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MESTRADO INTEGRADO EM MEDICINA

Human Embryonic Aortic and Umbilical Artery Doppler at very early clinical pregnancy

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2021

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Dissertação de candidatura ao grau de Mestre em Medicina, submetida ao Instituto de Ciências Biomédicas Abel Salazar – Universidade do Porto

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Porto, Maio de 2021

Ana Rita da Silva Morais

Dedication:

"Quem corre por gosto não cansa!". De facto, nenhuma frase se poderia adequar mais ao meu percurso académico do que esta.

Nenhum dos meus feitos teriam tido o mesmo valor, se não os tivesse conseguido partilhar com a minha família e amigos. Por esse motivo, a presente dissertação é dedicada a eles:

Aos meus pais e à minha irmã por acreditarem incondicionalmente em mim.

Aos meus avós por me terem transmitido os valores da vida.

Aos meus amigos, pelo percurso académico que fizemos juntos e por terem tornado esta jornada ainda melhor.

Obrigada.

Achnowledgments:

Ao Prof. Dr. Luís Guedes Martins pela incrível oportunidade de fazer parte da co-autoria do artigo de investigação, que está a ser desenvolvido e que será integrado na presente dissertação. Agradeço toda a disponibilidade tida ao longo deste percurso, a paciência, e acima de tudo, o voto de confiança que me foi concedido. Admiro a paixão que demonstra pela área de Medicina Fetal e toda a dedicação que apresenta no desenvolvimento de qualquer trabalho. Permitiu que me desafiasse todos os dias e descobrisse uma nova área da medicina, que até então, desconhecia.

À professora doutora Rita Gaio pelo apoio e disponibilidade tidos na compreensão da área estatista, que corresponde a um dos tópicos mais desafiantes no desenvolvimento desta tese.

Resumo:

Objetivos: Análise dos índices doppler da dinâmica circulatória do embrião (índice de pulsatilidade, índice de resistência e razão sistólica/diastólica) na artéria aorta e umbilical embrionária. Criação de um protocolo para a avaliação dos valores de referência dos parâmetros referidos anteriormente, durante a fase embrionária (menos de 10 semanas de gestação, pós-concepção) e a sua reprodutibilidade na prática clínica. Abordagem da importância da criação de curvas de referência e revisão dos melhores métodos para o seu desenvolvimento, perante análise de parâmetros gestacionais.

Metodologia: A pesquisa bibliográfica foi realizada no PubMed, tendo por base as palavras chave referidas seguidamente. Recorreu-se também à bibliografia dos artigos selecionados, quando necessário, para obter informação adicional relevante. A segunda parte da tese corresponderá à análise de um artigo de investigação, que está a ser desenvolvido pelo Prof. Luís Guedes Martins, juntamente com alguns docentes do Centro Materno Infantil do Porto, da qual farei parte da sua co-autoria. Irei abordar a necessidade de criação de protocolos, assim como apresentarei o protocolo utilizado na investigação, para a avaliação dos fluxos na artéria umbilical e aorta, uma vez que é fundamental para permitir a reprodutibilidade dos resultados e demostração da validade externa da investigação.

Resultados: Durante o primeiro trimestre é observada ausência de fluxo diastólico nas artérias umbilicais, sendo que este achado sofre alteração com a evolução do desenvolvimento fetal. A manutenção da ausência do fluxo diastólico, com o decorrer da gestação, está associada à presença de um circuito de alta resistência a nível placentar, que comprometerá o correto desenvolvimento do futuro recém-nascido. De facto, como a impedância vascular não pode ser diretamente medida com recurso a eco-doppler, a sua avaliação é feita com base na variação dos índices de pulsatilidade, índice de resistência e razão sistólica/diastólica. Verificou-se que, com o decurso da gestação, todos estes índices sofrem uma diminuição acentuada, que se inicia desde o final do primeiro trimestre, tanto na artéria umbilical como na artéria aorta.

Conclusão: As informações obtidas, com base nos índices doppler, calculados a partir das curvas de velocidade-fluxo das artérias aorta e umbilical, ao longo do desenvolvimento embrionário, permitem-nos avaliar alterações funcionais que, futuramente, representarão malformações. Por esta razão, é essencial a criação de curvas de referência para que seja possível avaliar as variações que ocorrem nestes vasos. Porém, é necessário o desenvolvimento de protocolos que permitam a reprodutibilidade das medições.

Palavras-chave: “dorsal aorta”; “umbilical arteries”; “embryo circulation”; “embrionic structures”; “Doppler embryonic”; “reference curves in obstetrics”

Abstract:

Objective: Evaluation of circulatory dynamics in the embryo, based on the calculation of Doppler indices (pulsatility index, resistance index and systolic/diastolic ratio), in the aortic and umbilical arteries. Creation of a protocol for the determination of the indices referred previously, during the embryonic period (less than ten weeks of gestation, post-conceptional), and its reproducibility in the clinical practice. The importance of reference chart, in obstetrics, and determination of the best method for its development.

Methods: The bibliographic research was made on PubMed based on certain key words. The bibliography of the selected articles was also used, when necessary, to obtain additional information. The second part of the present work is based on the analysis of an investigation article, developed by Professor Luís Guedes Martins and other teachers of Centro Materno Infantil do Porto, of which I will be part of its co-authorship. I will address the need to create protocols, as well as present the protocol used in the investigation, for the evaluation of umbilical artery and aorta flow, since it is essential to allow the reproducibility of the results.

Results: During the first trimester, there is an absence of end diastolic flow in the umbilical arteries, which changes with fetal development. The maintenance of end diastolic absence flow, with the course of pregnancy, is associated with the presence of a high resistance circuit at the placental circulatory vessels, which will compromise the correct development of the future newborn. In fact, since the vascular impedance cannot be directly measured using an Doppler ultrasound, its evaluation is based on the variation of the pulsatility indices, resistance index and systolic/diastolic ratio. Furthermore, it was observed that, with the course of pregnancy, all these indices suffer a marked decrease, which starts from the end of the first trimester, both in the umbilical artery and in the aorta.

Conclusion: The information obtained, based on the doppler indices, calculated from the flow velocity waveforms of the aorta and umbilical arteries, during embryonic development, allows the evaluation of functional changes that, in the future, will represent malformations. For this reason, it is essential to have reference charts to evaluate the change in these vessels, which can only be correctly obtained if we developed protocols that allow the reproducibility of the data collected for its creation.

Key words: “dorsal aorta”; “umbilical arteries”; “embryo circulation”; “embrionic structures”; “Doppler embryonic”; “reference curves in obstetrics”

List of abbreviations

Ao: aorta
DA: ductus arteriosus
DV: ductus venosus
EDV: end diastolic velocity
FO: foramen ovale
GA: gestational age
LA: left atrium
LV: left ventricle
PS: pulsatility index
PSV: peak systolic velocity
RA: right atrium
RV: right ventricle
RI: resistance index
SDR: systolic/diastolic ratio
TAMX: time average maximum velocity
UmbA: umbilical arteries

Index:

The Development Of The Embryo: Cardiovascular System

The development of the cardiovascular system starts at three weeks of gestation (post-conception).[1] The two most important steps in cardiovascular system development are vasculogenesis and angiogenesis. Vasculogenesis is the phenomenon that prevails during the early development of the fetus, since it's characterized by the formation of vessels from scratch, whereas angiogenesis is based on the formation of new vessels from pre-existing ones, which will be essential for the growth of the fetus. [2]

The first manifestation of vascular formation, in embryo development, occurs in the mesoderm of the extraembryonic yolk sac, with the creation of blood islands (day seventeen of gestation, post-conception).[3] These blood islands are composed of precursors of different types of cells, such as erythroblasts and angioblasts. Unlike the erythroblasts, the angioblasts initiate the migration from the blood islands early during development, giving rise to the formation of the dorsal aorta.[2],[3] This is the vessel that will later lead to the formation of the descending aorta.

Blood circulation is only possible after the lumenization of the vessels, since it allows the creation of a functional circuit. It is not yet established whether this process occurs before or after the development of cardiac function. [3]

1.1 The Aorta

One of the most important arteries in our organism is the aorta, since it is a major vessel that makes it possible for the blood to get to every part of the body. For this reason, the development of this vessel occurs early in development.[4] In the beginning of embryo development, the distribution of nutrients in the body occurs by diffusion.[5] However, with the growth of the embryo, this mechanism stops being efficient, because the body needs more nutrients than those that is receiving. At the same time, the cardiovascular system is being developed so that this issue can be resolved, firstly by using the blood supply from the yolk sac, and after by the placenta.[5]

The aorta, as we know it, goes through a lot of phases to be totally developed. One of the primitive precursors of this vessel is called the dorsal aorta. Initially, the embryo has two dorsal aortas, one right and one left, that suffer a lateral-medial translocation, which will lead to the fusion of both in a single dorsal aorta, just below the branchial arches.[4],[6] This phenomenon normally happens during the fourth week of gestation, post-conception.[7] These vessels have two connections established, the anterior one, with the primitive heart, and the posterior one, that is

established with the vessels that form the vitelline sac.[4] At the beginning of gestation, the yolk sac has a crucial role in the blood supply of the embryo, since the placenta is not yet formed, corresponding to an alternative form of nutrient supply, during organ formation. With continuous embryonic development, the arterial signals in the yolk sac circulation start to disappear, as the blood flow in the placenta unit starts to increase. This variation of blood flow in the different vessels, is assessed through the use of Doppler ultrasound, in the early stage of development. [5]

The connection of the dorsal aorta with the heart and the umbilical arteries creates the primary vascular network in the embryo.[4]

The final aorta is divided into five anatomical segments, aortic root, ascending aorta, aortic arch, descending aorta and the abdominal aorta.[8] The dorsal aorta, which corresponds to the precursor of the descendent and abdominal portion of the final aorta, is located dorsally to the primitive gut tube. [9]

Normally, the proximal portion of the primitive aorta is associated with more abnormalities, since the embryologic development is more complex in this section.[8] In the beginning of the vascular system, the primitive heart is connected to the truncus arteriosus. This structure will give rise to the portion of the aorta connected to the heart, as well as to the pulmonary artery. This septation occurs at the beginning of week five (post-conception) of gestation. During this week, the endocardial ridges are invaded by cells of the neural crest, separating the truncus arteriosus into the aorta and pulmonary channels. In the absence or alteration of endocardial ridges formation, this septation does not occur, or occurs in an unequal form, which will lead to severe complications in the final aorta and pulmonary arteries.[8] The truncus arteriosus and the aortic sac correspond to the embryological precursors of the ascending aorta. The aortic branch formation is associated with the pharyngeal arches, which in humans are called aortic arches. The embryo has six aortic arches, but only the third, fourth and six will contribute to the formation of the great branches from the aorta arch. The third is responsible for the formation of the common carotid arteries. The fourth aortic arch will form the subclavian artery on the right, whereas on the left, it will contribute to the formation of the aortic branch. The sixth aortic arch is associated with the formation of both pulmonary arteries, as well as, the ductus arteriosus. [10] All aortic arches are connected dorsally to the left and right dorsal aorta and to the aortic sac in the center.

The dorsal aorta is initially formed by two vessels. At week five of gestation, the right dorsal aorta suffers a regression, from the ventral extremity to the level marked by the fourth thoracic vertebra.[8,9] The portion of the dorsal aorta below this level, will also form only one vessel, by the fusion of the right and left dorsal aorta, a process previously explained. [8,10] By the end of this embryologic development, the portion of the descending aorta is formed.

As the gestation proceeds and the different organs start to form, inter-segmental arteries are formed from the dorsal aorta to give rise to major vessels in adult circulation, such as the celiac artery, the inferior and superior mesenteric artery and the renal arteries. [7],[8] During intra uterine life there is also another connection established with the dorsal aorta, that corresponds to the connection associated with placental circulation. This link is made, firstly by the vitelline vessels, and then by the umbilical arteries.[9]

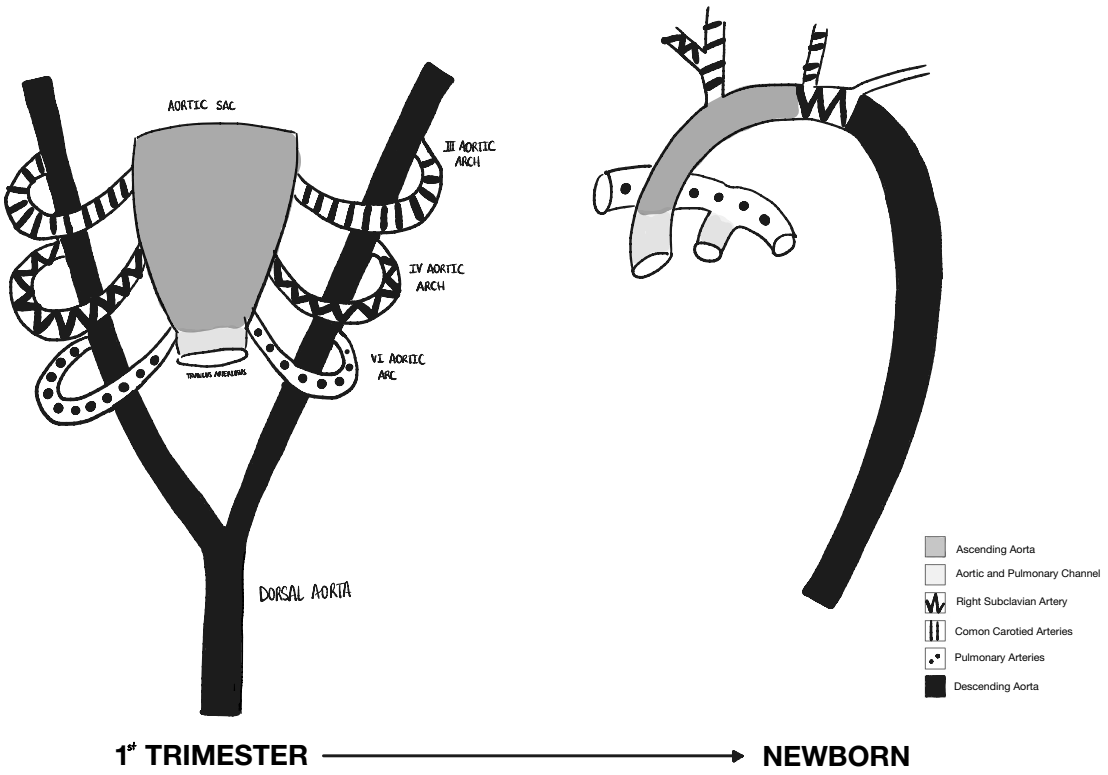

Figure 1: *Representation of the aorta from early embryonic development, before the definitive vascular pattern transformation, until the final form in the newborn. The different colors represent the precursor and final anatomical segment of the aorta and its branches. This scheme was based on information collected from the textbook Thomas W Sadler. Langman's Medical Embryology. 11th ed. Vol. 1. Wolters Kluwer Lippincott Williams & Wilkins, c2010.; 325 p.*

1.2 The Umbilical Arteries

The development of the umbilical cord begins at the fourth week of gestation (post-conception).[11] This structure is responsible for the connection between the mother, by the decidua basalis, and the embryo, by the connecting stalk, corresponding to the primitive umbilical cord.

At week two of gestation, the blastocyst is formed by two types of differentiated cells, the trophoblast, that will give rise to the placenta, and the embryoblast. The embryoblast divides itself in two layers, the epiblast, which is responsible for the formation of the amniotic fluid, and the hypoblast, that will form the yolk sac, which has the vitelline fluid. During week four of gestation, the embryo disk starts to fold in two directions, ventral and caudal, which leads to a round shape. Since the yolk sac is just beneath the embryo disc, this movement will lead to its separation. One of the parts, the one inside the embryo, will lead to the formation of the primitive gut tube, whereas the other part will remain outside, forming the final yolk sac.[12],[13] These two portions are connected by the vitelline duct, which will be part of the umbilical cord. As the amniotic fluid is produced around the embryo, the space occupied by the chorionic cavity is reduced until the amniotic cavity contacts with the chorion. This will lead to the approximation of the connecting stalk, which contains the allantois that, with the vitelline duct, will lead to the formation of the primitive umbilical cord.[12],[13]

The vitelline duct and the allantoic duct are both surrounded by extraembryonic mesoderm, that will give rise to the development of the vascular system in the umbilical cord, with the formation of the vitelline arteries and veins. As many studies show, the vitelline vessels are responsible for the initial blood supply in the embryo, but as the embryo develops, the primary circulation is taken by the allantoic vessels, the umbilical arteries and vein.[7],[13],[11] Normally the yolk sac tends to regress by the end of the embryonic period, which corresponds to the first ten weeks of gestation (post-conception).[14] At first, the umbilical cord is only made up of one umbilical artery, which is formed by a plexus of arteries formed in allantois. This vessel is then divided into two umbilical arteries with the development and elongation of the connecting stalk.[15] These two umbilical arteries will establish a connection between the two ramifications of the dorsal aorta, in the pelvic region, that with time will correspond to the internal iliac arteries.[7],[13],[11] During the initial formation of the umbilical cord, there are two umbilical veins. During week eight to twelve, with the asymmetric development of the liver and the heart, only the left umbilical vein remains, giving rise to the only umbilical vein in the cord, whereas the right umbilical vein regresses. [16]

The definitive umbilical cord contains two umbilical arteries and one umbilical vein, as well as wharton's jelly between them, and a single amniotic layer surrounding all structures.[7]

The Fetal Circulation

The fetal circulation has four main organs associated, which are the liver, heart, brain and the placenta, and four shunts, that are responsible for the typical blood circulation that occurs in the embryo.[17] The four shunts are the ductus venosus, foramen ovale, ductus arteriosus and the umbilical arteries.[17] All these structures suffer a regression after birth, since the major oxygen supply stops being the placenta, to become the pulmonary system, that does not have an important role during embryo development.

The blood supply enters the fetus by an established connection with the umbilical vein, where the blood has an oxygen concentration of 70-80%, corresponding to the place of the fetal circulation with the highest concentration of oxygen.[18] From here, half of the blood supply passes directly to the inferior vena cava, by the ductus venosus, without having to pass through the liver.[18] The other half of the blood, that goes through the liver, will then connect to the inferior vena cava, just before its entrance in the right atrium, by the left and right hepatic veins.[17],[19]

From the moment the blood is inside the right atrium, most of it will pass immediately through the foramen ovale to the left atrium, since the pressure variation between these two cavities favors this movement. The blood in the left atrium will then be associated with a portion of the blood that comes from the pulmonary circulation, passing subsequently to the aorta, from the left ventricle. In the ascending aorta, there is another shunt, the ductus arteriosus, that will establish the connection between the pulmonary truncus and the aorta. Only a small portion of the blood, coming from the venous system, will reach the pulmonary tract, because most of it will suffer a deviation to the aorta, by the ductus arteriosus.[18],[19]

The blood then passes through the fetus by the descending aorta. The common iliac arteries, which are a ramification of the descending aorta, are divided into two other arteries each, the internal and external iliac arteries. The internal iliac artery corresponds to the vessel that will establish the connection with the placenta, by its association with the umbilical arteries. At this point the blood can flow into the umbilical arteries, as well as, continuing the irrigation of the inferior part of the body. For this reason, it is important that the placental circulation has a low resistance circuit, so that a portion of the blood can flow into the umbilical arteries, instead of just continuing the irrigation of the inferior body. [17]

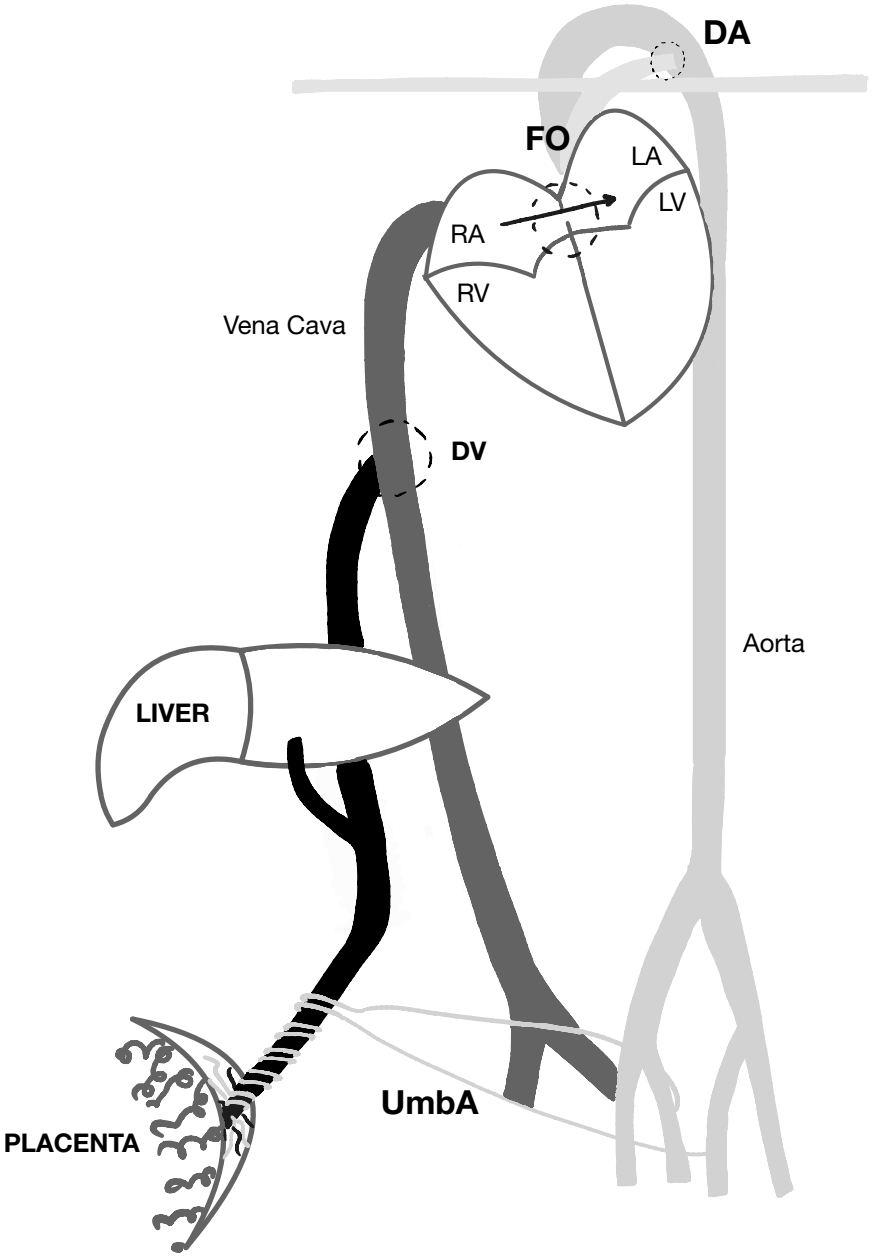

Figure 2: *This scheme summarizes the Fetal Circulation. It represents the four shunts that are responsible for the typical blood circulation that occurs in the fetus, ductus venosus (DV), foramen ovale (FO), ductus arteriosus (DA) and the umbilical arteries (UmbA), as well as the heart and its chambers (RA:right atrium; RV: right ventricle; LA: left atrium; LV: left ventricle), the liver and placenta. This scheme was based on information collected from Beckmann CRB, et al.; Obstetrics and Gynecology. Beckmann CRB, et al. (Eds). Lippincott Williams & Wilkins 2018. (8th edition)*

Doppler

The Doppler ultrasound is a non-invasive method which allows the evaluation of the circulatory dynamics in the embryo. With the creation of the flow velocity waveforms, it is possible to calculate a pulsatility index, resistance index and systolic/diastolic ratio, that allows the analysis of vascular impedance in the embryo.[20] The calculation of these indices, using the variables obtained in Doppler ultrasound, is useful to determine alterations in the fetus's development. This will allow a gestation with alterations to have closer surveillance, with the objective of creating the best environment for the fetus's development. [21]

During the first trimester, it is possible to notice an absence in the end of diastolic flow in the umbilical arteries. This phenomenon is only present during the beginning of pregnancy, whereby its maintenance is associated with a high resistance circuit. As seen in multiple investigations, as the gestation proceeds and the embryo and placenta develop, we have a decrease in the pulsatility index of the umbilical artery, as well as, a positive end diastolic flow, that suggests a reduction in the vascular resistance.[12],[22],[23] To have an alteration in the Doppler ultrasound, it is necessary to have a large number of abnormal tertiary villous arteries in the placenta.[20] In fact, if more than half of the villous arteries, formed from the ramifications of the umbilical arteries and vein, have alterations, like atrophy, thrombosis or reduced production of normal villi, it is possible that the end diastolic flow, obtained in the doppler ultrasound, is absent or reversed.[24],[25]

As described before, the dorsal aorta is the precursor of the descending aorta. The analysis of the flow velocity waveforms of this vessel gives additional information about the vascular impedance in placenta circulation. However, since it is more difficult to obtain quality waveforms from the descending aorta, when compared to the umbilical arteries, the analysis of descending aorta waveforms, in clinical practice, is rarely used.[24] The umbilical arteries are connected to ramifications that originate from the aorta, the internal iliac arteries. As a result of these different vessels being summited to the same environment, the presence of increasing resistance in the fetoplacental circulation will induce change in the flow of not only the umbilical arteries, but also de descending aorta.[24]

For the analysis of vascular impedance, since it cannot be directly measured, it is necessary to calculate the different indices from flow velocity waveform. With its calculation, it is possible to infer the values of vascular impedance.[26] The increase in resistance and pulsatility index is associated with an increase in vascular impedance. Unlike the systolic/diastolic reason and resistance index, the pulsatility index has a linear correlation with vascular resistance.[27]

In every flow velocity waveform from an artery, it is possible to differentiate two moments, one corresponding to the systole, the highest peak, peak systolic velocity (PSV), and the diastole, end diastolic velocity (EDV). As said before, the end diastolic flow is absent in the first trimester, but with embryonic development, this parameter is no longer zero, leading to the decrease of systolic diastolic ratio. This parameter is calculated by the ratio between PSV and EDV, obtained from the Doppler ultrasound. The pulsatility index is calculated from the ratio between the difference of PSV and EDV, and the time average maximum velocity (TAMX). The resistance index corresponds to the ratio between the difference of PSV and EDV, and PSV.[24],[26] This last parameter varies between the location of the umbilical cord chosen to analyze, having the highest value in the abdominal insertion of the umbilical cord.[25] This variability does not occur with the pulsatility index, because the alteration of the location, where the measurements are obtained, is considered insignificant in the final result.[28] The pulsatility index corresponds to the index that suffers the smallest variation, being the best parameter to analyze arterial waveform.[24],[27]

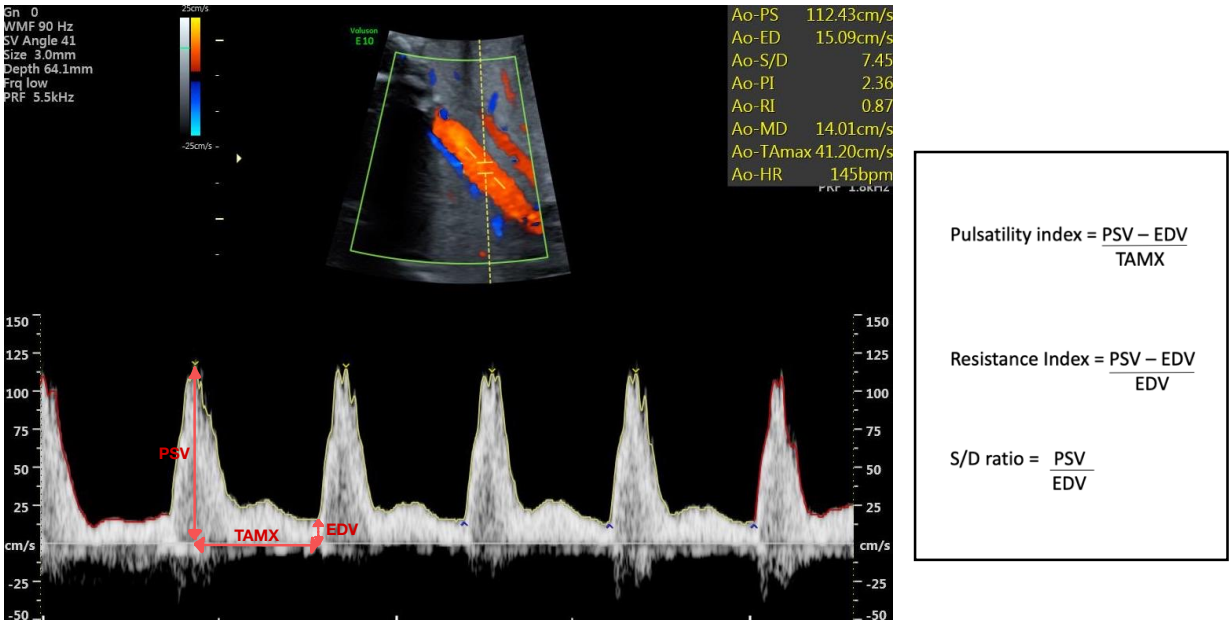

Figure 3: *Doppler ultrasound of an aortic artery with the x- axis measuring the time and the y-axis measuring the velocity. The values of PSV, EDV and TAMX are identified. These are essential for the calculation of the Doppler indices described previously. The peak systolic velocity corresponds to the maximum velocity value obtained during systole, which in this case corresponds to 112.43 cm/s. The end diastolic velocity is associated with the smallest velocity value just before the beginning of another systole, that is marked as 15.09 cm/s, in this case. The TAMX corresponds to the time average maximum velocity, which is the time between the PSV and EDV, that in this case is 41.20 cm/s*

The ultrasound has different types of flow modes, color flow imaging and spectral doppler, that can be used as a group in the analysis of a vessel, since they complement each other in different aspects. Color flow imaging is normally used to identify the vessel that is being exanimated, whereas the spectral doppler allows the detailed analysis of the vessel, with the calculation of the indices, that are essential for the application of the information in clinical practice.[29] It is also important that, during the recording of these measurements, the fetus is not breathing or moving, which is called the period of fetal quiescence, since this could induce alterations in the values obtained.[30] In the image above, it is possible to identify both of the ultrasound flow modes, the first one corresponding to the color flow and the one below corresponding to the spectral doppler.

The angle of insonation affects the image in the sonogram. It is important that the angle between the flow vessel and the bean is less than sixty degrees, because higher angles are associated with unclear values, which will affect the calculation of the indices.[29] The objective is to use the minimum angle of insonation possible.[30] The color flow imaging ensures that the best angle of insinuation is obtained.[30] This makes the angle of insonation one of the most important aspects in doppler ultrasound analysis. In fact, incorrect insonation angle use can result in false positives Doppler ultrasounds, because it can induce the absence of end diastolic flow, that is considered pathological from the second semester.[30] As we can see in the image above, the angle used is 41°, which is in the interval that is considered appropriate.

As seen in most of the articles published until now, the vascular impedance decreases from the second trimester onward. Since the vascular impedance cannot be calculated, this phenomenon is verified by the alterations on the Doppler indices. In fact, during gestational development, all the indices tend to reduce, especially during the third trimester, which can continue beyond term, even in pregnancies at forty weeks of gestation.[24],[29],[31] This alteration can be explained by the increase of end diastolic velocity, narrowing the difference between this parameter and the peak systolic velocity.

Aortic and umbilical Doppler parameters variation during pregnancy

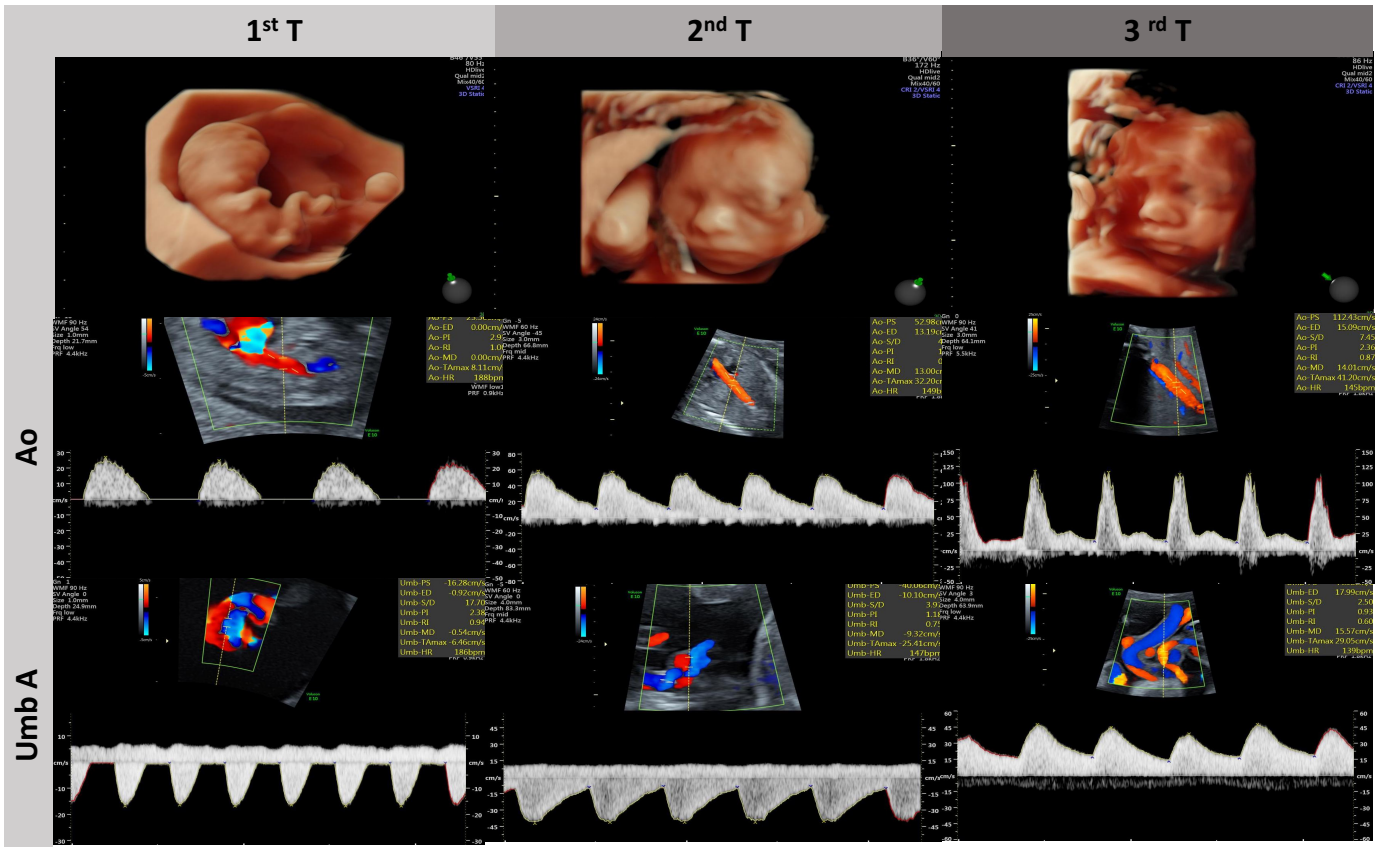

Figure 4: *Doppler ultrasound of the aortic (Ao) and the umbilical arteries (UmbA) during the thirst trimester (1st T), second trimester (2nd T) and third trimester (3rd T)*

1.1 The umbilical artery:

The image below lays out the alterations in doppler ultrasound during fetus development. In the first trimester, the absence of diastolic flow is considered normal. Towards the end of the first trimester, week eight to ten (post-conception), it is possible to have an increase in TMAX, with an unchanged pulsatility index. This phenomenon can be explained by the increase in the heart rate of the fetus, as well as, with the disappearance of the yolk sac circulation, which will lead to an increase in blood flow to the fetoplacental circulation. [(5),(16), (32)]

From the second trimester on, the absence of diastolic flow reflects abnormalities in the fetoplacental circulation.[(16)] This major alteration between trimesters is explained by the cytothrophoblasts invasion of the spiral arteries, which will lead to an increase diameter of these vessels, which is responsible for low resistance in the fetoplacental circulation. it. This occurs

because of alterations on the vascular wall, with gradual loss of some of the muscular component, culminating in a low resistance circuit. [24],[33],[34]

The doppler ultrasound presented below, illustrates a normal evolution of the different parameters evaluated, with a positive end diastolic flow, that appears at the beginning of the second trimester, week fourteen (post-conception). This will lead to a decrease in the pulsatility index as the gestation proceeds.[35] In fact, in this case, the value of PI in the second trimester is 1.18, but in the third the number decreases to 0.93, which is associated with the decrease in placenta flow impedance. The same will happen with resistance index and systolic/diastolic ratio.

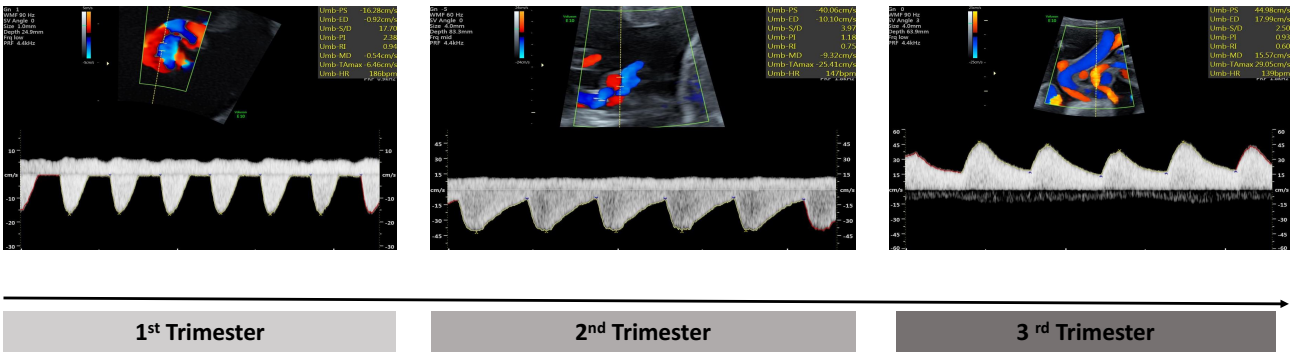

Figure 5: *Alterations in doppler ultrasound on the umbilical arteries during embryo-fetal development*

1.2 The Aorta:

The image below represents the doppler alterations in the aortic artery with fetus development. As previously seen, the aortic artery has no end diastolic flow during the first trimester, that is considered normal. The biggest doppler difference between the aortic artery and the umbilical arteries, during development, is associated with the pulsatility and resistance indices. In fact, in the aorta, these indices maintain their values constant, not suffering a decrease with gestational age (GA), as it happens in the umbilical arteries.[36],[37] This phenomenon can be explained by the presence of a compensatory vasoconstrictive mechanism that occurs in other branches of the aorta, like the renal artery and femoral artery.[36],[37] Although most of the blood from the aorta flows into the fetoplacental circulation, that is associated with a low resistance circuit, the other ramifications are also able to induce significant resistance in the aorta. [37] For these reasons, it is establish a balance between the two, which will lead to a stable pulsatility index, especially in the third trimester.[38] Because of this fact, if we compare the doppler ultrasound from the umbilical artery with the one from the aortic artery, it is possible to notice that the last one has a bigger pulsatility waveform, which means that the difference between the

PSV and EDV is higher.[37] Also, the value of PI, in the third trimester, in the umbilical artery is 0.93, whereas the PI in the aortic artery is 2.36.

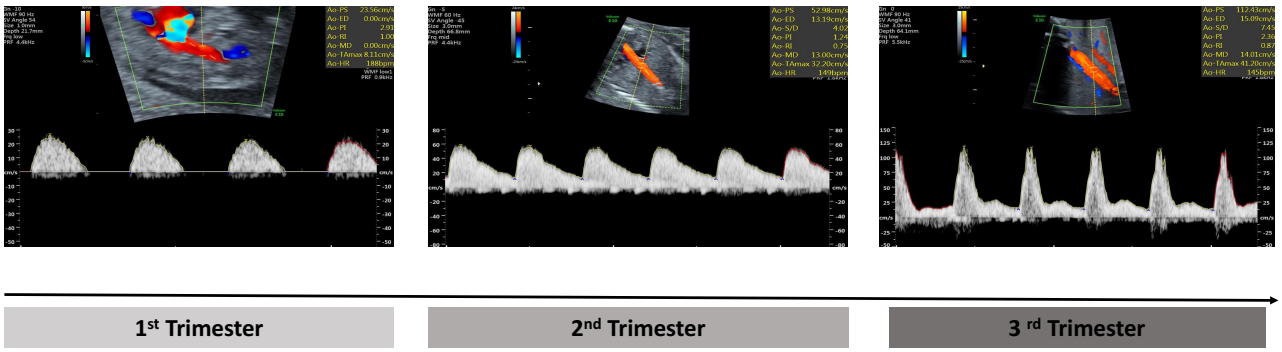

Figure 6: *Alterations in doppler ultrasound on the aortic artery during embryo-fetal development*

Derivation of Reference Curves in Obstetrics:

In obstetrics, reference charts for fetal size variables (head measurements, abdominal measurements, femur length, …) are important methods in clinical medicine. By direct comparison of measurements from a fetus with reference data for that GA, clinicians can evaluate if the fetus is having the expected development.[20] For example, a centile position above (respectively below) 50%, or equivalently a positive (respectively negative) Z-score, signifies a measurement greater (respectively lower) than average for that GA. Moreover, measurements corresponding to extreme centiles, such as below the 3rd or beyond the 97th centiles, greatly transcend the average patterns and may be associated with clinical complications.[39],[40]

Three main uses of charts of fetal measurements are the following: (1) to compare the size of a fetus (of known GA) on a single occasion with reference data; (2) to estimate GA from fetal size; (3) to compare the growth of a fetus between two occasions with reference data. This paper focuses on (1) and that goal will be assumed throughout if nothing is mentioned against it.

There are two types of study designs: prospective and retrospective. Prospective studies are those recommended for deriving centiles, since the data will be collected with the specific aim of constructing the reference charts. In the opposite way, retrospective designs use data already collected, most often from a selected group or from routine medical appointments. The sample may not represent the population being studied, and the study may be unsuitable if its purpose is not the research, but the patients follow-up.[39],[40]

Derivation of reference curves can be addressed by means of cross-sectional data (including a single measurement from each fetus) or longitudinal data (including more than one measurement per fetus, read at different time instants). Cross-sectional data is advocated for developing reference centiles of fetal size. [41],[42]

The design of a prospective cross-sectional study can be implemented in the following way: randomly allocate a date of measurement for each woman in the sample, so that, approximately, equal numbers are measured at each week of gestation. In particular, data collected later in gestation, should not be performed due to clinical suspicions of unusual events. The number of women to be measured in the latest weeks may have to be increased, as some will be delivered earlier. Women should always be collected from the population to which the chart will apply.

Sample size determination:

The question of sample size determination is a delicate issue. The larger the sample, the higher the precision of the estimates. Moreover, special attention should be paid to the tails of the distribution, for identification of extreme centiles since there are few observations there. A large sample is desirable so that the centiles can be estimated precisely, although it does not compensate for inappropriate samples or design deficiencies.[41] The total size of the sample should also account for observations that may be excluded due to clinical complications, early deliveries or drop-offs of the study.

Sample size calculations always follow from the statistical method used in the analysis. For the commonly used regression-based reference ranges, formulae were initially developed by Royston[43] and extended in 2007 by Bellera and Hanley. [44] Most recently, Hanley discussed sample size considerations, including adjustments for covariates of interest.[45] For data following a conditional normal distribution, the formula for the standard error of the pth centile is

$$SE_p = SD\sqrt{\left(1 + \frac{1}{2}{z_p}^2\right)/n}$$

where SD is the standard deviation of the measurement, z_p is the pth centile of the standard normal distribution, and n is the sample size. Researchers can use this formula for the determination of n for extreme centiles.

Once the sample is collected, a structured descriptive analysis is necessary, so that data can be critically analyzed (and modeled). A table showing how many women were recruited in each GA window, together with the mean and SD of the measurements, should be reported in publications.

Reference centiles can be estimated using parametric or non-parametric (empirical) statistical methods. While parametric approaches can be simpler to apply for a potential user of the charts, they require more assumptions than the non-parametric methods. Moreover, if those assumptions fail, the model conclusions may be compromised. On the other hand, the non-parametric approaches require choices for certain (smoothing) parameters, that are not totally objective. In this way, there is no recommended method that will work in any circumstances.

Generally, reference centiles should change smoothly with GA and result from a, as simple as possible, statistical model that takes into account the increasing variability among fetuses as gestation evolves, and that fits the raw data well. [40],[46] The simplicity of the model will allow the potential user to calculate the corresponding Z-score and centile for a given measurement.

The simplest parametric approach that has shown good results in the identification of reference centiles is the "mean and SD model". [47] It assumes that, at each GA, data comes from a normal population, and both mean of variance of the fetal size variable are modeled as a polynomial function of GA. A useful tool in assessing model fit are Z-scores, representing the observed values expressed on a standard normal scale (mean=0 and SD=1) with the mean and SD fitted from the model. More precisely: (1) a plot of the Z-scores against GA should be randomly scattered about zero for all GAs; (2) normality of the Z-scores should not be rejected from adequate normal plots, eg, Q-Q plots. If necessary, adequate transformations of the response variable (e.g., logarithmic) can be applied to ensure normality of residuals. An extension of the "mean and SD model", formalized by Royston and Altman in 1994, allows regression parameters to also take fractional powers. Over recent years, the use of fractional polynomials in the construction of centile curves has become more popular. [40]

Whenever the skewness in the measurements cannot be corrected for normality, by a suitable simple transformation, a Box-Cox transformation can be applied. The LMS method, introduced by Cole, uses that transformation to allow the skewness of the response variable, the median and variability to vary with GA. [48] Three parameters – λ, μ and σ – are respectively considered for that purpose, which initials give rise to the name of the method. [40],[46] Two extensions of the LMS method, that are able to also deal with kurtosis, are LMST and LMSP. [49] The difference between them relies on the ability to model kurtosis, LMSP can model any type of kurtoses, whereas LMST can only model excess kurtosis, named leptokurtosis.[40] The main drawback of these approaches is the complexity of the methods, with no simple formula for calculation of centiles, and the arbitrariness in the choice of the parameters. Nevertheless, the centiles can be displayed graphically or in tabular form.

Another method that makes no assumption about the nature of the measurements distribution, at a fixed GA, and that also does not return a succinct formula to the user, is the one based on empirical centile estimates, presented by Healy et al 7.[50] It imposes the condition that both the centiles themselves and the intervals between centiles, at a fixed GA, should behave smoothly. There is some degree of subjectivity in choosing the values of the parameters involved and the method is sensitive to the presence of outliers. It has not been recommended for derivation of fetal size charts, except when other methods are unsuccessful. [40]

The goodness-of-fit assessment of a chosen model can use numerical measures, depending on the statistical modeling used, but should always be also supported on a graphical approach. A simple plot, that should be included in publications, is the scatterplot of the observations across gestational age with the calculated centiles superimposed. The proportion of points outside the outer centiles should be coherent with the centiles and evenly spread throughout the range of gestation.[41] The same should happen to the proportion of observations falling between the fitted centiles.

Other information of interest to future users of the charts, is a table with selected fitted centile values (eg, 10^{th}, 50^{th} and 90^{th}) and, whenever possible, the explicit equation enabling the calculation of any desired centiles and/or z-scores.

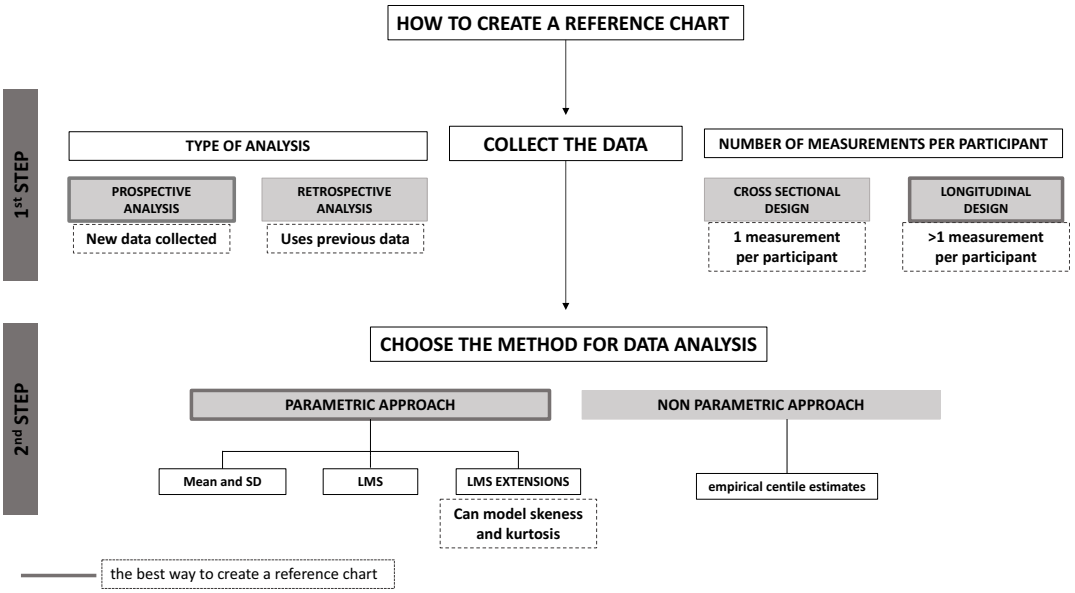

Figure 7- How to create a reference chart: *The scheme advocated if one of the parametric methods is useful in obstetrics. Firstly, data have to be collected. Based on the type of analyses that is used, we can have a prospective analysis, when the data is collected with the objective of constructing a reference chart, or retrospective analysis, that uses data already collected for other reasons. Also, depending on the number of measurements per participant, it is possible to have a cross-sectional design, formed only by one measurement per participant, and longitudinal design, which has more than one measurement. The next step, after collecting the data, is to choose the best method for the analysis. Inside the parametric approach, advocated if one of the parametric methods is a successful model, LMS or LMS extensions. The nonparametric approach is only used if the data cannot be converted into a normal distribution. To sum up, the best way to create a reference chart is to have data from a prospective analysis with longitudinal design, essentially according to the empirical distribution of the measurements.*

Protocol for assessment of aortic and umbilical embryonic arteries flow

Reference charts are an important tool used in obstetrics and in other areas of medicine. The correct development of these charts is essential for the determination of the true state of fetal development, making it possible for the improvement of clinical care in the fetus and the mother. In fact, reference charts allow the clinician to evaluate if a fetus is having the expected development for a certain gestational age. [39]

However, it is only possible to use reference charts if they are made based on a series of criteria, that make it possible for data to be reproduced. In fact, in the section below, a protocol for the assessment of aortic flow and umbilical artery flow, at very early clinical pregnancy, suggested a group of rules for its determination. These rules allow the replication of the conclusions obtained in the study. The only way for the study to have external validation, is to have a reproducibility of the method used. This variable is needed to access that the curves, developed in the study, can be applied to a new group of patients, giving them reproducibility and transportability.[51] The inter-operator and intra-operator variability are also an important variable in every study, which can only be evaluated if the sample is standardized. The objective of any study is to have a good reproducibility of the sample and a low inter-operator and intra-operator variability, so that the protocol developed can be used, with safety, in clinical practice.

5.1. Protocol for assessment of aortic flow at very early clinical pregnancy

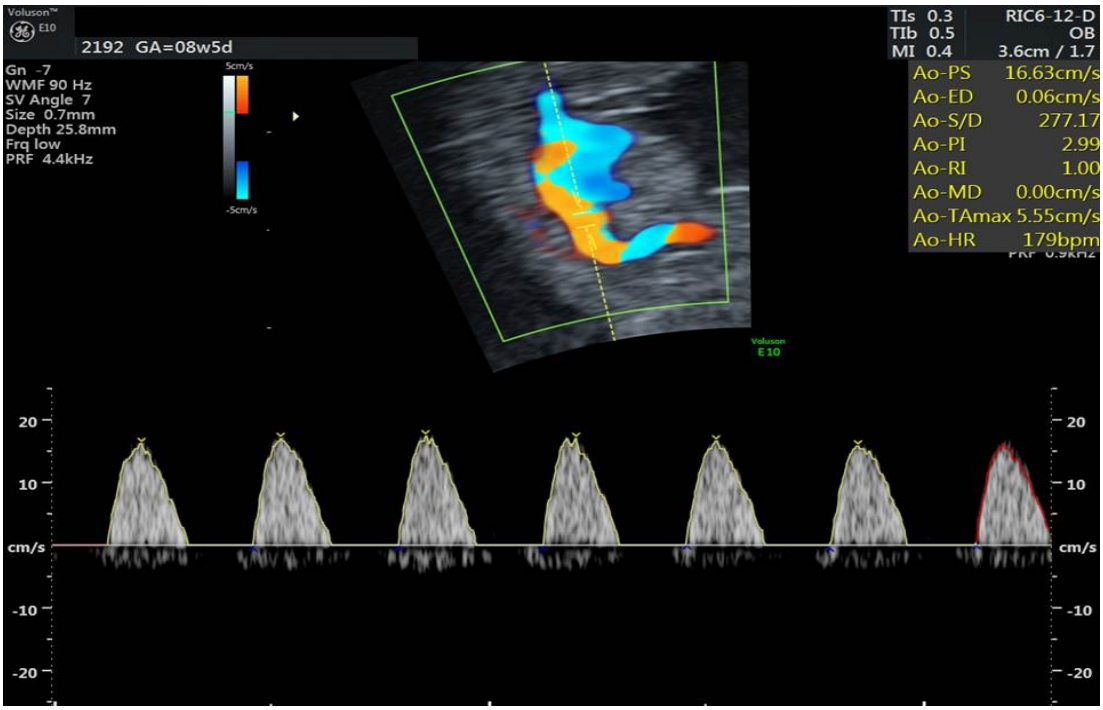

***Figure 8**- Embryonic aortic flow analysis*

The gestational period must be 7 to 10 weeks of gestation;

The examination should be undertaken during embryonic quiescence (especially during the ninth and tenth weeks, when the embryo shows its first movements);

The magnification of the image should be such that the embryonic abdomen occupies the whole image;

A mid-sagittal view of the embryo trunk should be obtained and color flow mapping should be undertaken to demonstrate the dorsal aorta;

The pulsed Doppler sample volume should be small (0.7-1mm) to avoid contamination from the adjacent veins, and it should be placed in the middle portion of the dorsal aorta;

The insonation angle should be less than 30 degrees;

The filter should be set at 70-120 Hz;

The sweep speed should be high (2-3 cm/s) so that the waveforms are spread allowing better assessment of the diastolic flow;

The Aortic Doppler parameters are measured by the machine after automatic tracing of the outline of the waveform.

5.2. Protocol for assessment of umbilical artery flow at very early clinical pregnancy

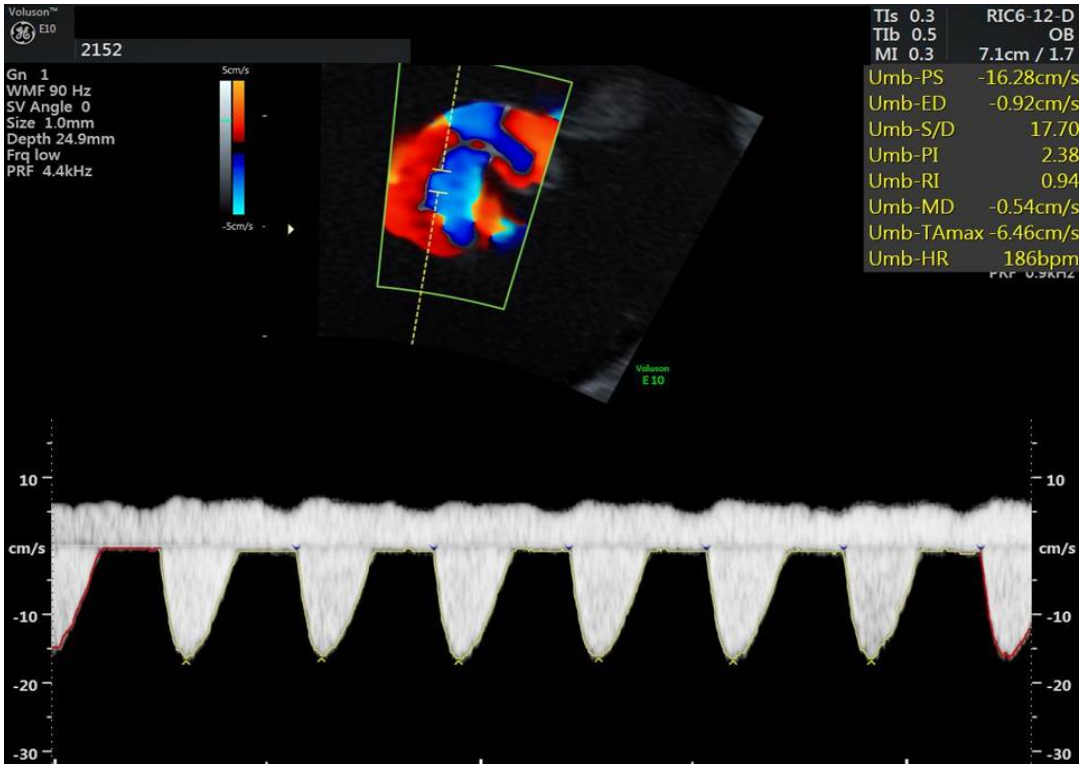

***Figure 9**- Embryonic umbilical flow analysis*

The gestational period must be 7 to 10 weeks of gestation;

The examination should be undertaken during embryonic quiescence (especially during the ninth and tenth weeks, when the embryo shows its first movements);

The magnification of the image should be such that the umbilical cord occupies the whole image;

Color flow mapping should be undertaken to demonstrate the umbilical artery in a free loop of the umbilical cord;

The pulsed Doppler sample volume should be small (0.7-1mm) to avoid contamination from the adjacent umbilical vein, and it should be placed parallel to the umbilical artery;

The insonation angle should be less than 30 degrees;

The filter should be set at 70-120 Hz;

The sweep speed should be high (2-3 cm/s) so that the waveforms are spread allowing better assessment of the diastolic flow;

The umbilical Doppler parameters are measured by the machine after automatic tracing of the outline of the waveform.

Conclusion

Nowadays, the investigation in Fetal Medicine is increasing, however most of the studies made until now are from the tenth week onward, being rare the investigations associated with the embryonic age.

The first trimester of pregnancy is associated with the development of all the systems that will, posteriorly, form the newborn. This fact demonstrates the importance of continuous investigation and determination of new knowledge associated with this period of development. The continuous technological progress in doppler ultrasound is, nowadays, responsible for the early detection of fetal and placental functional alterations, that will, in the future, be associated with malformations. For these reasons, the investment in studies associated with very early gestational periods is essential, since it makes possible the early detection of functional alterations, even when they are not yet expressed, improving gestation management.

Reference charts are, also, an important tool used in clinical practice, especially in obstetrics. It allows the clinician to evaluate if a fetus is having the expected development for a certain gestational age. However, it is necessary that the method used, for the construction of reference curves, is simple and systematic, which can be difficult to find. At the present time, none of the methods developed for the creation of a reference range, can be used in all circumstances. If the chosen method is not suitable for the type of data used, the reference chart created will lead to an incorrect analysis of the fetus, leading to a decrease in the clinical care quality.

The present work summarizes the principles of doppler ultrasound and the indices that can be calculated, based on the measurement of flow velocity waveforms. The indices, calculated from the umbilical artery and aortic artery flow, have a direct relationship with the impedance in the fetoplacental circulation. In fact, early determination of these indices will allow the differentiation between expected development and detachment of expected development of a fetus, in very early gestation. For these reasons, it is presented a protocol for the assessment of these arteries, that suggests a group of rules to be followed, so that this method can be applied universally. Without rules, it would not be possible to have a reproducibility of the results and conclusions, not being possible to achieve external variability.

To summarize, the investigation in the embryonic period, before ten weeks of gestation (post-conception), should be promoted and encouraged, because there is a lot of ground to be explored. This type of investigation could revolutionize Fetal Medicine, since the early detection of a functional alteration, could be the key for the improvement of pregnancy outcomes that are associated with alterations.

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