ΤΙΤΛΟΣ ΔΙΠΛΩΜΑΤΙΚΗΣ ΕΡΓΑΣΙΑΣ

"Μονήρης ομφαλική αρτηρία. Συχνότητα και συσχέτιση με επιδημιολογικούς παράγοντες, διαταραχές αύξησης και συγγενείς διαμαρτίες σε νεκροτομικό υλικό εμβρυϊκών θανάτων από τη Βόρεια Ελλάδα"

"Single umbilical artery: Frequency and correlation with demographic data, growth and congenital malformations on a series of fetal autopsies from Northern Greece"

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Contents

ΕΥΧΑΡΙΣΤΙΕΣ

Η παρούσα διπλωματική εργασία εκπονήθηκε στο πλαίσιο του Προγράμματος Μεταπτυχιακών Σπουδών της Ιατρικής Σχολής του Καποδιστριακού Πανεπιστημίου Αθηνών σε συνεργασία με το τμήμα της Μαιευτικής του Τ.Ε.Ι Αθήνας με τίτλο ``Έρευνα στη Γυναικεία αναπαραγωγή''. Πραγματοποιήθηκε κάτω από την καθοδήγηση της κ. Μεδίτσκου-Ευθυμιάδου Σουλτάνας, αναπληρώτριας καθηγήτριας στο εργαστήριο Ιστολογίας-Εμβρυολογίας του τμήματος Ιατρικής της Σχολής Επιστημών Υγείας του Αριστοτελείου Πανεπιστημίου Θεσσαλονίκης. Ως την ελάχιστη δυνατή μνεία, με την παρούσα παράγραφο οφείλω να ευχαριστήσω όλους όσους συνέβαλαν στην εκπόνησή της και ιδιαίτερα:

Την επιβλέπουσα καθηγήτριά μου, κυρία Μεδίτσκου-Ευθυμιάδου Σουλτάνα, για την ανεκτίμητη συμβολή της, τον πολύτιμο χρόνο της, τις καίριες υποδείξεις της και το εξαιρετικό κλίμα συνεργασίας που διαμόρφωσε, καθώς και την εμπιστοσύνη που επέδειξε στο πρόσωπό μου για την ανάθεση του υλικού του εργαστηρίου Ιστολογίας-Εμβρυολογίας της Ιατρικής Σχολής ΑΠΘ.

Επίσης, είμαι ευγνώμων στον Καθηγητή κύριο Μηλιαρά Δημοσθένη και την Αναπληρώτρια Καθηγήτρια κυρία Νόννη Αφροδίτη, που ήταν πρόθυμοι να συμμετάσχουν ως μέλοι της τριμελούς εξεταστικής επιτροπής και να μου προσφέρουν ουσιώδεις υποδείξεις και συμβουλές.

Ευχαριστώ θερμά και τον συντονιστή του μεταπτυχιακού προγράμματος «Έρευνα στη Γυναικεία Αναπαραγωγή», κύριο Μαστοράκο Γεώργιο και τους καταξιωμένους συνεργάτες, ειδικούς βασικής και κλινικής έρευνας, που με ενέπνευσαν και μου έδωσαν τη δυνατότητα να διευρύνω τους ορίζοντές μου μέσω της εις βάθος εκπαίδευσης και επιμόρφωσης σε θέματα της γυναικείας αναπαραγωγής και της ενδοκρινολογίας.

Τέλος, θα ήθελα να ευχαριστήσω τους συναδέλφους μου, μεταπτυχιακούς φοιτητές, για τη γόνιμη ανταλλαγή απόψεων, το ειλικρινές ενδιαφέρον τους και για την αξιοσημείωτη συμβολή τους σε όλα τα στάδια εκπόνησης της παρούσας εργασίας.

ABSTRACT

Introduction:

The umbilical cord consists normally of three vessels: two umbilical arteries and one umbilical vein, which are surrounded by a gelatinous substance called Wharton's jelly. The physiological role of the umbilical vein is to transfer oxygenated blood to the fetus, whereas the umbilical arteries carry deoxygenated blood from the fetus to the placenta. (1) The presence of a single umbilical artery (SUB) is one of the most common congenital malformations that have been described in humans (approximately 0.2-2% of deliveries) (2). This frequency is approximately 2-4 times more in autopsy cases (3) (4). The role of SUB remains controversial. Multiple mechanisms have been implicated in the pathogenesis of this disorder, such as aplasia or atresia of one of the umbilical arteries during embryogenesis or persistence of a single anomalous vessel of omphalomesenteric origin in the body stalk of the embryo. (2) (5) (6) SUB can be an isolated finding or it can be associated with other congenital anomalies that affect the cardiovascular, genitourinary, musculoskeletal, nervous and gastrointestinal systems. Poly- or oligohydramnios, velamentous insertion of the cord and placental abnormalities have been described at a greater frequency in embryos with SUB. (6)Furthermore, these pregnancies carry a higher risk of preterm delivery, perinatal mortality, intrauterine growth restriction and chromosomal anomalies. (7).

Aim:

The aim of this study is to determine the prevalence of SUB in autopsy cases obtained from the Laboratory of Histology and Embryology of the Medical School of Aristotle University of Thessaloniki, Greece and to correlate this malformation with multiple epidemiologic parameters, such as fetal weight, maternal age, structural defects, sex and age of the fetuses.

Materials and Methods:

We retrospectively retrieved 649 fetal and neonatal autopsy cases from the archives of Histopathology lab of Hippocrateion General Hospital and the laboratory of Pathology of General Clinic of Thessaloniki, Greece during the period of 1992 to 2008. The data were reviewed and analyzed at the Laboratory of Histology and Embryology of Medical Faculty of Aristotle University of Thessaloniki.

Results:

The data were categorized according to the maternal and gestational age, fetal and neonatal gender and weight, presence or absence of congenital anomalies and the histopathology of the umbilical cord. The statistical analysis software used was SPSS with a significance level set at 5%. An association between the presence of single umbilical artery and demographic and histopathologic data is being sought. The presence or absence of association between two categorical variables in the sample was determined with the use of Pearson's chi-squared ($\chi 2$) test. Single umbilical artery was the most commonly observed congenital anomaly in the examined material with a relative frequency of 4.46%. Statistically significant association was found between the presence of single umbilical artery and growth restriction, presence of other congenital anomalies and perinatal mortality. No association was described between the presence of the anomaly and maternal and fetal age.

Conclusion:

Our data suggest an increased risk between the presence of single umbilical artery and co-existing congenital anomalies. The results of the study were derived from autopsy material and not from prenatal data, so no information can be given about the prognosis of the pregnancies with single umbilical artery. We hope that the creation of a national registry containing demographic and biometric information about the characteristics of fetal abortuses and stillborns will increase our understanding about the correlation and prevalence of congenital anomalies.

ΠΕΡΙΛΗΨΗ

Εισαγωγή:

Ο ομφάλιος λώρος αποτελείται φυσιολογικά από τρία αγγεία: δύο ομφαλικές αρτηρίες και μία ομφαλική φλέβα, οι οποίες περιβάλλονται από μία ουσία ζελατινοειδούς σύστασης που ονομάζεται γέλη του Wharton. Ο ρόλος της ομφαλικής φλέβας είναι να μεταφέρει οξυγονωμένο αίμα στο έμβρυο, ενώ των ομφαλικών αρτηριών να απάγουν αποοξυγονωμένο αίμα από το έμβρυο στον πλακούντα (1). Η παρουσία της μονήρους ομφαλικής αρτηρίας εμφανίζεται με συχνότητα περίπου 0.2-2% στον τοκετό, (2) ενώ είναι περίπου 2-4 φορές μεγαλύτερη σε νεκροτομικά παρασκευάσματα. (3) (4) Ο ρόλος της μονήρους ομφαλικής αρτηρίας παραμένει αδιευκρίνιστος. Διάφοροι μηχανισμοί έχουν ενεπλακεί στην παθοφυσιολογία της διαταραχής, όπως αγενεσία ή ατρησία ενός εκ των δύο ομφαλικών αρτηριών. (2) (5) (6) Η απουσία μίας ομφαλικής αρτηρίας έχει συσχετιστεί με διαμαρτίες του καρδιαγγειακού, ουροποιητικού, μυοσκελετικού, νευρικού και γαστρεντερικού συστήματος, ενώ οι κυήσεις εμφανίσουν σε μεγαλύτερη συχνότητα πρόωρο τοκετό, αυξημένη περιγεννητική θνησιμότητα, ενδομήτριο περιορισμό της αύξησης και χρωμοσωμικές ανωμαλίες. (7)

Σκοπός:

Σκοπός της παρούσας εργασίας ήταν η εκτίμηση του επιπολασμού της μονήρους ομφαλικής αρτηρίας σε αρχειακό υλικό εμβρυϊκών θανάτων και η συσχέτιση της διαταραχής με διάφορες επιδημιολογικές παραμέτρους, όπως βάρος κύησης, ηλικία μητέρας, διαταραχές διάπλασης, φύλο και ηλικία του εμβρύου.

Μέθοδοι:

Στην παρούσα μελέτη συμπεριλήφθηκαν 649 περιπτώσεις εμβρυϊκών και νεογνικών θανάτων από τα αρχεία του εργαστήρια Ιστοπαθολογίας του Ιπποκρατείου Γενικού Νοσοκομείου και της Γενικής Κλινικής Θεσσαλονίκης. Η καταγραφή και επεξεργασία των

δεδομένων έγινε στο εργαστήριο Ιστολογίας και Εμβρυολογίας του Αριστοτελείου Πανεπιστημίου Θεσσαλονίκης.

Αποτελέσματα:

Τα δεδομένα κατηγοριοποιήθηκαν με βάση την ηλικία μητέρας, την ηλικία κύησης, το φύλο εμβρύου-νεογνού, το βάρος κυήματος, την παρουσία ή απουσία συγγενών ανωμαλιών και τα ιστοπαθολογικά χαρακτηριστικά του πλακούντα. Το πρόγραμμα στατιστικής ανάλυσης που χρησιμοποιήθηκε ήταν το SPSS με επίπεδο στατιστικής σημαντικότητας 5%. Στην παρούσα μελέτη αναλύθηκε η συσχέτιση μεταξύ της παρουσίας μονήρους ομφαλικής αρτηρίας και δημογραφικών-ιστοπαθολογικών δεδομένων. Η παρουσία συσχέτισης μεταξύ ποιοτικών μεταβλητών του δείγματος καθορίστηκε με τη στατιστική δοκιμασία χ2. Η μονήρης ομφαλική αρτηρία ήταν η πιο συχνή συγγενής ανωμαλία που παρατηρήθηκε στο δείγμα με σχετική συχνότητα 4.46%. Στατιστικά σημαντική σχέση παρατηρήθηκε μεταξύ της παρουσίας μονήρους ομφαλικής αρτηρίας και υπολειπόμενης αύξησης, παρουσίας άλλων συγγενών ανωμαλιών και περιγεννητικής θνησιμότητας. Δεν παρατηρήθηκε συσχέτιση μεταξύ της μονήρους ομφαλικής αρτηρίας και της ηλικίας μητέρας-εμβρύου.

Συμπεράσματα:

Τα δεδομένα μας περιγράφουν αυξημένη συσχέτιση μεταξύ της παρουσίας μονήρους ομφαλικής αρτηρίας και άλλων συγγενών ανωμαλιών. Τα αποτελέσματα της παρούσας μελέτης προέκυψαν από τη μελέτη νεκροτομικών παρασκευασμάτων και όχι από δεδομένα προγενετικού ελέγχου, με αποτέλεσμα να μην μπορεί να καθοριστεί η πρόγνωση των κυήσεων με μονήρη ομφαλική αρτηρία. Ευελπιστούμε ότι η δημιουργία εθνικού αρχείου καταγραφής εμβρυϊκών θανάτων που θα περιγράφει τα δημογραφικά και βιομετρικά χαρακτηριστικά των εμβρυϊκών θανάτων θα συμβάλλει στη κατανόηση των συσχετίσεων και επιπολασμού των συγγενών ανωμαλιών.

GENERAL SECTION

CHAPTER 1.DEVELOPMENT AND STRUCTURE OF THE UMBILICAL CORD

Embryogenesis of the umbilical cord

The umbilical cord is a helical structure that connects the developing embryo with the placenta. It usually develops between the 4th and the 8th week of development. (8) At the end of the 3rd week the fetus is connected to the chorion by the body or connective stalk. The body stalk is a structure derived from extraembryonic mesoderm. Originally it contains two umbilical arteries and two umbilical veins. Atresia of the second umbilical vein at the end of the 6th week results in the final form of the umbilical cord with two arteries and one vein. Another component of the connecting stalk is the allantois. The allantois is a diverticulum of the hindgut that later in development is continuous with the urinary bladder. (8) (9) (10) As the embryonic disk begins to fold the amniotic cavity expands. The umbilical cord develops as the expanding amnion engulfs the components of the body or connecting stalk and the yolk sac on the ventral surface of the embryo. (11) (12)
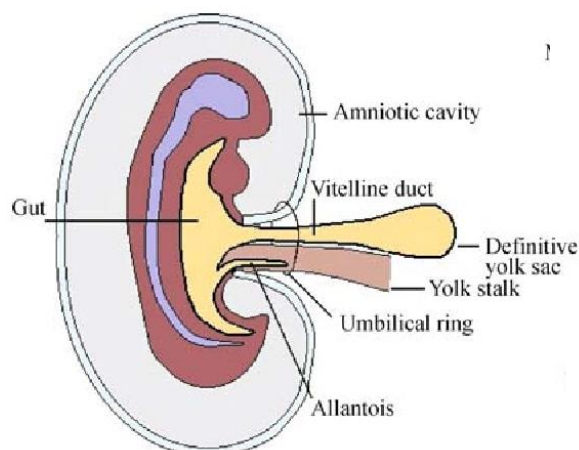

Formation of the umbilical cord (13)

This folding divides the yolk sac into two parts: one part inside the fetal body which leads to the formation of the gut and one part outside the fetal body, which is called definite yolk sac. The two parts are connected through the vitelline or omphalomesenteric duct.

(13) As the fetal gut develops it normally protrudes into the umbilical cord. Enlargement of the abdominal cavity by the 12th week of gestation enables the accommodation of the intestines and reduces this physiologic herniation. During fetal development the allantois regresses and ultimately forms the median umbilical ligament. (14) (11)

<u>Anatomy and histology</u>

In its fully developed form the umbilical cord is a tubular structure that is surrounded by the amnion and contains two umbilical arteries and one umbilical vein that are contained within a gelatinous substance called Wharton's jelly. The umbilical vein is responsible for carrying oxygenated blood and nutrients to the embryo, whereas the umbilical arteries are responsible for the transport of deoxygenated blood and metabolism by-products from the fetus to the placenta. (8) (14)
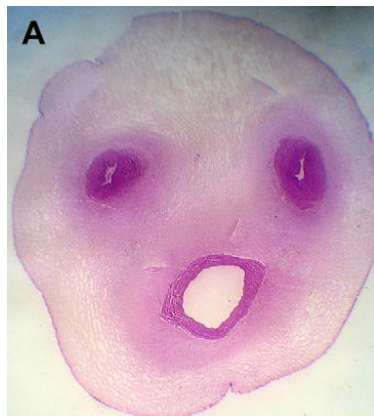
Normal umbilical cord cross-section containing two arteries and one vein (15)

Major differences exist between the umbilical and the adult vessels. Since the umbilical vein is responsible for the transport of oxygenated blood, its wall contains a thick muscularis layer as well as an internal elastic lamina. On the contrary, umbilical arteries lack the external and internal elastic lamina. (16) (17)

A.   The umbilical arteries

Normal umbilical cords contain two arteries. They are branches of the internal iliac arteries in the embryo. (18) Their lumen is surrounded by endothelial cells which contain prominent nuclei. (15) Unlike the endothelium of other vessels, the endothelium of umbilical arteries is rich in organelles. (16) The media, which is the thickest layer, contains two arrangements of muscle cells, the outer, which is circular and the inner, which is

longitudinal. (19) The inner media contains few myofilaments and is poorly differentiated. (16)

Cross section of a umbilical artery showing a pale intima layer and a thick muscular coat with two layers, an outer circular and an inner longitudinal (15)

Located within 0.5 to 6cm from the placental insertion there is an anastomosis between the umbilical arteries called Hyrtl anastomosis. Its function is to equalize the pressure and flow within the two umbilical arteries in order to ensure an even distribution of blood volume within the placenta. (20) (21) (22)
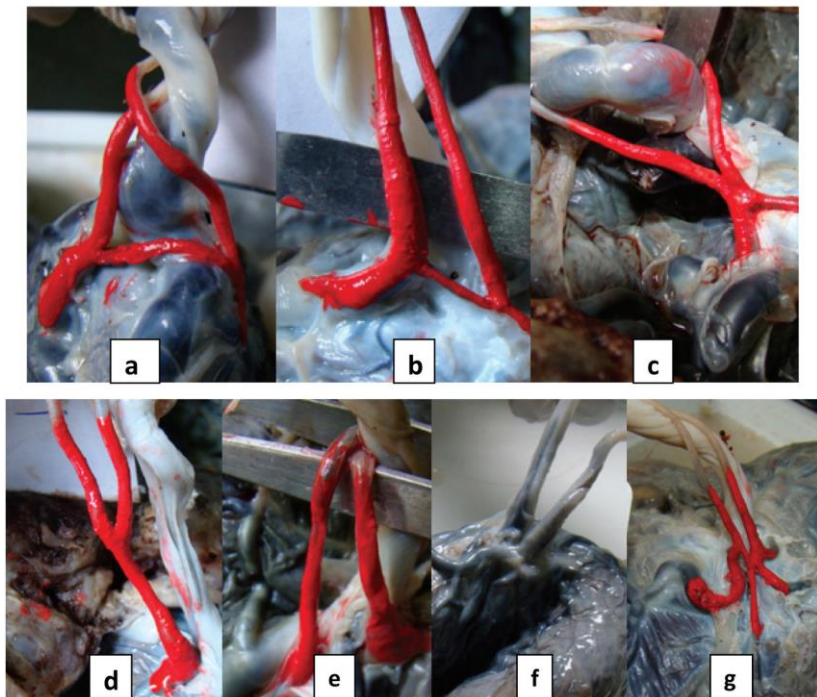
Hyrtl anastomosis: multiple anatomic variations (23)

B.   The umbilical vein

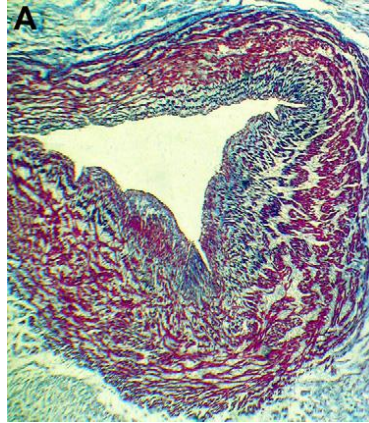Unlike the umbilical arteries the umbilical vein contains a thin tunica media which is comprised of more discrete layers of longitudinal and circular fibers. (16) (19)

Because the umbilical arteries have a thicker muscular wall, they always have a diameter that is less than 50% of the diameter of the vein. (24)

Cross section of a umbilical vein (15)

The adventitia of both arteries and veins is replaced by a loose layer of spiraled collagen fibers. The umbilical vessels contain no vasa vasorum and are not innervated. They only respond to endocrine stimuli, such as prostaglandins, serotonin, oxytocin and angiotensin which contribute to the contraction of their muscle wall after delivery. (16)

The umbilical vessels are embedded in a gelatinous, loose matrix called Wharton jelly. Wharton jelly is the connective tissue of the cord and is rich in proteoglycans, glycosaminoglycans, hyaluronic acid and mesenchymal stromal cells. (25) (26) It serves as a protective layer which cushions the vessels and acts against any vibratory or stretching forces. (14)   Wharton's jelly is derived from the extraembryonic mesoblast. (27) Other than the umbilical vessels no other vessels or nerves are contained. (28)

The umbilical cord is covered by the amnion. Near the umbilical ring the epithelium is stratified squamous, while further away it is stratified columnar containing 2-8 layers of cells. (16) (19) The amniotic epithelium is continuous with the epithelium of the placenta and the skin of the embryo. (19) The amniotic epithelium of the cord is largely unkeratinized, except in the region close to the embryo. (29) Unlike the amnion that surrounds the placenta, the amniotic epithelium of the umbilical cord is attached firmly to its surface and cannot get detached. (19)

The normal umbilical cord is a pearl-white structure that is approximately 50-60cm long and has a diameter of 1-2cm at term. (11) (30) The two umbilical arteries usually coil

around the large-diameter umbilical vein. The normal cord has one coil per 5 cm of umbilical cord length. (31) Although the true etiology of the coiling remains unclear it is believed that it exerts a protective function against compression and torsion of the umbilical vessels. (32) (33)


Connection with the placenta

The umbilical cord connects the developing embryo with the fetal portion of the placenta called chorionic plate. Mature placenta consists of two parts:

A.   The fetal part or chorionic plate. The fetal part is derived from the syncytiotrophoblast, cytotrophoblast and the somatic extraembryonic mesoderm. On its fetal surface it is covered by the amnion and the amniotic mesenchyme which is an avascular connective tissue. The chorionic plate contains multiple chorionic villi that project into the intervillous space. (14) (34) The umbilical cord usually inserts at or near the center of the placenta. (16) The vessels of the chorionic plate are continuous with the vessels of the cord. More specifically, the two umbilical arteries branch in a centrifugal pattern in order to give rise to the chorionic arteries. The chorionic veins, when they coalesce, they give rise to the single umbilical vein. (34) The fetal part of the placenta has a smooth, shiny appearance.

B.   The maternal part or basal plate. The maternal part is derived from the maternal endometrium, and more specifically from the decidua basalis. Contained within the maternal part of the placenta are extravillous trophoblasts, decidual stroma cells and cells of the immune system, such as macrophages and natural killer cells. (34) During the fourth and fifth month of development septa that arise from maternal tissue protrude at several sites in the intervillous space. These septa divide the maternal part of the placenta into 10–40 slightly elevated lobes, called cotyledons. (25) Cotyledones give a cobblestone appearance to the maternal surface.
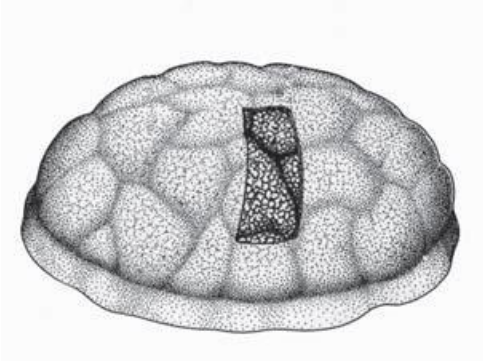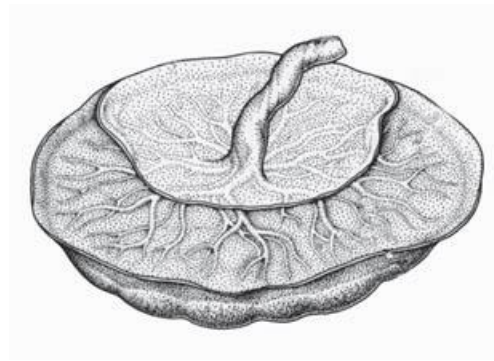
A.   Maternal part of the placenta divided into cotyledons by the decidua septa

B.   Fetal part of the placenta covered by the amnion (11)

Placental circulation

Umbilical arteries drain deoxygenated blood from the embryo to the placenta. (14) (25) As the arteries enter the placenta they branch into an extensive network of chorionic arteries that ultimately enter the chorionic villi. In the chorionic villi the arteries give rise to arterioles and capillaries with ultimately drain into venules. When these venules coalesce they form a single umbilical vein which then enters the umbilical cord. (14) (35)The fetal and maternal blood circulates in two different compartments, so there is no mixing of maternal and fetal blood. (36) The exchange of substances occurs through the placental membrane. The composition of the placenta membrane is altered in the course of pregnancy. Until 20 weeks gestation the placenta membrane consists of four layers: the syncytiotrophoblast, the cytotrophoblast, connective tissue and the endothelium of fetal capillaries. In late pregnancy the placental membrane is composed of two layers: the syncytiotrophoblast and the endothelium of the fetal capillaries. (14) (34) (11)
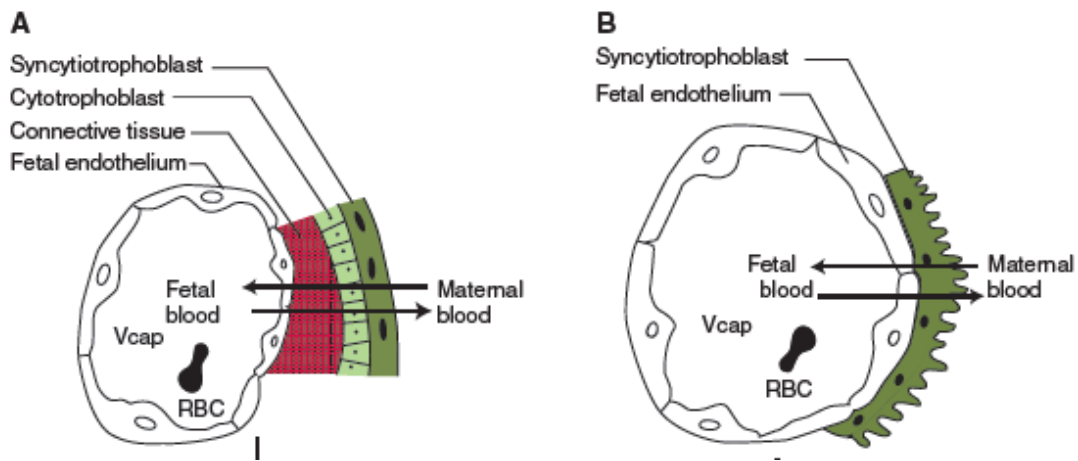
A. Placental membrane during early pregnancy

B. Placental membrane during late pregnancy (11)

At the maternal side the cotyledons receive blood through spiral arteries. During normal pregnancy spiral arteries undergo remodeling that transforms them into low-resistance vessels that can accommodate a large amount of blood volume. Remodeling occurs as extravillous trophoblasts migrate into the decidua and the myometrium, invade the maternal vasculature and replace their endothelial and smooth muscle lining. (37) (38) Failure in this remodeling process leads to high-resistance vessels and can contribute to the development of pre-eclampsia and intrauterine growth restriction. (39) (40) (41)
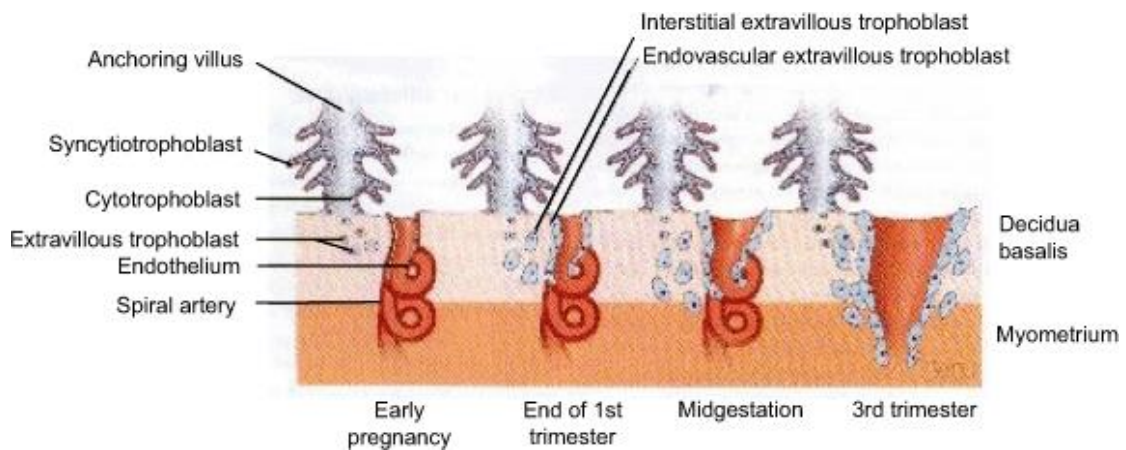
The process of spiral artery remodeling (38)

Maternal blood enters the intervillous space where it comes in contact with the chorionic villi. After the exchange takes place, the blood returns to the maternal systemic circulation through the uterine veins. (38)

The complex placental circulation can be reviewed in the image (14):
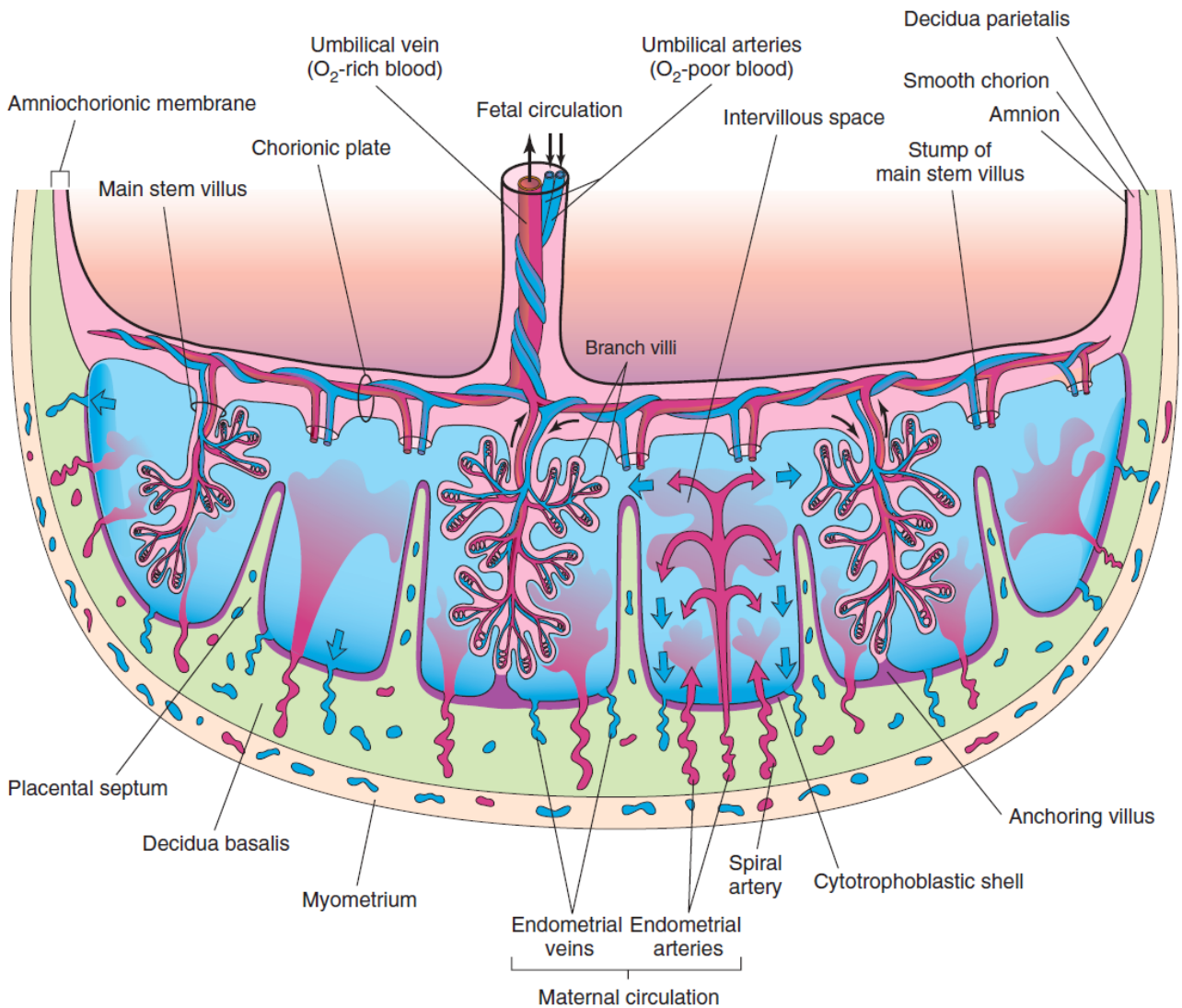
Fetal circulation

Fetal circulation differs significantly from that of the newborn. Unlike the newborn fetal circulation is characterized by high pulmonary vascular resistance as the alveoli are filled with amniotic fluid. (42) Umbilical vein brings oxygenated blood to the fetus. Through the umbilical vein the blood divides and flows through the portal circulation or the ductus venosus. Through the ductus venosus blood passes through the inferior vena cava and enters the right atrium. The greatest proportion of this blood is shifted through the foramen ovale to the left atrium, left ventricle and ultimately into the aorta. Deoxygenated

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blood from the superior vena cava enters the right atrium and then passes through the tricuspid valve into the right ventricle where it is pumped into the pulmonary artery. Because of high resistance in the pulmonary circulation the blood enters the descending aorta through the ductus arteriosus. The descending aorta ultimately bifurcates to the iliac arteries which give rise to the umbilical arteries that bring deoxygenated blood to the placenta. (43) (44) (45) (46) (47)
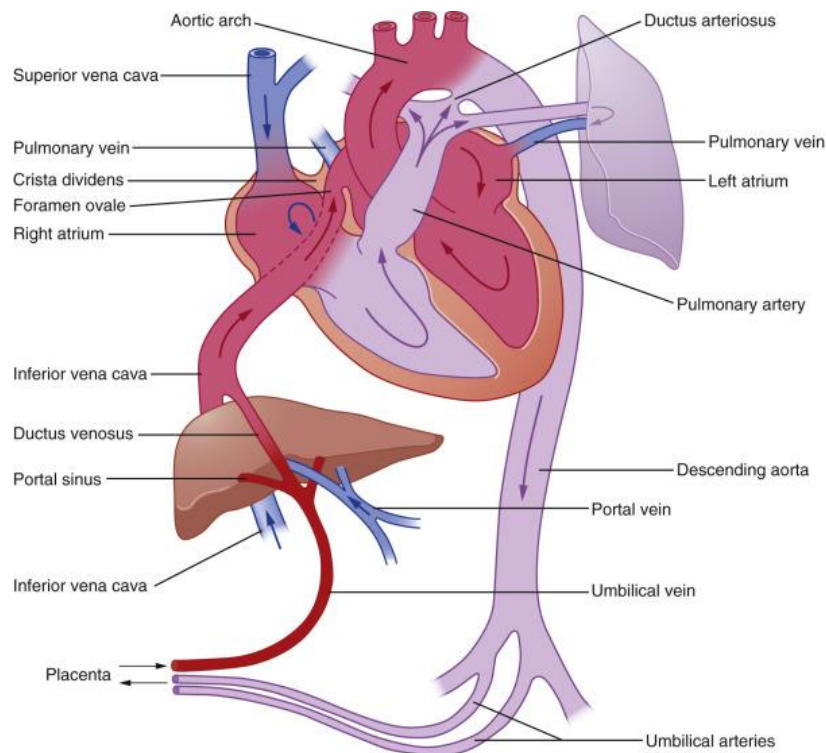
Fetal circulation (43)

CHAPTER 2.UMBILICAL CORD PATHOLOGY

Umbilical cord abnormalities are widely recognized are risk factors for increased perinatal morbidity and mortality. More specifically, any disruption to the blood flow can lead to intrauterine hypoxia and anoxia, hypoxic brain injury and fetal demise. (48) Umbilical cord anomalies can be sorted in the following categories:

A.   Abnormalities of position and insertion:

The most common site of insertion of the umbilical cord is at the center or near the center of the chorionic plate. Marginal cord attachment which occurs in approximately 7% of term placentas refers to an insertion at the edge of the placenta. Velamentous cord insertion affects approximately 1% of term placentas. The term is used to describe an umbilical cord that is inserted into the membranes rather than the placenta. (49) As the umbilical cord enters the membranes it loses the support of the protective Wharton jelly and carries an increased risk for hemorrhage, compression and thrombosis. (49) (50) (51) The term vasa previa describes velamentous vessels that cross near the internal os of the cervix and below the fetal presenting part. (52) Laceration of these vessels during spontaneous or artificial rupture of membranes leads to fetal exsanguination and rapid fetal demise.

A
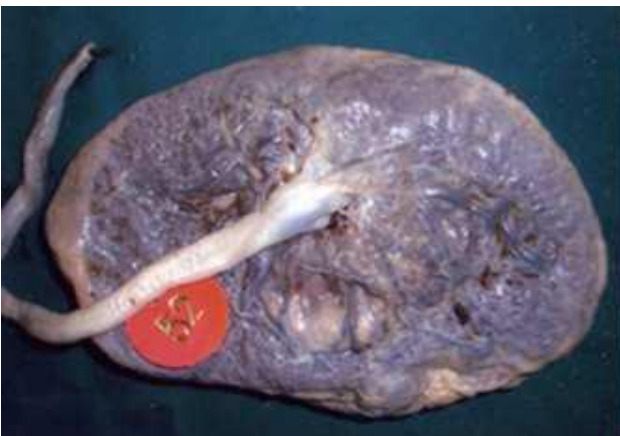
B
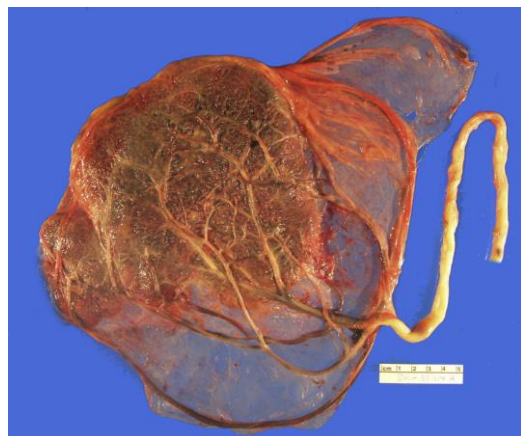
C
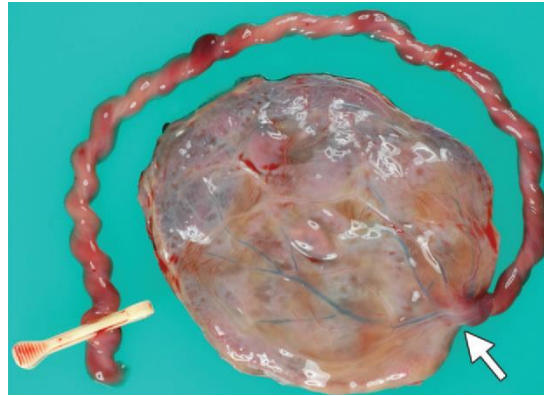
A. Normal central insertion of the cord (53)

B. Velamentous inrsetion of the cord (49)

C. Marginal insertion of the cord (54)

B.   Length and diameter abnormalities

Excessively long cords (>80cm) can form a loop around the neck of the embryo (commonly referred to as nuchal cords) or they can prolapse and thus carry an increased risk for fetal demise. (8) Furthermore, long cords have a higher vascular resistance and carry an increased risk for thrombosis. (49) On the contrary, very short cords (<35cm) are associated with chromosomal anomalies, abnormal position in utero, intrauterine growth restriction (IUGR), poor mental and motor development, abdominal wall defects and limb deformities. (55) (56) (57) Short cords are also associated with premature separation of the placenta.

A   B

A.   Extremely long umbilical cord.   B. Extremely short umbilical cord (19)

Congenital absence of the umbilical cord is rarely described and is associated with abdominal wall and cardiac anomalies. (58)

Normal term umbilical cords usually have a diameter of approximately 3-5 cm. Excessively thin cords can result from deficiency of the Wharton jelly. This makes the umbilical vessels more prone to mechanical damage. Thin umbilical cords have been associated with intrauterine growth restriction, preeclampsia and fetal demise. (16) (59)

C.   Vascular anomalies

Single umbilical artery is the most common congenital abnormality in humans. It is going to be discussed in great detail in the next chapter.

The presence of a supplementary umbilical vessel is rarely reported. It most commonly arises due to failure of the right umbilical vein to regress. This leads to the formation of a four-vessel cord which is associated with multiple congenital anomalies, such as ectopia cordis, atrial septal defect, symmetrical bifid liver, cleft lip and palate, malrotation and arteriovenous fistulas of the placenta. (60)
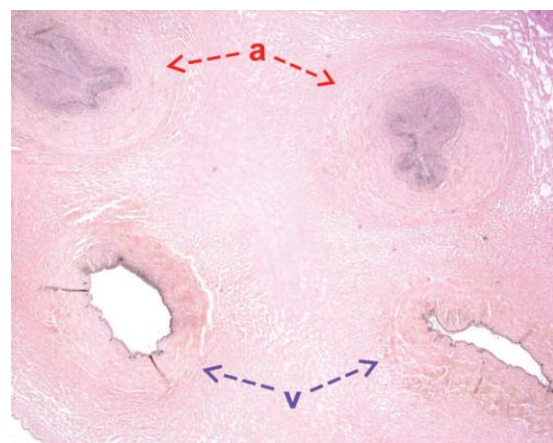
Four-vessel umbilical cord containing two arteries (a) and two veins (b) (61)

Persistent right umbilical vein (PRUV) is another anomaly that results when the left umbilical vein regresses while the right remains patent. (62) PRUV can be associated with congenital absence of the ductus venosus and other severe anomalies that affect the cardiovascular, gastrointestinal, musculoskeletal and urogenital system. If it occurs as an isolated finding the prognosis is usually benign. (63)

D. Cord knots

True and false knots can form in the umbilical cord. False knots are variations of the normal cord structure and are not associated with any adverse event. (18) They are the result of a formation of a varicosity or exaggerated looping in the umbilical vessels (usually the vein) in order to accommodate the length of the cord. (64) True knots, on the other hand, affect approximately 0.3-2% of pregnancies and are associated with adverse outcomes due to compromised blood flow when the knot tightens. (65) (66) (67) Risk factors include multiple pregnancy (esp. monoamniotic twins), advanced maternal age, long or hypercoiled umbilical cords, polyhydramnios, small-sized embrya and male gender of the embryo. (68) (69)
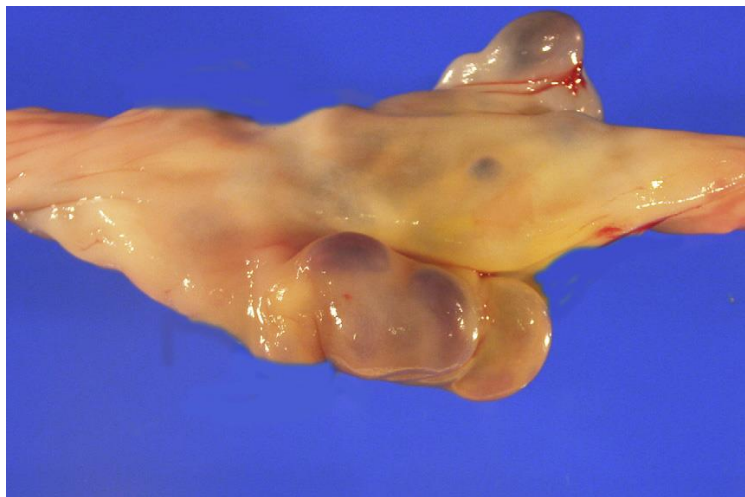
False knot (49)

True knot (68)

E.   Over- and undercoiling

Normal umbilical cords have an average of coiling of 1 spiral per 5cm cord length. (31) Coiling exerts a protective role against external forces that might compromise the umbilical bood flow. Umbilical cord coiling can be evaluated with the umbilical cord index. The umbilical cord index is calculated by dividing the total number of complete umbilical vascular coils by the umbilical cord length (in centimeters). (70) Most authors define hypocoiling as number of coils below the 10th percentile and hypercoiling as above the 90th percentile. (70) (54) The prevalence of hypocoiled or non-coiled cords is approximately 4-5%. (71) Hypocoiling is associated with increased risk for mechanical damage, meconium staining, low APGAR scores, chromosomal anomalies, preeclampsia, placental abruption and chorioamnionitis. (72) (73) (74) Hypocoiling is also associated with a higher risk of abnormal cord insertion. (75) The prevalence of hypercoiling ranges from 10 to 20%. Hypercoiled cords can create a constriction with subsequent thrombosis which can lead to fetal demise and intrauterine growth restriction. (49) (76)
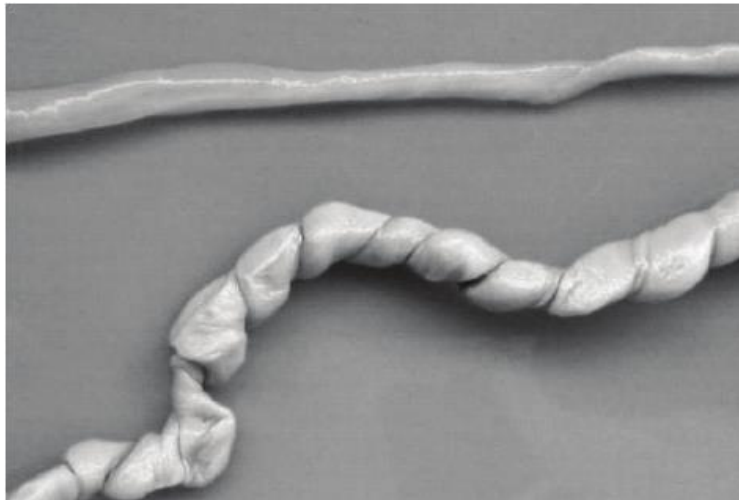
Hypocoiled (top) and hypercoiled (bottom) umbilical cord (77)

F.   Cord hematomas, cysts and tumors

Cord hematomas result from exravasation of blood within the umbilical cord and carry approximately 50% of fetal mortality rate. (49) The majority of these cases is iatrogenic and is lined with intrauterine procedures, particularly cordocentesis. (54)

Cord hematoma (78)

Cord cysts can either be true or false. True cysts have an epithelial lining and are embryologic remnants of the vitalline duct or the allantois. In the majority of cases allantoic remnants are of no significance. In rare occasions if the allantoic duct has not obliterated, it is connected with the bladder and can lead to urination through the umbilical stump. (49) Cysts in the vitelline duct can be associated with abdominal wall defects and Meckel's diverticulum. (79) False cysts or pseudocysts are not lined by epithelium and are created by local liquefaction of the Wharton jelly. They can be single or multiple. When they are large, they can compress the cord vessels or rupture, thus carrying an increased risk for fetal distress. (80)

True umbilical cord cyst (54)

Umbilical cord tumors are rare entities with hemangiomas and teratomas being the most common. (19) Hemangiomas are benign endothelial neoplasms that are characterized by the proliferation of capillaries surrounded by a connective tissue stroma. Hemangiomas are associated with increased risk for intrauterine bleeding, high-output cardiac failure,

elevated α-fetoprotein levels in the maternal serum, fetal malformations and fetal demise. (49)
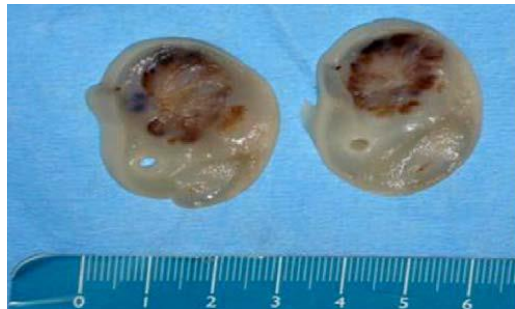
Cross-section of a umbilical cord hemangioma (81)

Umbilical cord termatomas are rare. As with other sites they may contain tissue from all three germ layers. (49)

CHAPTER 3.SINGLE UMBILICAL ARTERY

<u>Introduction</u>

Single umbilical artery (SUB) refers to the presence of one umbilical artery instead of two resulting in the formation of a two-vessel cord. Single umbilical artery is the most common congenital anomaly in humans. Its prevalence ranges from 0.5 to 1% in singletons and 6-11% in multiple pregnancies depending on the population studied. (82) (83) The presence of SUB is up to four times more common in autopsy series. (84) (85) (86)
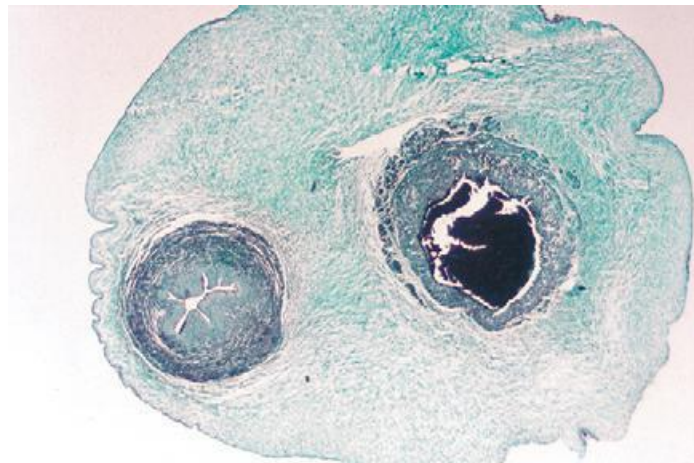
Cross-section of a umbilical cord containing one artery (left) and one vein (right) (14)

<u>Embryogenesis</u>

Three mechanisms have been proposed for the development of single umbilical artery:

A.   Primary agenesis of one of the umbilical arteries

B.   Secondary atresia or atrophy of one of the umbilical arteries

C.   Persistence of an original allantoic artery of the connective stalk (87) (88) (89)

The left umbilical artery is more commonly absent than the right. (90) (89) (91) The underlying etiology is not clear. It is presumed that it is linked with the asymmetry in the size of the arteries, with the right artery being larger than the left. (91)

Blackburn and Cooley have suggested four types of single umbilical artery based on its developmental etiology (92) (93):

Type I SUA: Occurs in approximately 98% of cases. This type is characterized by one vein (left) and one artery, the artery being of allantoic origin from either the left or the right iliac artery. It is associated with abnormalities of the lower gut, acardia, central nervous system defects and short cord syndrome (characterized by the presence of a short umbilical cord combined with exencephaly or encephalocele, thoracic and/or abdominal wall defect, thoraco- and/or abdomin-oschisis, and limb anomalies). (55)

Type II SUA: Occurs in approximately 1.5% of cases. This type is characterized by the presence one vein (left) and one artery, the artery being of vitalline origin either from the aorta or from the superior mesenteric artery. Abdominal aorta is hypoplastic distal to the origin of the umbilical artery. Type II SUA is associated with severe fetal malformations, such as caudal regression, OEIS complex (omphalocele, exstrophy, imperforate anus and spinal defects), exstrophy of the bladder and sirenomyelia (rare congenital anomaly in which the legs are fused together resembling the tail of a mermaid).

Type III SUA: It is characterized by the presence of one umbilical artery of either allantoic or vitelline origin and two umbilical veins. This type is extremely rare and is associated with severe malformations, such as renal agenesis, anencephaly, ipsilateral limb reduction, unicornuate uterus and total anomalous pulmonary venous return.

Type IV SUA: It is characterized by the presence of one umbilical artery of either allantoic or vitelline origin and one umbilical vein (right). Spontaneous abortion or fetal demise is expected in these embryos.

Risk factors

The underlying etiology is not well understood. Multiple maternal and fetal factors have been associated to the development of single umbilical artery including white ethnicity, smoking, alcohol abuse, obesity, advanced maternal age, multiparity, diabetes mellitus, hypertension, preeclampsia, antepartum hemorrhage, hydramnios, oligohydramnios and epilepsy. Drug use such as vitamin A, phenytoin, thalidomide and levothyroxine has also been implicated in the pathophysiology of this condition. (54) (94) (95) (96) It has been observed that the prevalence of single umbilical artery is greater in aneuploid embryos compraired to euploid (9-11% vs. 0.2-1.6%) (97). The role of environmental and genetic factors remains unknown. However, association of the anomaly with chromosomal

abnormalities (most notably with trisomy 18) may imply the presence of a genetic component in the development of the anomaly. (86) No familial tendency of the condition has been reported so far. (98)

Clinical significance

A. Single umbilical artery and structural abnormalities

Single umbilical artery can be an isolated finding or it can be combined with other congenital abnormalities. The clinical significance of single umbilical artery is directly linked to the presence or absence of other structural anomalies. Associated fetal anomalies can occur in every organ system. No abnormality has been described to be pathognomonic for this condition. (99) (100) Congenital abnormalities of the urogenital, cardiovascular, gastrointestinal and nervous systems occur in approximately 7-46% of pregnancies with SUA. Several authors have reported the strongest association between the single umbilical artery and urological abnormalities, followed by abnormalities of the cardiovascular and finally the nervous system. (101) The presence of multiple abnormalities is usually lethal. (102) (103)

Structural anomalies associated with single umbilical artery (104)	
1. Urogenital	Renal agenesis, hypoplasia or dysplasia
	Horseshoe kidney
	Hydroureter and hydronephrosis
	Müllerian duct agenesis or anomalies
	Wolffian duct agenesis or anomalies
	Malformed external genitalia
2. Cardiovascular	Ventricular septal defects
	Atrial septal defects
	Patent ductus arteriosus
	Truncus anomalies
	Valvular anomalies
	Acardia

3. Musculoskeletal	Cleft lip and palate
	Clubfeet /rocker bottom feet
	Polydactyly, clino- syndactyly
	Vertebral anomalies
	Rib and sternal anomalies
	Sirenomyelia
4. Gastrointestinal	Esophageal atresia or stenosis
	Tracheo - esophageal fistula
	Imperforate anus
	Malrotation of gut
	Omphalocele and gastroschisis
5. Central nervous system	Anencephaly
	Meningomyelocele
	Holoprosencephaly
	Hydrocephalus
	Microcephaly
6. Miscellaneous	Pulmonary hypoplasia or aplasia
	Diaphragmatic hernia
	Cystic hygroma — web neck
	Hydrops fetalis

B.   Single umbilical artery and fetal karyotype

Fetuses with SUA carry a 9-11% risk of being aneuploid. (105) (106) The most common aneuploidies in embryos with SUA are trisomy 18 and 13. (54) Interestingly, fetuses with trisomy 18 have 10-50% risk of having SUA. All aneuploid fetuses have one or more structural defects. The risk for chromosomal abnormalities increases from about 4% for a single defect to 50% for multiple defects. (107) (108) (109) In both the chromosomally normal and abnormal fetuses, those with an SUA have an increased risk for major defects than fetuses with a three-vessel cord. (110) If after careful examination all structural anomalies have been excluded and the presence of a single umbilical artery is an isolated finding, no increased risk for chromosomal anomalies has been reported. (107) (111) (112)

C.   Single umbilical artery and intrauterine growth restriction

Embryos with SUA carry an increased risk for intrauterine growth restriction (IUGR) with subsequent low birth weight. (99) This is in accordance with experimental studies in lamb fetuses in which one of the umbilical arteries was ligated. (113) The association of IUGR and isolated single umbilical artery is still unclear. Some authors have found no clinically important association between an isolated SUB and IUGR suggesting that the remaining umbilical artery undergoes vasodilation in order to deliver the same amount of blood. (114) (115) (116) A large retrospective cohort study of 72.373 pregnancies conducted by Hua et al found a 2.1 greater risk of IUGR in pregnancies with single umbilical artery even in the absence of other abnormalities. (117) Mailath-Pokorny et al studied 209 cases of single umbilical artery and resulted in an 11-fold increase of IUGR in pregnancies with isolated SUA. (118) Raio et al reported decreased amount of Wharton jelly in umbilical cords with one artery resulting in decreased support of blood flow and hypoperfusion of the developing embryo. This can lead to subsequent reduction of cytoplasmic mass due to decreased delivery of nutrients. (119)

D.   Single umbilical artery and prematurity

Whether an association between the presence of single umbilical artery and prematurity exists is still unclear. In the case of association with chromosomal and anatomical abnormalities the timing of delivery mainly depends on the coexisting structural defects. Some studies have shown an increased risk for preterm delivery (94) (120) (121) (116) while others (122) (123) (124) have shown no association. A meta-analysis conducted by Kim et al that examined the association between prematurity and isolated SUA in 127.742 pregnancies showed that pregnancies with isolated SUA carry a 2.1 greater risk for preterm delivery. (125)

E.   Single umbilical artery and perinatal mortality

Perinatal mortality is increased among infants with single umbilical artery mainly due to low birth weight, intrauterine growth retardation, prematurity and congenital anomalies. Although structural defects are the main cause that attributes to high perinatal mortality, infants in which single umbilical artery is an isolated finding still carry a significant risk for increased mortality. (126) Burshtein et al found a significant association between isolated SUA and perinatal mortality (OR 3.91, 95% CI 2.06–7.43) in a population-based study of

194.809 deliveries (88). A meta-analysis conducted by Kim et al that examined the association between perinatal mortality and isolated SUA in 127.742 pregnancies showed that pregnancies with isolated SUA carry a 2.29 greater risk for preterm delivery. (125) It is not clear why embryos with an isolated SUA still have an increased risk for perinatal death. It has been hypothesized that this may be linked with the decreased amount of spirals and lesser quantity of Wharton jelly in the umbilical cords of these embryos which make them susceptible to mechanical damage. (127)

Diagnosis

A single umbilical artery can be detected through prenatal ultrasonography by visualization of two vessels in a transverse section of the umbilical cord. Color Doppler may facilitate the diagnosis. (18) Imaging can be achieved as early as 12 weeks gestation (90), although the highest rates of detection are at 17 to 35 weeks. (128) Since the umbilical vessels often fuse near the placental insertion site, images should be taken at the fetal insertion site. (54) However, the most reliable way to examine the number of vessels is to identify the intra-abdominal portion of the umbilical artery near the urinary bladder with color Doppler. (129) Factors that limit the accuracy of the study include oligohydramnios, multiple pregnancy and fetal position. One important clue is that in the case of single umbilical artery the diameter of the artery is greater than 50% of the diameter of the vein resulting in a umbilical vein to artery ratio of ≤2. (24)
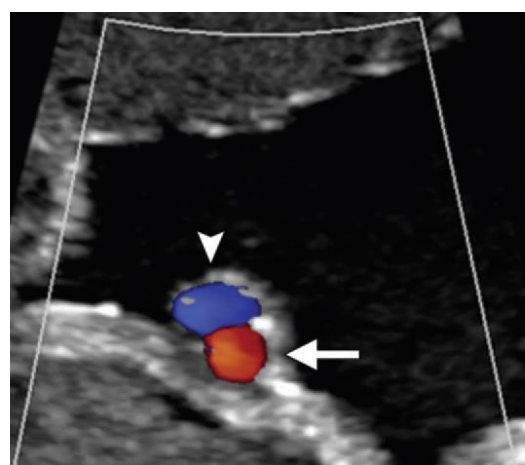
Cross-section of a umbilical cord containing one artery and one vein (54) (127)

The presence of single umbilical artery during routine ultrasonography mandates a detailed anatomic survey with fetal echocardiography in order to detect other associated fetal anomalies. (99) (102) (127) (89) (130) If other structural abnormalities are identified, amniocentesis with karyotyping should be offered due to high rate of aneuploidy. (54) (124) In all pregnancies with single umbilical artery increased surveillance should be initiated due to increased incidence of non-reassuring fetal heart rate tracings and perinatal mortality (124)

Single umbilical artery and multiple gestation

Single umbilical artery occurs approximately four times more frequently in twin compared to singleton pregnancies. (131) No difference between monochorionic and dichorionic twins has been found. (90) (117) (132) (133) This risk may be higher in twins conceived with assisted reproductive technologies. (134) Although the incidence of congenital malformations is expected to be two times greater in twins compared to singletons, twin SUA infants have no greater incidence of associated malformations than SUA singletons. The majority of SUA twins are discordant for the anomaly with SUA occurring in the smaller twin. (98)

SPECIAL SECTION

<u>OBJECTIVE</u>

Histopathologic data of fetuses and embryos with single umbilical artery are scarcely reported in Greece. In the laboratory of Histology and Embryology of the Medical School of Aristotle University of Thessaloniki we maintain a registry of fetal and neonatal deaths coming primarily from Hippocrateion General Hospital and General Clinic of Thessaloniki, Greece. The purpose of this study is to collect and analyze the data of fetal and neonatal deaths, calculate the prevalence of single umbilical artery in the histopathologic material of the laboratory and determine the presence of a correlation with multiple demographic and epidemiologic parameters, such as fetal and neonatal weight, maternal and gestational age, presence or absence of malformations and fetal and neonatal gender.

MATERIALS AND METHODS

Anonymous data from 649 aborted fetuses and stillborns from 1992 to 2008 were retrospectively retrieved from the archives of histopathology lab of Hippocrateion General Hospital of Thessaloniki and histopathology laboratory of General Clinic of Thessaloniki. The data were reviewed and statistically analyzed at the Laboratory of Histology and Embryology of Medical Faculty of Aristotle University of Thessaloniki.

The data were categorized and analyzed according to the following parameters:

- Maternal age in years
- Fetal age in weeks and neonatal age in days
- Fetal and neonatal gender
- Fetal and neonatal weight
- Presence and nature of congenital anomalies
- Histopathology of the placenta and umbilical cord

The past medical and obstetrical history written in the histopathologic reports was also utilized in the analysis.

Congenital anomalies were further categorized based on the International Classification of Diseases (ICD) 10-congenital malformations and deformations in the following groups:

Congenital malformations of the nervous system
Congenital malformations of eye, ear, face and neck
Congenital malformations of the circulatory system
Congenital malformations of the respiratory system
Cleft lip and cleft palate
Other congenital malformations of the digestive system
Congenital malformations of genital organs
Congenital malformations of the urinary system
Congenital malformations and deformations of the musculoskeletal system
Other congenital malformations
Chromosomal abnormalities, not elsewhere classified

The statistical analysis software was SPSS. The defined statistical significance level was set to be 5%. The presence or absence of association between two categorical variables in the sample was determined with the use of Pearson's chi-squared (χ^2) test.

Frequency and relative frequency were used to describe categorical variables. The results are often portrayed in bar and circle charts. The mean value and the standard deviation where used to describe continuous variables. The distribution of continuous variables is depicted in histograms.

RESULTS

A. SAMPLE CHARACTERISTICS

We retrospectively retrieved 649 fetal and neonatal autopsy cases from the archives of Histopathology lab of Hippocrateion General Hospital and the laboratory of Pathology of General Clinic of Thessaloniki, Greece during the period of 1992 to 2008. The data were reviewed and analyzed at the Laboratory of Histology and Embryology of Medical Faculty of Aristotle University of Thessaloniki. The frequency and the relative frequency of fetal and neonatal deaths in their respective year can be reviewed in the table below:

Year	Frequency	Relative frequency
1992	44	6,7%
1993	53	8,17%
1994	28	4,31%
1995	11	1,69%
1996	9	1,39%
1997	39	6,01%
1998	45	6,93%
1999	51	7,86%
2000	34	5,24%
2001	32	4,93%
2002	47	7,24%
2003	45	6,93%
2004	69	10,63%
2005	23	3,54%
2006	45	6,93%
2007	29	4,47%
2008	45	6,93%
Total	649	100%
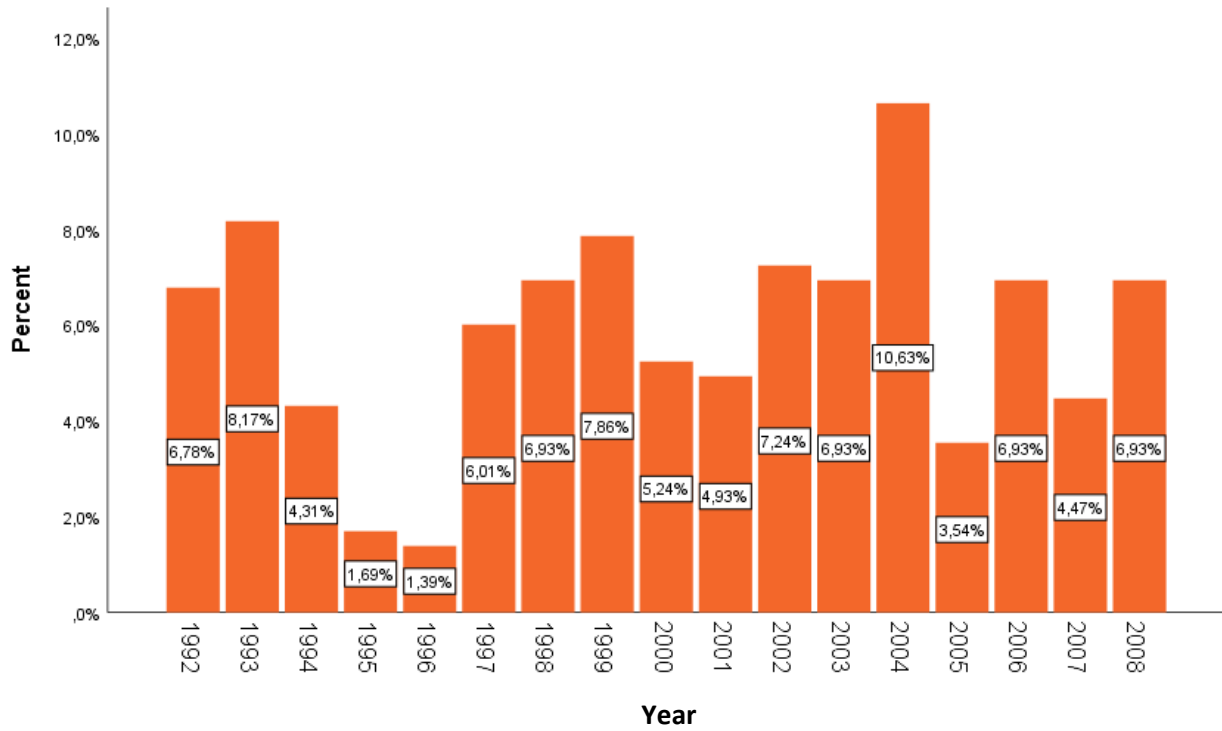
Maternal age

Data regarding maternal age could be retrieved in 580 cases. The youngest maternal age was 16 years and the oldest 46 years with a mean value of 29,86 years and a standard deviation of 5,653. The histogram of the maternal age is depicted below:

Mean = 29,86
Std. Dev. = 5,653
N = 580
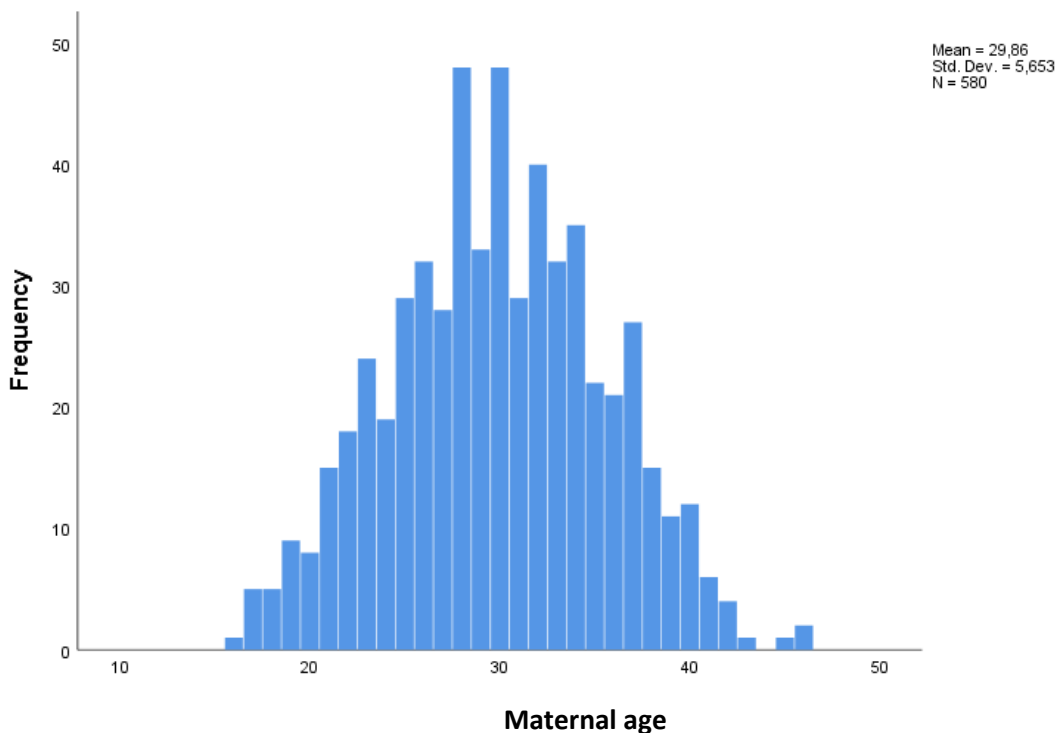
Based on the Kolmogorov-Smirnov test maternal age does not follow normal distribution (p<0.01).

458 mothers (78,9%) had an age of <35 years, while 122 (21%) had an age ≥35 years.

Gestational and neonatal age

Gestational age was recorded in 608 cases. The mean value for gestational age is 24,98 weeks with a standard deviation of 7,5. The histogram for gestational age is depicted below:
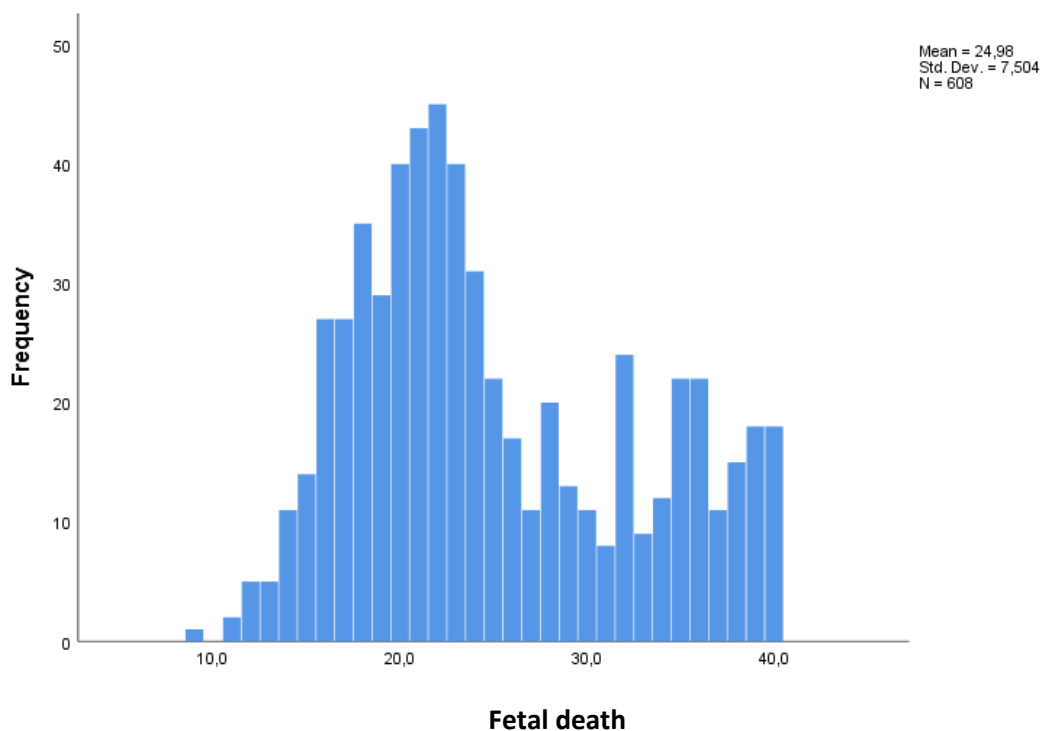
Fetal death

Based on the Kolmogorov-Smirnov test gestational age does not follow normal distribution (p<0,01).

In the reported sample we recorded 16 cases of neonatal death with the youngest age being 1 hour and the oldest age being 34 days postpartum.

Fetal and neonatal gender

The fetal and neonatal gender was known in 607 of the reported autopsy cases. 341 cases of the identified embryos were male (56,17%) and 266 (43,82%) female.

Fetal and neonatal gender

43,82%

56,17%

- Male
- Female
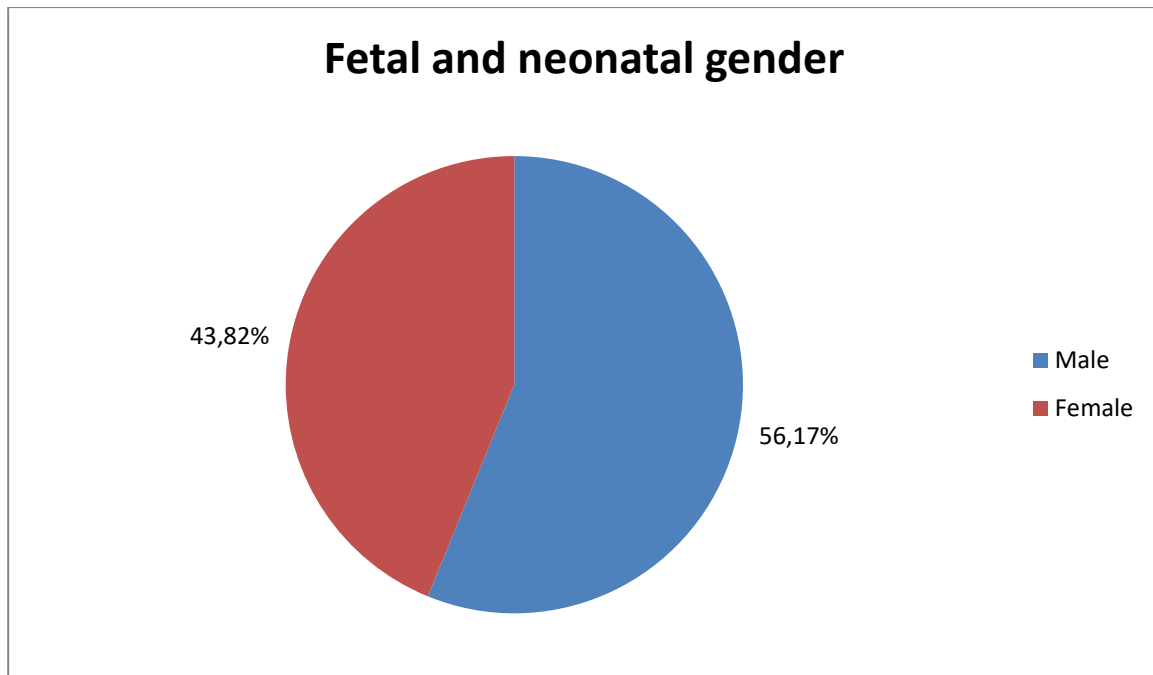
Fetal and neonatal weight

Data regarding fetal and neonatal weight were retrieved in 547 autopsy cases. The mean value is 975,4g with a minimum of 5g and a maximum of 5500g. The standard deviation is 1054,307. The histogram for the fetal and neonatal weight is portrayed below:

Mean = 975,4
Std. Dev. = 1054,307
N = 547

**Fetal and neonatal
weight**
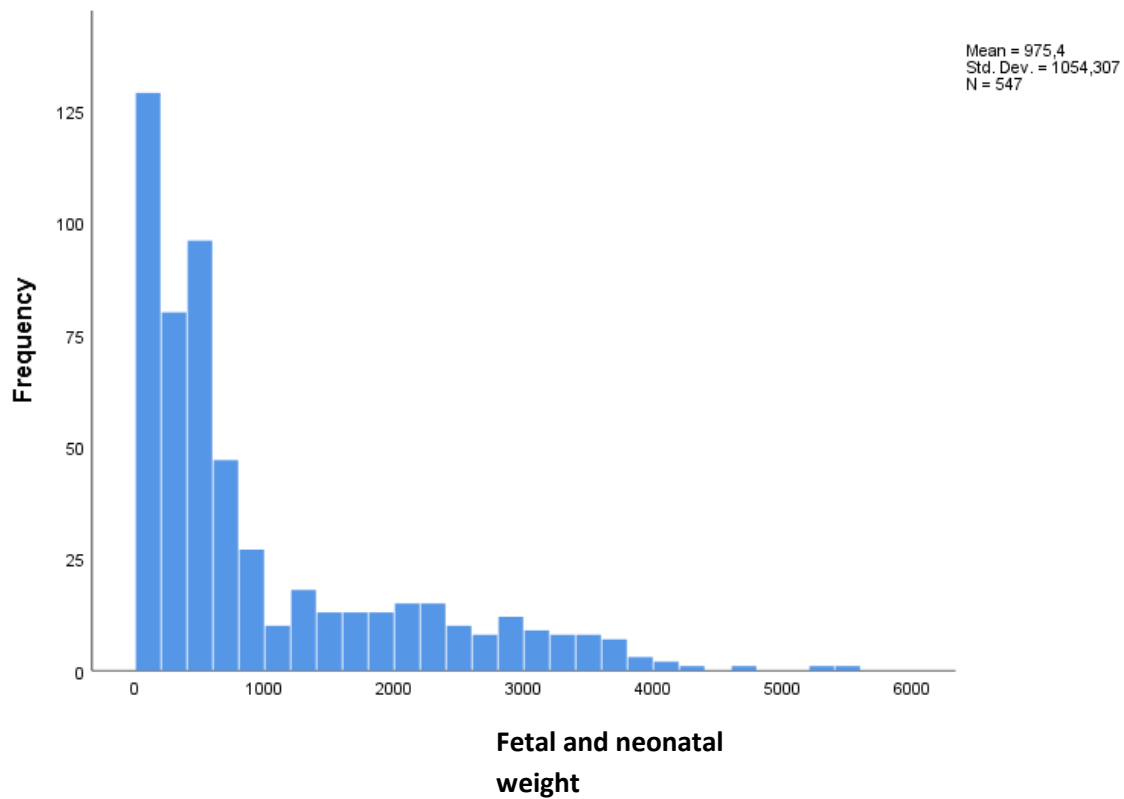
Based on the histogram and the Kolmogorov-Smirnov test the specimen's weight does not follow normal distribution (p<0,001).

Congenital anomalies

Congenital anomalies were recognized in 256 autopsy cases (39,44%). The remaining 393 showed no congenital anomalies (60,55%).

Congenital anomalies

39,44%

60,55%

■ Present
■ Absent
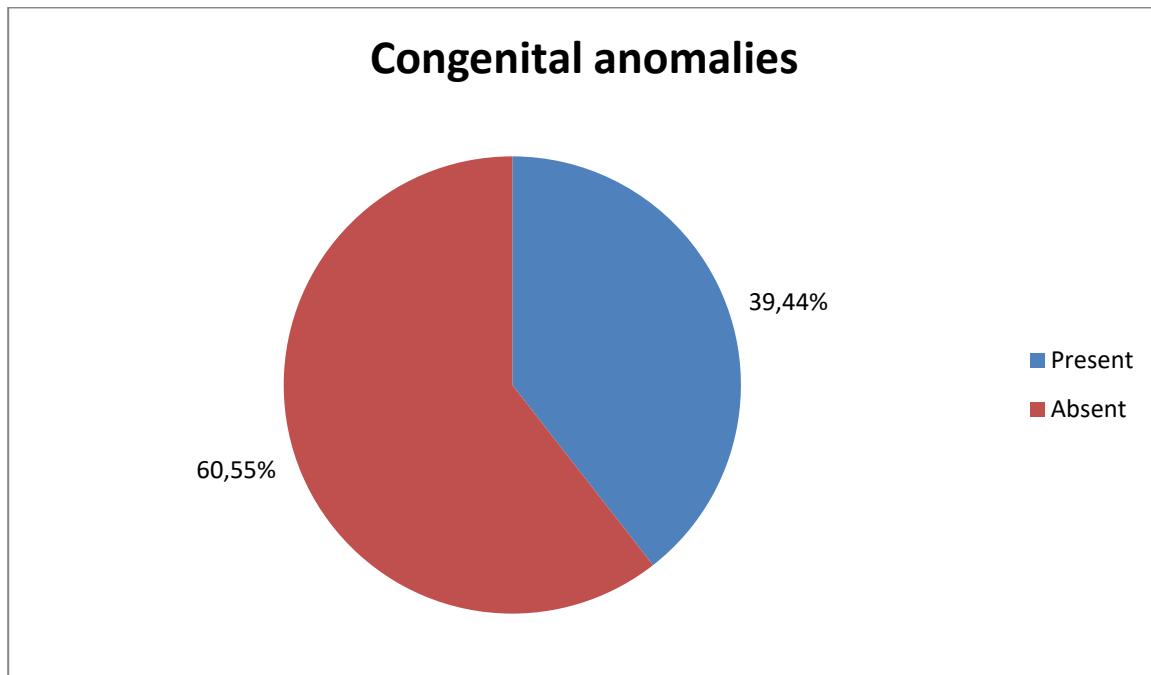
Multiple pregnancies

30 of the autopsy cases were derived from multiple pregnancies (4,62%). Two of these embryos were conjoined twins.

B. UMBILICAL CORD MALFORMATIONS

In a total of 649 histopathologic specimens (N) of fetal and neonatal deaths umbilical cord malformations were recognized in 101 cases (n) (15,56%). The absolute value and the relative frequency of umbilical cord malformations are analyzed in the following table:

Umbilical cord malformations	n=101	
	Frequency	Relative frequency
Single umbilical artery	29	28,71%
Marginal-velamentous insertion	18	17,82%
Thrombosis	9	8,9%
Inflammation	13	12,87%

Umbilical cord looping	21	20,79%
True knots	4	3,96%
Twin-to-twin transfusion syndrome	2	1,98%
Short length	3	2,97%
Allantoic duct remnant	3	2,97%
Hematoma	2	1,98%
Stenosis	2	1,98%

One case was characterized by the presence of a umbilical vein and a single umbilical artery which was thrombosed. It is been hypothetized that the thrombosis of the single artery was the inciting event that led to fetal demise at the age of 38 weeks.

In the total population of 649 autopsy specimens the relative frequency of umbilical cord malformations is as follows:

Umbilical cord malformations	N=649	
	Frequency	Relative frequency
Single umbilical artery	29	4,46%
Marginal-velamentous insertion	18	2,77%
Thrombosis	9	1,38%
Inflammation	13	2%
Umbilical cord looping	21	3,23%
True knots	4	0,61%
Twin-to-twin transfusion syndrome	2	0,3%
Short length	3	0,46%
Allantoic duct remnant	3	0,46%
Hematoma	2	0,3%
Stenosis	2	0,3%

Umbilical cord malformations were recognized in 48 male fetuses and 48 female fetuses.

Umbilical cord malformations	Male	Female	Total
Yes	48 *(53.93)*	48 *(42.07)*	96
No	293 *(287.07)*	218 *(223.93)*	511
Total	341	266	607

Expected values are displayed in italics. Using the chi-squared test no statistical significance was found between the gender of the fetus and the presence of umbilical cord malformations (x^2=1.768, df=1, p= 0. 0.183)

C. SINGLE UMBILICAL ARTERY

Single umbilical artery and fetal gender

Single umbilical artery was present in 15 male and 12 female fetuses. The gender could not be determined in 2 fetuses with single umbilical artery.

Of the total 48 male fetuses with umbilical cord malformations 15 had single umbilical artery (31,25%). Of the total 48 female fetuses with umbilical cord malformations 12 had single umbilical artery (25%).

Single umbilical artery in fetuses with umbilical cord anomalies
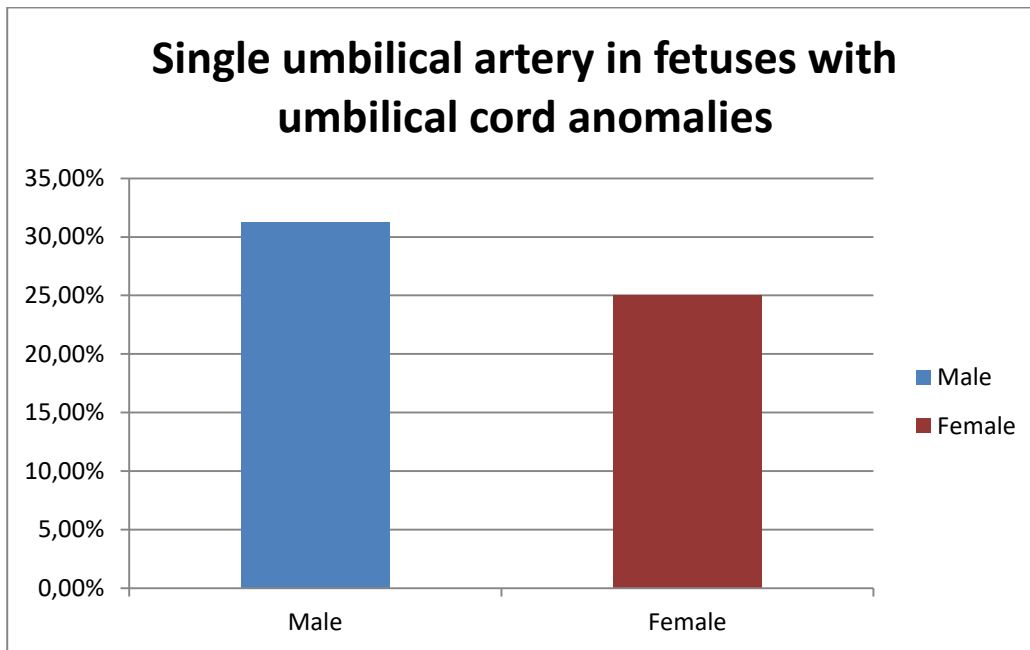
The results are portrayed in the following two-way contingency table:

Single umbilical artery	Male	Female	Total
Yes	15 *(13.5)*	12 *(13.5)*	27
No	33 *(34.5)*	36 *(34.5)*	69
Total	48	48	96

Expected values are displayed in italics. Using the chi-squared test no statistical significance was found between the gender of the fetus with a umbilical cord anomaly and the presence of a single umbilical artery (x^2= 0.464, df=1, p= 0.4959)

Single umbilical artery and fetal growth

Growth restriction (weight $\leq 10^{th}$ percentile for fetal age) was observed in 204 autopsy cases. The weight was within normal limits in 385 fetuses. Of the 204 fetuses with growth restriction 15 had a single umbilical artery (7,35%). Of the 385 fetuses with normal growth 10 had a single umbilical artery (2,59%).

	Single umbilical Artery	Two umbilical arteries	Total
Normal growth	10 *(16.34)*	375 *(368.66)*	385
Intrauterine growth restriction	15 *(8.66)*	189 *(195.34)*	204
Total	25	564	589

Using the chi-squared test there is statistical significance between the presence of single umbilical artery and growth restriction (χ^2 = 7.42, df=1, p=0.0065).

Based on our data the odds ratio for intrauterine growth restriction in fetuses with single umbilical artery is: Odds ratio= 15*375/10*189=2,98. This means that fetuses with single umbilical artery have 2,98 times greater risk for intrauterine growth restriction compared to fetuses with two umbilical arteries.

Single umbilical artery and maternal age

Maternal age was <35 years in 20 fetuses with single umbilical artery (71,4%). 8 fetuses had a mother aged ≥35 years (28,57%). Data considering maternal age could not be retrieved in one case of single umbilical artery.

	Maternal age in years		
Single umbilical artery	<35	≥35	Total
Yes	20 *(22.11)*	8 *(5.89)*	28
No	438 *(435.89)*	114 *(116.11)*	552
Total	458	122	580

Using the chi-squared test there is no statistical significance between the presence of single umbilical artery and maternal age (χ^2 1,006, df=1, p= 0.3158).

Single umbilical artery and congenital malformations

Of the 29 embryos with single umbilical artery 18 had simultaneous congenital anomalies (62,06%). In the whole series of 649 specimens 2,77% of embryos had a single umbilical artery combined with other anomalies.

	Congenital anomalies		
Single umbilical artery	Yes	No	Total
Yes	18 (11.44)	11 (17.56)	29
No	238 (244.56)	382 (375.44)	620
Total	256	393	649

The chi-squared test revealed a statistical correlation between the presence of single umbilical artery and other congenital anomalies (χ^2 = 6.505, df= 1, p= 0.0108).
The odds ratio in this table can be calculated as follows: 18*382/11*238=2,63. This means that fetuses with single umbilical artery have 2,63 times greater risk of having other congenital anomalies compared with fetuses with two umbilical arteries.

The nature and the frequency of the congenital malformations are portrayed in the following chart:

Congenital malformations in fetuses with single umbilical artery
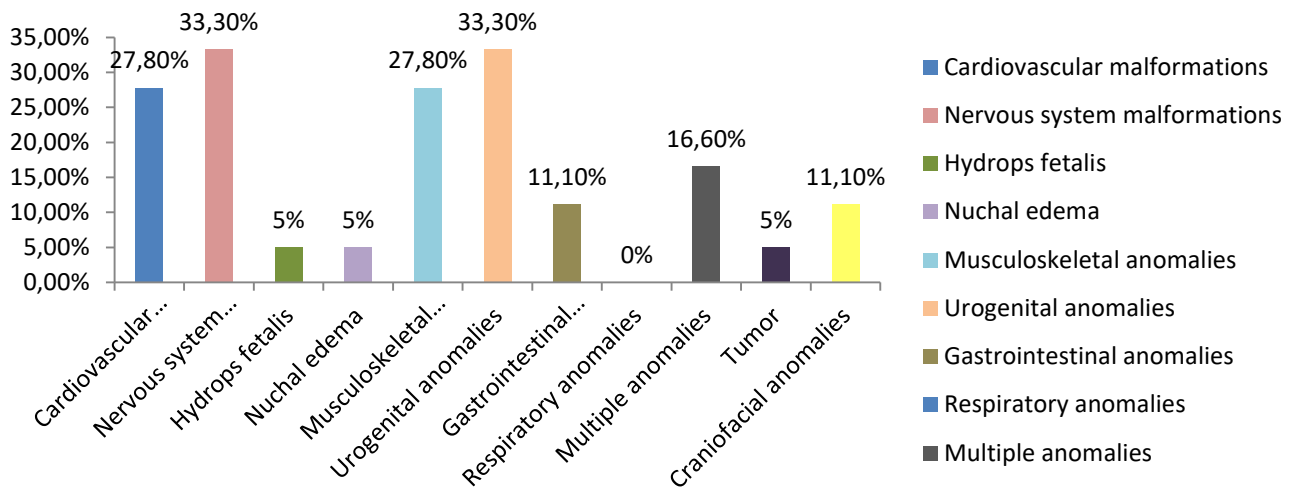
Category	
Cardiovascular malformations (27,8%)	• Tetralogy of Fallot • Ventricular septal defect • Ventricular hypoplasia • Transposition of the great vessels •
Nervous system malformations (33,3%)	• Lateral ventricular enlargement • Aplasia of the tentorium cerebelli • Malformation of the cerebral skull • Spina bifida • Myelomeningocele • Hydrocephalus
Musculoskeletal anomalies (27,8%)	• Limb hypoplasia • Hip bone aplasia • Sirenomelia • Pectus excavatum • Clubfoot • Caudal regression syndrome
Urogenital anomalies (33,3%)	• Hydroureteronephrosis • Renal agenesis • Obstruction of the urethra

	• Potter sequence	
	• Aplasia of external genitalia	
	• Horseshoe kidney	
	• Uterus aplasia	
Gastrointestinal anomalies (11,1)	• Omphalocele	
	• Imperforated anus	
	• Malrotation	
	• Tracheoesophageal fistula	
Tumor (5%)	• Neuroblastoma	
Craniofacial anomalies (11,1%)	• Micrognathism	
	• Cleft lip and palate	
	• Low-set ears	
Chromosomal anomalies (5%)	• Mosaicism (46XX/46XY)	

<u>Single umbilical artery and perinatal mortality</u>

19 embryos with single umbilical artery suffered spontaneous abortion or fetal demise, 9 underwent induced abortion, while 1 died during birth.

Umbilical artery	Spontaneous abortion/ Fetal demise	Induced Abortion	Delivery	Total
Yes	19 *(23.47)*	9 *(3.58)*	1 *(1.95)*	29
No	499 *(494.53)*	70 *(75.42)*	42 *(41.05)*	611
	518	79	43	640

The difference in the three groups reached statistical significance (χ^2 = 9.973, df=2, p=0.0068)

Of the 19 fetuses with single umbilical artery that suffered intrauterine demise 11 had a gestational age <26 weeks (57,9%) and 8 had a gestational age ≥26 weeks (42,1%).

Single umbilical artery	Gestational age in weeks		
	<26	≥26	Total
Yes	11 *(11.46)*	8 *(7.54)*	19
No	290 *(289.54)*	190 *(190.46)*	480
Total	301	198	499

Based on the chi-squared test no statistical significance was found between the age of fetuses with intrauterine demise and the presence of single umbilical artery (x^2= 0,049, df=1, p= 0.826).

Of the 19 fetuses that suffered intrauterine demise 9 were males and 8 were females. The gender was not known in 2 cases.

Single umbilical artery	Fetal gender		
	Male	Female	Total
Yes	9 *(9.18)*	8 *(7.82)*	17
No	254 *(253.82)*	216 *(216.18)*	470
Total	263	224	487

According to the chi-squared test no statistical significance was found in the fetal demise of male and female fetuses with single umbilical artery (x^2=0.008, df=1, p= 0.94).

Single umbilical artery and placental anomalies

The placenta was studied in 13 cases with single umbilical artery (44,8%). 4 of the examined cases had normal placentas (30,76%). Ischemic changes were recognized in 3 cases (23,1%). Evidence of placental hematomas was found in 1 embryo (7,6%). The rest of the specimens was characterized by the presence of inflammatory changes and fibrin deposition in the perivillous space (30,76%).

Placental morphology in specimens with single umbilical arteries

Category	Percentage
Inflammation	30,76%
Hematoma	7,60%
Ischemia	23,10%
Normal	30,76%

Legend:
- Normal
- Ischemia
- Hematoma
- Inflammation
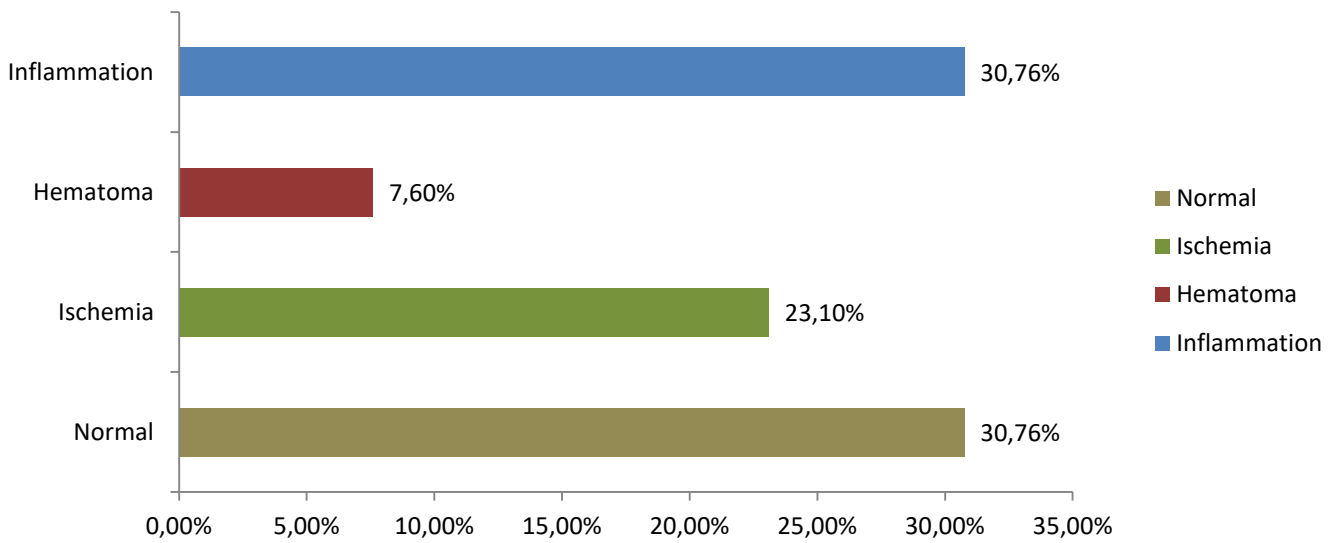
DISCUSSION

Single umbilical artery is the most commonly observed congenital anomaly in humans with a prevalence ranging from 0.5 to 1% in singletons and 6-11% in multiple pregnancies depending on the population studied. (1) (135) (2) (136) Single umbilical artery is a relatively common finding during fetal ultrasonography and its significance is largely dependent on the presence of other congenital anomalies in the developing embryo (117) (137) (138) (94) (100)

The goal of this study was to gather and analyze data of autopsy cases of the histopathologic archive of the Laboratory of Histology and Embryology of Aristotle University of Thessaloniki and determine the prevalence and significance of single umbilical artery in abortuses and dead embryos. Of the studied material umbilical cord malformations were identified in 15,56% of the examined cases. The most commonly observed congenital anomaly was the single umbilical artery with a relative frequency of 28.71% among the fetuses with umbilical cord malformations and a relative frequency of 4.46% in the total population studied. This result is in accordance with the literature findings that suggest that single umbilical artery is the most common anomaly not only of the umbilical cord but among all birth defects. (135) (136)

It is clear from the literature that single umbilical artery is more commonly associated with other birth anomalies rather than being an isolated finding. In our study 62.2% of the examined fetuses had one or more co-existing congenital anomaly. Fetuses with single umbilical arteries were observed to carry a 2.63 greater risk of having another anomaly. The most frequent defects in fetuses with single umbilical artery affect the urogenital and nervous system. One large retrospective cohort study by Hua et al that compared 392 cases of single umbilical artery in a population of 72.373 pregnancies revealed that embryos with SUA have threefold increased risk of renal anomalies compared with embyos with a three-vessel cord. (141) Because of the high incidence of renal malformations Leung and Robson recommended in their study in 1989 renal ultrasonography in all fetuses with single umbilical artery. (96) It is generally accepted that pregnancies with single umbilical arteries should undergo a comprehensive sonographic evaluation of the cardiac, renal, nervous, gastrointestinal and musculoskeletal systems. (86) (142)

Significant correlation was observed between the presence of single umbilical artery and growth restriction. In our study we determined that fetuses with single umbilical artery have a 2.98 greater risk for intrauterine growth restriction compared to fetuses that have a normal umbilical cord. Bibliographic data are controversial about the presence of association between the single umbilical artery and growth restriction. In a study performed by Tulek et al pregnancies with SUA had a three times higher risk of SGA compared to pregnancies with three-vessel cords. (123) A large systematic review and meta-analysis performed by Kim et al in 2017 that included 1.731 pregnancies with isolated SUA revealed significant association between the presence of SUA and growth restriction (OR=2.75, CI=95%, 1.97-3.83). This is in contrast with a meta-analysis performed by Voskamp et al in 2013 which included 928 cases with isolated single umbilical artery that described non-statistically significant association between the presence of the anomaly and fetal growth. (143)

No significance was observed between the presence of single umbilical artery and fetal gender or maternal age, as shown in previous studies (144) (145) (146)

In our study placental abnormalities were found in 69.2% of cases with single umbilical artery. Previous studies also confirm this finding. In a large cohort study performed by Battarbee et al the presence of single umbilical artery was associated with SGA placenta, maternal and fetal placental vascular anomalies and chronic villitis. (147) In our study the most commonly observed placental anomaly was the presence of inflammatory changes in the perivillous space. Unfortunately the number of cases in which the placenta was examined was too low in order for statistical significance to be established.

With this study we attempted to analyze and correlate the data regarding the presence of single umbilical artery in autopsied fetuses. It is important to emphasize that these results were derived from autopsy specimens and not from prenatal data concerning the prevalence of demographic and biometric factors in unborn embryos. Another study limitation concerns the lack of data regarding fetal karyotype since cytogenetic analysis was not performed in the majority of cases.

Our study material was grossly obtained from the laboratory of Hippocrateion General Hospital that examines histopathologic specimens of three obstetrics/gynecology departments and is a tertiary public center for gynecologic cases in Northern Greece and the laboratory of General Clinic of Thessaloniki that examines specimens from two of the largest obstetric/ gynecology clinics in the same area. Although our material is derived from two different centers without uniform data registration, we consider our results to be representative of the population of Northern Greece. We strongly believe that the creation of a database that uniformly analyzes the characteristics of fetal autopsy cases will largely contribute to our knowledge of congenital anomalies and its associations.

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