This study aims to analyze the histological and morphometric characteristics of the proximal umbilical cord vessels in normal and malformed human fetuses at different gestational ages.

MATERIAL AND METHOD

Ethical Considerations

This study was conducted in accordance with Brazilian Federal Law no. 8.501/1992, which permits the use of unclaimed cadaveric material for research and teaching purposes. The use of fetal specimens was authorized by the board of the Department of Morphology at Universidade Federal Fluminense (UFF). All procedures complied with the principles of the Declaration of Helsinki (1995; revised in Edinburgh, 2000) (World Medical Association, 2000).

Sample Selection

Ninety-seven human fetuses preserved in 10 % buffered formalin were selected from the anatomical collection of the Morphology Department at UFF. Only specimens with umbilical cord segments measuring at least 3 cm from the fetal abdominal wall were included, ensuring the absence of vascular anastomoses or embryonic remnants that could interfere with the analysis.

The sample was divided into two groups based on external morphological assessment. The normal group (N) consisted of 51 fetuses without visible malformations, while the malformed group (M) comprised 46 fetuses with evident anatomical abnormalities, such as microcephaly, ectromelia, gastroschisis, or omphalocele. Gestational age was determined in post-conceptional weeks (PCW) by measuring the right foot length from the heel to the tip of the longest toe using a digital caliper (Streeter, 1920). Each measurement was taken three times and averaged. In one malformed fetus with lower-limb agenesis, cranial dimensions were used to estimate fetal age.

Based on PCW, fetuses were distributed into the following subgroups: 13–16, 17–20, 21–24, and 25–28 PCW for the normal and malformed groups (NG and MG, respectively).

Histological Procedures

Segments from the proximal portion of each umbilical cord were processed using standard paraffin embedding protocols (Figs. 1 to 3). Three histological sections were obtained per specimen and stained using Weigert's resorcin-fuchsin and Picrosirius Red for collagen fiber typification under polarized light.

Photomicrographs were taken using an Olympus light microscope equipped with a digital camera. Images were acquired at 4X, 10X, 20X, and 40X magnifications to assess both general morphology and fine structural details. In cords with larger cross-sectional areas, additional images were obtained using a stereomicroscope (DinoCapture 2.0) to capture complete views of the cord sections.

Histomorphometric Analysis

Quantitative morphometric measurements were performed using ImageJ software with calibrated scales for each magnification. The variables analyzed included wall thickness and area of the umbilical cord, umbilical vein and umbilical arteries, while collagen type was assessed qualitatively by the Picrosirius Red staining viewed under polarized light. Each measurement was performed in triplicate, and the arithmetic mean was used for analysis.

Statistical Analysis

All statistical analyses were performed using GraphPad Prism version 6. Descriptive statistics were

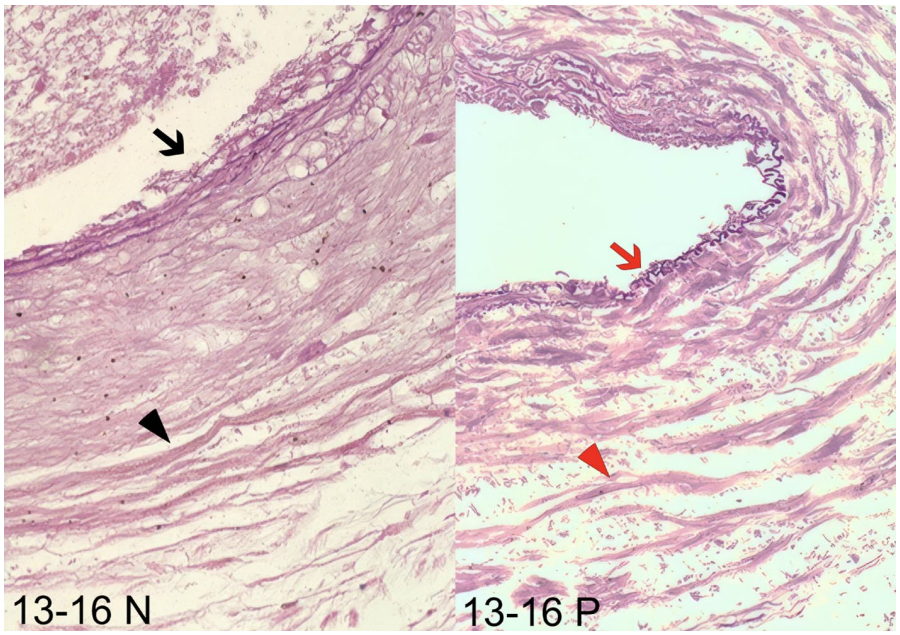

Fig. 1. Histological Analysis of Umbilical Arteries (Weigert's Stain, 40X). Umbilical arteries from human fetuses at 13–16 and 17–20 post-conception weeks. Left panels: normal specimens (N); right panels: malformed specimens (P). Weigert's stain reveals elastic fibers within the tunica media and the internal elastic lamina. Normal arteries display well-defined and continuous elastic lamellae, whereas malformed specimens show fragmentation, discontinuity, or reduction of elastic fiber content.

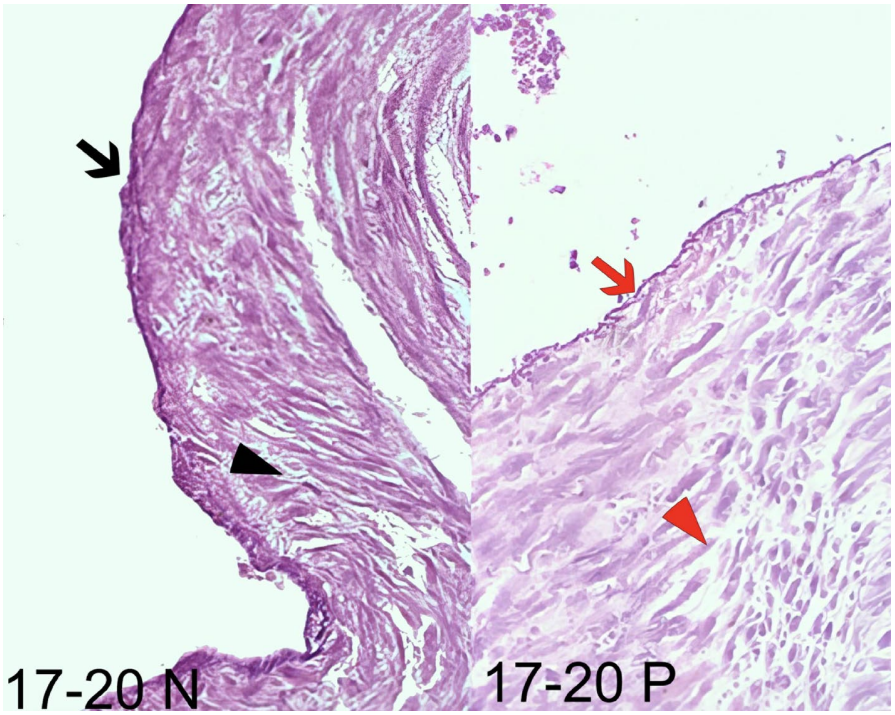

Fig. 2. Histological Analysis of Umbilical Veins (Weigert's Stain, 40X). Umbilical veins from human fetuses at 13–16 and 17–20 post-conception weeks. Panels compare normal (N) and malformed (P) specimens. Weigert's stain demonstrates elastic fiber distribution and venous wall architecture. Compared with normal veins, malformed specimens exhibit decreased fiber density and disorganization of the tunica media and adventitia.

reported as mean values (mm^2 or mm) with corresponding standard deviations (SD). The Kolmogorov-Smirnov test was used to assess the normality of data distributions.

For intragroup comparisons across gestational age intervals, the Kruskal-Wallis test was applied, followed by Dunn's post hoc test when appropriate. Intergroup comparisons between normal and malformed fetuses were conducted using the unpaired Student's t test for normally distributed variables or the Mann-Whitney U test for non-parametric data.

To evaluate the association between gestational age (PCW) and morphometric parameters (Table I) - including umbilical cord area, vein and artery cross-sectional areas, and vessel wall thickness - Spearman's rank correlation coefficient (rho, r) was calculated separately for normal and malformed groups (Table II). A p-value less than 0.05 was considered statistically significant for all analyses.

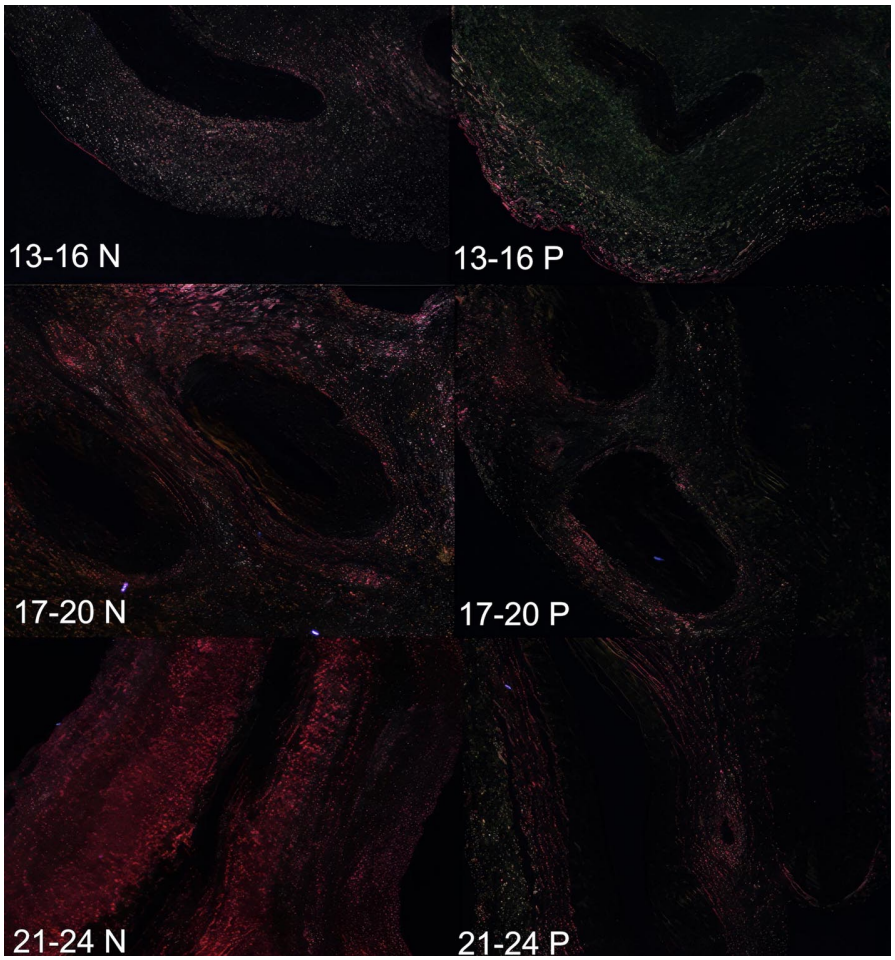

Fig. 3. Collagen Organization in Umbilical Cord Vessels (Picrosirius Red under Polarized Light, 13–24 SPC) Arteries and veins from normal (N) and malformed (P) human fetuses stained with Picrosirius Red and examined under polarized light. Type I collagen appears red-orange, and Type III collagen appears greenish-yellow. Normal vessels exhibit densely packed and well-organized collagen, particularly in the adventitia and perivascular regions. Malformed cords show irregular collagen arrangement and reduced birefringence intensity, more pronounced at earlier stages of post-conception development.

Table I. Morphometric parameters (Mean ± SD) for area (mm^2) and wall thickness (mm) of the umbilical arteries and vein between groups and subgroups (according to postconception weeks, PCW).

Group	**PCW Interval**	**Vessel**	Area	Wall thickness
Normal	13-16	Artery 1	0.590 ± 0.200	0.170 ± 0.030
Normal	13-16	Artery 2	0.630 ± 0.180	0.160 ± 0.030
Normal	13-16	Vein	1.320 ± 0.320	0.130 ± 0.020
Normal	17-20	Artery 1	1.360 ± 0.360	0.300 ± 0.050
Normal	17-20	Artery 2	1.240 ± 0.330	0.310 ± 0.050
Normal	17-20	Vein	2.550 ± 0.650	0.244 ± 0.035
Pathological	13-16	Artery 1	0.868 ± 0.240	0.196 ± 0.040
Pathological	13-16	Artery 2	0.836 ± 0.220	0.181 ± 0.050
Pathological	13-16	Vein	2.200 ± 0.600	0.180 ± 0.040
Pathological	17-20	Artery 1	1.560 ± 0.420	0.159 ± 0.050
Pathological	17-20	Artery 2	1.220 ± 0.300	0.177 ± 0.040
Pathological	17-20	Vein	2.954 ± 0.880	0.141 ± 0.040

Table II. Spearman correlation coefficients between morphometric and histological variables.

Subgroup	**ρ (Area *vs* Wall Thickness)**	**ρ (Wall Thickness *vs* EFS)**	**ρ (EFS *vs* LEI)**
13–16 N	-0,45	-0,04	0,26
13–16 P	-0,18	-0,26	0,64
17–20 N	-0,35	-0,18	0,03
17–20 P	-0,06	0,37	0,16

r (rho) = Spearman's rank correlation coefficient.

RESULTS

A total of 97 human fetal umbilical cords were analyzed, comprising 51 morphologically normal fetuses and 46 with visible malformations. Fetuses were stratified into gestational age subgroups by post-conceptional weeks (PCW), and histomorphometric comparisons were performed both within and between groups. Stratified into post-conceptional age subgroups as follows (Tables I and II):

Normal group (n = 51):
-13–16 PCW: n = 21
-17–20 PCW: n = 22
-21–24 PCW: n = 5
-25–28 PCW: n = 3

Malformed group (n = 46):
-13–16 PCW: n = 20
-17–20 PCW: n = 15
-21–24 PCW: n = 7
-25–28 PCW: n = 4

Intragroup findings in normal fetuses

A statistically significant difference in umbilical cord cross-sectional area was observed across the gestational age groups (Kruskal-Wallis, $p < 0.01$). Post hoc analysis using Dunn's test revealed that the 13-16 PCW group (16.45 ± 6.31 mm^2; n = 21) had significantly lower umbilical cord area compared to both the 17-20 PCW group (27.79 ± 9.74 mm^2; n = 22; $p = 0.0022$) and the 21-24 PCW group (44.45 ± 16.61 mm^2; n = 5; $p = 0.0006$). Although the mean in the 25-28 PCW group (32.00 ± 8.53 mm^2; n = 3) was higher than that in the 13-16 PCW group, this difference did not reach statistical significance ($p > 0.05$).

A significant increase in umbilical vein cross-sectional area was observed with advancing gestational age (Kruskal-Wallis, $p < 0.001$). Dunn's post hoc test revealed that the 13-16 PCW group (1.33 ± 1.11 mm^2; n = 21) had significantly smaller areas compared to the 17-20 PCW group (2.50 ± 1.29 mm^2; n = 21; $p = 0.010$), the 21-24 PCW group (5.42 ± 1.47 mm^2; n = 5; $p = 0.0002$), and the 25-28 PCW group (6.02 ± 0.84 mm^2; n = 3; $p = 0.0026$).

Umbilical vein wall thickness demonstrated a significant increase with gestational age (Kruskal-Wallis, $p < 0.05$). According to Dunn's post hoc test, the 13-16 PCW group (mean ± SD: 0.134 ± 0.073 mm; n = 21) exhibited significantly lower thickness compared to the 17-20 PCW group (0.234 ± 0.104 mm; n = 21; $p = 0.0091$). Although mean values were higher in the 21–24 PCW group (0.250 ± 0.183 mm; n = 5) and 25-28 PCW group (0.197 ± 0.071 mm; n = 3) compared to the 13-16 PCW group, these differences did not reach statistical significance ($p > 0.05$).

A statistically significant difference in umbilical artery cross-sectional area was found among gestational age groups (Kruskal-Wallis, $p < 0.001$). Post hoc comparisons using Dunn's test showed that the 13-16 PCW group (mean ± SD: 0.646 ± 0.273 mm^2; n = 21) had significantly smaller arterial areas than the 17-20 PCW group (1.309 ± 0.596 mm^2; n = 22; $p = 0.0016$), the 21-24 PCW group (2.560 ± 0.993 mm^2; n = 5; $p = 0.0004$), and the 25-28 PCW group (2.030 ± 0.455 mm^2; n = 3; $p = 0.0119$).

Umbilical artery wall thickness increased significantly across gestational age groups (Kruskal-Wallis, $p < 0.001$). Dunn's post hoc analysis showed that the 13-16 PCW group (mean ± SD: 0.171 ± 0.065 mm; n = 21) had significantly lower values than the 17-20 PCW group (0.306 ± 0.069 mm; n = 22; $p < 0.0001$) and the 25-28 PCW group (0.353 ± 0.091 mm; n = 3; $p = 0.0150$). A significant difference was also observed between the 17–20 and 21–24 PCW groups (mean ± SD: 0.322 ± 0.055 mm; n = 5; $p = 0.0066$).

In normal fetuses, all morphometric parameters showed strong and statistically significant positive correlations with gestational age, indicating regular and coordinated vascular growth. The highest correlations were observed for vein area (r = 0.735), artery thickness (r = 0.734), and artery area (r = 0.725).

Intragroup findings in malformed fetuses

No statistically significant differences were found in umbilical cord cross-sectional area among the gestational age groups (Kruskal-Wallis, $p = 0.108$). Mean values ranged from 20.75 ± 8.21 mm^2 in the 13-16 PCW group (n = 20) to 32.33 ± 8.28 mm^2 in the 25-28 PCW group (n = 4), with intermediate values of 23.67 ± 11.85 mm^2 (n = 15) and 28.84 ± 10.48 mm^2 (n = 7) in the 17-20 and 21-24 PCW groups, respectively.

A significant difference was found in umbilical vein cross-sectional area across PCW intervals (Kruskal-Wallis, $p = 0.0039$). Dunn's post hoc test revealed a significant increase between the 13-16 PCW group (2.20 ± 1.19 mm^2; n = 18) and the 21-24 PCW group (4.31 ± 0.15 mm^2; n = 7; $p = 0.004$). The 17-20 and 25-28 PCW groups showed mean areas of 2.95 ± 1.37 mm^2 (n = 15) and 3.88 ± 1.58 mm^2 (n = 4), respectively, but did not differ significantly from the 13-16 PCW group.

No significant differences were observed in umbilical vein wall thickness between gestational age groups (Kruskal-Wallis, $p = 0.0711$). Mean values ranged from 0.137 ± 0.068 mm in the 17-20 PCW group (n = 15) to 0.548 ± 0.461 mm in

the 25-28 PCW group (n = 4). The 13-16 and 21-24 PCW subgroups had means of 0.177 ± 0.060 mm (n = 18) and 0.196 ± 0.075 mm (n = 7), respectively.

A statistically significant difference was found in arterial cross-sectional area between PCW groups (Kruskal-Wallis, p = 0.005). Dunn's post hoc analysis indicated a significant decrease from the 13-16 PCW group (0.844 ± 0.355 mm^2; n = 20) to the 17-20 PCW group (1.455 ± 0.702 mm^2; n = 15; p = 0.0460), followed by a significant increase from 13-16 to the 21-24 PCW group (1.971 ± 1.024 mm^2; n = 7; p = 0.0159). The 25-28 PCW group had a mean of 0.940 ± 0.343 mm^2 (n = 4), not significantly different from other intervals.

Arterial wall thickness increased significantly across gestational age groups (Kruskal-Wallis, p = 0.0006). Dunn's post hoc tests showed a significant increase between the 13-16 PCW group (0.189 ± 0.052 mm; n = 20) and the 25-28 PCW group (0.390 ± 0.052 mm; n = 4; p = 0.0012), as well as between the 17-20 PCW group (0.218 ± 0.058 mm; n = 15) and the 25-28 PCW group (p = 0.0235). The 21-24 PCW group had a mean of 0.261 ± 0.044 mm (n = 7), not differing significantly from other groups.

In malformed fetuses, gestational age correlated positively with umbilical cord area (r = 0.341, p = 0.020), vein area (r = 0.526, p < 0.001), artery area (r = 0.392, p = 0.007), and artery wall thickness (r = 0.570, p < 0.001). However, no significant correlation was found between gestational age and vein wall thickness (r = 0.086, p = 0.580).

Intergroup Comparisons

At 13-16 PCW, a statistically significant difference in umbilical vein cross-sectional area was observed between groups. Malformed fetuses (n = 18) exhibited a larger vein area (2.20 ± 1.19 mm^2) compared to normal fetuses (n = 21; 1.33 ± 1.11 mm^2; Mann-Whitney test, p = 0.0041). No significant differences were found in umbilical cord area (p = 0.060), vein wall thickness (p = 0.0550), artery cross-sectional area (p = 0.0726), or artery wall thickness (p = 0.3468).

At 17-20 PCW, statistically significant reductions were observed in both umbilical vein and artery wall thickness in malformed fetuses compared to normal controls. Vein wall thickness was lower in the malformed group (n = 15; 0.14 ± 0.07 mm) than in the normal group (n = 21; 0.23 ± 0.10 mm; Mann–Whitney test, p = 0.0027). Arterial wall thickness also showed a significant decrease in the malformed group (n = 15; 0.21 ± 0.05 mm) compared to normal fetuses (n = 22; 0.31 ± 0.06 mm; Student's t test, p = 0.0003). No significant intergroup differences were observed in umbilical cord area (p = 0.254; Student's t test), vein area (p = 0.293; Mann–Whitney test), or artery area (p = 0.498; Student's t test).

At 21-24 PCW, no statistically significant differences were observed between normal (n = 5) and malformed (n = 7) fetuses in any of the measured parameters. Umbilical cord area (p = 0.0732), umbilical vein area (p = 0.1490), vein wall thickness (p = 0.7235), artery cross-sectional area (p = 0.2677), and artery wall thickness (p = 0.0732) did not differ significantly between groups. The Mann-Whitney test was used for all comparisons at this interval.

Likewise, at 25-28 PCW, no statistically significant differences were found between normal (n = 3) and malformed (n = 4) fetuses for any of the analyzed parameters. Umbilical cord area (p = 0.8571), vein cross-sectional area (p = 0.1143), vein wall thickness (p = 0.4000), artery cross-sectional area (p = 0.0571), and artery wall thickness (p = 0.8571) did not differ significantly between groups. All tests were performed using the Mann-Whitney method.

Qualitative analysis (Picrosirius red staining)

Qualitative analysis of collagen fibers using Picrosirius red staining under polarized light revealed distinct differences in the organization, distribution, and maturation of collagen fibers between normal and malformed umbilical cords across the analyzed gestational intervals.

In the 13–16 PCW subgroup, normal samples exhibited predominantly greenish birefringence with a loosely arranged collagen network, consistent with immature, thin collagen fibers. Malformed samples in this interval displayed a more irregular and disorganized pattern with attenuated birefringence, suggesting impaired collagen deposition in early development.

In the 17–20 PCW subgroup, normal cords showed an increased presence of yellow to red birefringence and a more compact, concentric arrangement of fibers around vessel walls, reflecting progressive fiber maturation. Malformed cords, however, retained a disorganized architecture with heterogeneous birefringence and disrupted layering, indicating a delay or alteration in collagen fiber development.

By 21–24 PCW and 25-28 PCW, normal cords demonstrated intense red birefringence and a well-organized distribution of collagen, particularly surrounding arterial and venous walls, indicative of mature type I collagen. In contrast, malformed samples showed fragmented, unevenly distributed fibers with weaker birefringence, suggesting persistent structural disorganization and deficient maturation of the extracellular matrix.

DISCUSSION

This study provides a comprehensive histomorphometric and qualitative assessment of the human umbilical cord and its vascular components in both normal and malformed fetuses across mid-gestation (13–28 postconceptional weeks, PCW). Our findings reveal consistent morphometric growth trends in normal fetuses, whereas malformed specimens show variable or impaired development in several parameters. These differences are corroborated by qualitative histological changes, particularly in collagen maturation.

Across all normal fetuses, there was a progressive and statistically significant increase in umbilical cord and vessel dimensions with advancing gestational age. The most robust correlations with postconceptional weeks were observed in artery wall thickness (r = 0.734), artery area (r = 0.725), and vein area (r = 0.735), consistent with coordinated vascular growth and matrix deposition. These observations align with prior morphometric studies that describe exponential increases in cord diameter and vessel size as gestation progresses (Togni *et al*., 2007; Barbieri *et al*., 2011). Adaptations in fetoplacental circulation, such as increased vessel caliber and reduced resistance, are believed to support the growing fetus by ensuring efficient oxygen and nutrient delivery (Divon & Hsu, 1992; Coenen *et al*., 2022).

In contrast, malformed fetuses demonstrated more heterogeneous growth patterns. Although the umbilical vein area also increased significantly with gestational age (r = 0.526), other metrics such as cord area (r = 0.341) and artery area (r = 0.392) showed weaker correlations. Notably, vein wall thickness failed to correlate significantly with gestational age (r = 0.086), indicating a potential impairment in vascular remodeling or connective tissue support. The delayed or irregular maturation of vascular walls in malformed fetuses may contribute to suboptimal perfusion, a phenomenon previously described in cases of intrauterine growth restriction (Chillakuru *et al*., 2020; Gayatri *et al*., 2023) and various congenital anomalies (Qin *et al*., 2021). These differences became particularly evident in intergroup comparisons. At 13-16 PCW, malformed fetuses exhibited significantly larger umbilical vein areas compared to normal controls. While this might appear paradoxical, it may reflect altered hemodynamic demands or compensatory venodilation secondary to underlying cardiac or vascular malformations (Contro *et al*., 2023; Kozadinos *et al*., 2025). By 17-20 PCW, both vein and artery wall thicknesses were significantly reduced in malformed fetuses, suggesting impaired extracellular matrix deposition and structural instability. These results mirror findings in diabetic and hypertensive pregnancies, where altered metabolic or inflammatory environments disrupt normal vascular wall development (Chakraborty & Banu, 2013; Chandrakar *et al*., 2025).

From 21-28 PCW, no significant intergroup differences were observed, though small sample sizes may have limited statistical power. Nevertheless, qualitative analysis of collagen fibers under Picrosirius red staining revealed persistent structural disorganization in malformed cords, even at later gestational ages. Whereas normal cords showed progressive maturation toward thick, red birefringent Type I collagen with concentric layering, malformed cords retained immature or fragmented collagen networks with weak birefringence. These findings corroborate reports of disrupted extracellular matrix architecture in congenital anomalies and gestational pathologies (Junek *et al*., 2000; Salem *et al*., 2020; Dubetskyi *et al*., 2023).

The morphometric trends observed in normal fetuses - progressive increases in vessel area and wall thickness - are consistent with previous research on fetal vascular development. Togni *et al*. (2007) and Barbieri *et al*. (2011) reported significant correlations between gestational age and the cross-sectional areas of the umbilical cord and its vascular components, highlighting the role of Wharton's jelly and vessel expansion in supporting fetal growth. The structural role of Wharton's jelly is supported by experimental data in newborn rats, where it accounted for over 60 % of umbilical cord volume and housed abundant mesenchymal stromal cells with active cytological profiles (Altunkaynak & Yahyazadeh, 2021).

Our observations of progressive increases in vessel area and wall thickness are further corroborated by ultrastructural findings from Sexton *et al*. (1996), who documented progressive arterial and venous wall thickening, collagen accumulation, and smooth muscle maturation throughout gestation.

In malformed fetuses, our findings mirror those described in pregnancies complicated by diabetes (Salem *et al*., 2020; Yousif *et al*., 2023), hypertension (Chillakuru *et al*., 2020; Chandrakar *et al*., 2025), and growth restriction (Sharony *et al*., 2016; Gayatri *et al*., 2023), all of which showed disorganized vessel wall architecture and impaired collagen deposition. Moreover, Junek *et al*. (2000) documented elastic fiber system disruption in preeclamptic cords, a structural abnormality that may underlie the lack of vascular wall thickening observed in our malformed cases.

Additionally, our findings of aberrant vein enlargement in malformed fetuses during early gestation echo studies on venous remodeling in response to abnormal fetal hemodynamics, including altered umbilicocerebral

ratios (Coenen *et al.*, 2022) and the presence of supernumerary or absent vessels (Kurakazu *et al*., 2019; Qin *et al.*, 2021). These morphologic changes may be compensatory in nature, or a result of intrinsic anomalies in vascular development (Kozadinos *et al*., 2025).

Histologically, the progressive maturation of collagen fibers in normal cords and their persistent disorganization in malformed cases is consistent with descriptions by Sharony *et al*. (2016), who emphasized the role of collagen integrity in maintaining vascular tensile strength and fetal-placental resilience. Stehbens *et al*. (2005) also documented ultrastructural abnormalities in vessel walls, including matrix degeneration, that may compromise vascular integrity in compromised pregnancies.

The umbilical cord is not merely a passive conduit but a dynamically remodeling structure essential to fetal homeostasis. Normal morphometric progression - reflected in the strong correlation between gestational age and vessel dimensions - supports adequate blood flow, oxygenation, and mechanical protection of the vasculature (Ferguson & Dodson, 2009; Alfirevic *et al*., 2017).

The disrupted growth patterns and wall remodeling observed in malformed fetuses likely indicate impaired fetal-placental hemodynamics. Thinner vascular walls, delayed collagen maturation, and absence of correlation between vein thickness and gestational age may compromise vascular resilience and flow regulation. These abnormalities may not only be secondary to systemic fetal malformations but could contribute to suboptimal fetal development, highlighting a bidirectional relationship between form and function. In this context, the absence of expected smooth muscle differentiation and matrix maturation in malformed cords contrasts sharply with the structured, progressive remodeling, as observed in normal vessels (Sexton *et al*., 1996). Similar patterns of altered wall thickness and vein narrowing have been reported in preeclampsia, where increased wall-luminal ratios and smooth muscle proliferation were associated with adverse fetal outcomes (Lan *et al*., 2018).

Clinically, our findings suggest that morphometric assessment of the umbilical cord and vessels - both prenatally via Doppler and postnatally via histology - could serve as complementary tools in the risk stratification of pregnancies complicated by congenital anomalies. This is supported not only by studies in preeclampsia, which stated that quantitative vessel parameters, such as wall/lumen ratios and medial thickening, can reveal distinct fetal vascular phenotypes (Blanco *et al*., 2011; Lan *et al*., 2018) but in other diseases as well (Yousif *et al*., 2023; Souza *et al*., 2024; Chandrakar *et al*., 2025).

Strengths and limitations

One of the major strengths of this study is its systematic and simultaneous evaluation of both morphometric and histological features across multiple gestational intervals. By including well-defined normal and malformed fetal groups, and standardizing measurement protocols for cross-sectional area and wall thickness, the study enhances internal validity. The use of Picrosirius red staining with polarized light microscopy provided high-resolution insight into collagen maturation - an underreported but critical aspect of umbilical vessel development. Moreover, the application of robust statistical methods - including non-parametric testing and Spearman correlation - allowed for nuanced detection of trends even in small subgroups.

As limitations we can highlight the sample size for later gestational intervals (especially 25-28 PCW) was relatively small, which may have limited statistical power in some comparisons. The classification of "malformed" included a heterogeneous group of congenital anomalies without stratification by etiology, severity, or syndromic involvement, which may confound the interpretation of vascular changes. Additionally, postmortem artifacts cannot be entirely excluded, although fixation and histological processing were standardized. Lastly, the cross-sectional nature of the study prevents longitudinal tracking of morphometric changes within individual fetuses.

CONCLUSION

This study demonstrates that the histological and morphometric development of the proximal umbilical cord follows a predictable maturational pattern in normal human fetuses, marked by progressive increases in vessel wall thickness, vascular area, elastic fiber organization, and collagen Type I predominance. In malformed fetuses, these parameters were frequently disrupted, with stagnation or regression of vessel growth, reduced or disorganized elastic components, and persistent Type III collagen expression even in later gestational ages.

The significant intergroup differences, particularly in vessel wall thickness and elastic fiber scores, suggest that congenital anomalies may interfere with normal vascular remodeling during fetal development. These findings highlight the importance of structural assessment of the umbilical cord as a potential marker of fetal pathology and may contribute to future understanding of the tissue-level mechanisms underlying adverse perinatal outcomes in malformed fetuses. Further investigations using immunohistochemistry could enhance the understanding of the molecular alterations underlying the structural changes observed in malformed umbilical cords.

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RESUMEN: El cordón umbilical es esencial para el desarrollo fetal, y sus vasos experimentan un remodelado continuo para sostener el crecimiento. Las alteraciones en la estructura de la pared vascular y en la organización de la matriz extracelular se han vinculado con un desarrollo deficiente y con malformaciones congénitas. Sin embargo, los análisis sistemáticos de la histomorfometría de los vasos del cordón y de la maduración del colágeno a lo largo de la gestación siguen siendo escasos. Se estudiaron 97 fetos humanos (51 normales, 46 malformados), estratificados en subgrupos por semanas postconcepcionales (13–16, 17–20, 21–24, 25–28). Los segmentos proximales del cordón fueron procesados con resorcina-fucsina de Weigert y Picrosirius Red. El área y el grosor del cordón y de los vasos se midieron con ImageJ. La distribución del colágeno se evaluó cualitativamente bajo luz polarizada. La normalidad se verificó con Kolmogorov-Smirnov; las comparaciones intragrupo se realizaron con Kruskal-Wallis y post hoc de Dunn; las intergrupales con t de Student o Mann-Whitney U; y las correlaciones con rho de Spearman. Se consideró significativo un valor de $p < 0,05$. En los fetos normales, todos los parámetros morfométricos se correlacionaron positivamente con la edad gestacional ($p < 0,001$), incluyendo el área del cordón ($r = 0,502$), área venosa ($r = 0,735$), área arterial ($r = 0,725$) y grosores de la pared. Tanto áreas transversales como grosores aumentaron significativamente en los intervalos. Por ejemplo, el grosor arterial pasó de $0,17 \pm 0,07$ mm a las 13–16 SPC a $0,35 \pm 0,09$ mm a las 25–28 SPC ($p < 0,05$). En los malformados, solo el área arterial ($r = 0,392$, $p = 0,007$) y el grosor arterial ($r = 0,570$, $p < 0,001$) se correlacionaron con la edad; el área del cordón y el grosor venoso no mostraron progresión. Entre grupos, el área venosa fue mayor en malformados a las 13–16 SPC ($p = 0,0041$), mientras que a las 17–20 SPC el grosor venoso y arterial fue menor ($p = 0,0027$ y $p = 0,0003$). No hubo diferencias a las 21–28 SPC. Picrosirius Red mostró maduración progresiva del colágeno en cordones normales, frente a desorganización y retraso en los malformados. Los vasos umbilicales normales presentaban patrones predecibles de maduración morfométrica y de colágeno, mientras que los fetos malformados mostraron un remodelado deficiente, lo que respalda el estudio histológico del cordón como posible marcador de patología fetal.

PALABRAS CLAVE: Cordón umbilical; Malformación fetal; Grosor de la pared vascular; Fibras elásticas; Colágeno; Histomorfometría.

REFERENCES

Alfirevic, Z.; Stampalija, T. & Dowswell, T. Fetal and umbilical Doppler ultrasound in high-risk pregnancies. *Cochrane Database Syst. Rev., 6(6)*:CD007529, 2017.

Altunkaynak, B. Z. & Yahyazadeh, A. Stereological and histological assessment of the umbilical cord in newborn rat. *J. Microsc. Ultrastruct., 9(3)*:108-13, 2021.

Barbieri, C.; Cecatti, J. G.; Surita, F. G.; Costa, M. L.; Marussi, E. F. & Costa, J. V. Area of Wharton's jelly as an estimate of the thickness of the umbilical cord and its relationship with estimated fetal weight. *Reprod. Health, 8*:32, 2011.

Blanco, M. V.; Vega, H. R.; Giuliano, R.; Grana, D. R.; Azzato, F.; Lerman, J. & Milei, J. Histomorphometry of umbilical cord blood vessels in preeclampsia. *J. Clin. Hypertens. (Greenwich), 13(1)*:30-4, 2011.

Chakraborty, S. K. & Banu, L. A. Microscopic impacts of gestational diabetes mellitus on the umbilical cord. *Mymensingh Med. J., 22(4)*:755-60, 2013.

Chandrakar, D.; Goyal, M.; Dhurandhar, D.; Khare, S.; Chakraborty, K. & Agrawal, J. Umbilical cord structural changes in pregnancy-induced hypertension: histological and morphometric insights. *Cureus, 17(2)*:e78951, 2025.

Chillakuru, S.; Velichety, S. D. & Rajagopalan, V. Human umbilical cord and its vessels: a histomorphometric study in different severity of hypertensive disorders of pregnancy. *Anat. Cell Biol., 53(1)*:68-75, 2020.

Coenen, H.; Braun, J.; Koster, H.; Mollers, M.; Schmitz, R.; Steinhard, J. & Oelmeier, K. Role of umbilicocerebral and cerebroplacental ratios in prediction of perinatal outcome in FGR pregnancies. *Arch. Gynecol. Obstet., 305(6)*:1383-92, 2022.

Contro, E.; Larcher, L.; Lenzi, J.; Valeriani, M.; Farina, A. & Jauniaux, E. Changes in artery diameters and fetal growth in cases of isolated single umbilical artery. *Diagnostics (Basel), 13(3)*:571, 2023.

Divon, M. Y. & Hsu, H. W. Maternal and fetal blood flow velocity waveforms in intrauterine growth retardation. *Clin. Obstet. Gynecol., 35(1)*:156-71, 1992.

Dubetskyi, B. I.; Makarchuk, O. M.; Zhurakivska, O. Y.; Rymarchuk, M. I.; Andriets, O. A.; Lenchuk, T. L.; Delva, K. M.; Piron-Dumitrascu, M. & Bakun, O. V. Pregnancy and umbilical cord pathology: structural and functional parameters of the umbilical cord. *J. Med. Life, 16(8)*:1282-91, 2023.

Ferguson, V. L. & Dodson, R. B. Bioengineering aspects of the umbilical cord. *Eur. J. Obstet. Gynecol. Reprod. Biol., 144(Suppl. 1)*:S108-13, 2009.

Gayatri, R.; Crasta, J.; Thomas, T.; Pratibha, D.; Thomas, A.; Sridhar, T. S. & Kurpad, A. V. Structural analysis of the umbilical cord and its vessels in intrauterine growth restriction and pre-eclampsia. *J. Fetal Med., 4(2)*:85-92, 2023.

Junek, T.; Baum, O.; Läuter, H.; Vetter, K.; Matejevic, D. & Graf, R. Pre-eclampsia associated alterations of the elastic fibre system in umbilical cord vessels. *Anat. Embryol. (Berl.), 201(4)*:291-303, 2000.

Kim, H. J.; Kim, J. H.; Chay, D. B.; Park, J. H. & Kim, M. A. Association of isolated single umbilical artery with perinatal outcomes: systematic review and meta-analysis. *Obstet. Gynecol. Sci., 60(3)*:266-73, 2017.

Kozadinos, A.; Mylonakis, A.; Bekos, F.; Kydonakis, N.; Korovesis, G.; Kastanaki, P.; Despotidis, M.; Chrysikos, D. & Troupis, T. The development of the umbilical vein and its anatomical and clinical significance. *Cureus, 17(2)*:e79712, 2025.

Kurakazu, M.; Kurakazu, M.; Murata, M.; Miyamoto, T.; Takahashi, Y.; Hamasaki, M.; Ohta, E.; Yotsumoto, F. & Miyamoto, S. A partial supernumerary umbilical vein: a case report. *J. Med. Case Rep., 13(1)*:149, 2019.

Lan, Y.; Yang, Z.; Huang, M.; Cui, Z.; Qi, Y. & Niu, H. Morphological and structural changes of umbilical veins and clinical significance in preeclampsia. *Hypertens. Pregnancy, 37(3)*:105-10, 2018.

Mailath-Pokorny, M.; Worda, K.; Schmid, M.; Polterauer, S. & Bettelheim, D. Isolated single umbilical artery: evaluating the risk of adverse pregnancy outcome. *Eur. J. Obstet. Gynecol. Reprod. Biol., 184*:80-3, 2015.

Qin, Y.; Wen, H.; Liang, M.; Luo, D.; Zeng, Q.; Liao, Y.; Zhang, M.; Ding, Y.; Wen, X.; Tan, Y.; *et al.* A new classification of congenital abnormalities of UPVS: sonographic appearances, screening strategy and clinical significance. *Insights Imaging, 12(1)*:125, 2021.

Salem, M.; Abd El-Gawwad, S.; Mahmoud, E. & Elyamany, H. Histological study of human umbilical cord in diabetic pregnant females. *Egypt. J. Histol., 42(4)*:874-87, 2020.

Sexton, A. J.; Turmaine, M.; Cai, W. Q. & Burnstock, G. A study of the ultrastructure of developing human umbilical vessels. *J. Anat., 188(Pt. 1)*:75-85, 1996.

Sharony, R.; Eran, K.; Biron-Shental, T. & Kidron, D. Morphometric characteristics of the umbilical cord and vessels in fetal growth restriction and pre-eclampsia. *Early Hum. Dev., 92*:57-62, 2016.

Souza, K.; Hochberger, L. C.; Castrignano Camargo, F. E.; Silva, G. S.; Camargo, G. C.; Mello, J. P. L.; Alcantara dos Santos, F. C.; Giachini, F. R.; Lobato, N. S. & Souto, P. C. S. Umbilical vein remodeling is associated with pregestational maternal overweight. *Front. Endocrinol. (Lausanne), 15*:1483364, 2024.

Stehbens, W. E.; Wakefield, J. S.; Gilbert-Barness, E. & Zuccollo, J. M. Histopathology and ultrastructure of human umbilical blood vessels. *Fetal Pediatr. Pathol., 24(6)*:297-315, 2005.

Streeter, G. L. Weight, sitting height, head size, foot length and menstrual age of the human embryo. *Contrib. Embryol. Carnegie Inst. Wash. Publ., 11*:143-70, 1920.

Togni, F. A.; Araujo Junior, E.; Vasques, F. A.; Moron, A. F.; Torloni, M. R. & Nardozza, L. M. The cross-sectional area of umbilical cord components in normal pregnancy. *Int. J. Gynaecol. Obstet., 96(3)*:156-61, 2007.

World Medical Association. World Medical Association Declaration of Helsinki: ethical principles for medical research involving human subjects. *JAMA, 284(23)*:3043-5, 2000.

Yousif, A. I.; Waheed, I. N. & Abdulla, I. T. A comparison of the human umbilical cord's histomorphometric and histological structure in pregnant diabetic and non-diabetic women. *J. Biol. Agric. Healthc., 13(6)*:32-42, 2023.

Yu, J.; Wu, Q.; Kong, F. & Ning, Y. Diagnosis of single umbilical artery and risk of foetal congenital malformations by prenatal ultrasound: a retrospective study. *BMC Pregnancy Childbirth, 24(1)*:193, 2024.

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