UiT The Arctic University of Norway

Faculty of Health Sciences
Department of Clinical Medicine

Sex differences in placental circulation

Christian Widnes

A dissertation for the degree of Philosophiae Doctor – March 2020

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Women's Health and Perinatology Research Group
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Christian Widnes

LIST OF ABBREVIATIONS

AC	Abdominal circumference
ALARA	As low as reasonably possible
BP	Blood pressure
BPD	Biparietal diameter
BMI	Body mass index
CO	Cardiac output
CPR	Cerebro-placental ratio
CSA	Cross-sectional area
CTG	Cardiotocography
CV	Coefficient of variation
CVP	Central venous pressure
DBP	Diastolic blood pressure
DV	Ductus venosus
EDRF	Endothelium-derived relaxing factor
EDV	End-diastolic velocity
EFW	Estimated fetal weight
FL	Femur length
HCG	Human chorionic gonadotropin
HR	Heart rate
ICG	Impedance cardiography
IUGR	Intrauterine growth restriction
MAP	Mean arterial pressure
MCA	Middle cerebral artery
MI	Mechanical index
NO	Nitric oxide
PE	Preeclampsia
PI	Pulsatility index
PSV	Peak systolic velocity
Q_{ua}	Umbilical artery volume blood flow
Q_{UtA}	Uterine artery volume blood flow
Q_{uv}	Umbilical vein volume blood flow
RI	Resistance index

R_{UtA}	Uterine artery resistance
SBP	Systolic blood pressure
S/D ratio	Systolic/diastolic ratio
STV	Short-term variability
SV	Stroke volume
SVR	Systemic vascular resistance
TAMXV	Time-averaged maximum velocity
TAV	Time-averaged intensity weighted mean velocity
TI	Thermal index
TPR	Total peripheral resistance
UA	Umbilical artery
UtA	Uterine artery
UV	Umbilical vein
V_{max}	Maximum velocity
V_{mean}	Mean velocity

ABSTRACT

Introduction

Over the last decade there has been a growing consciousness about analyzing data stratified by sex. Sexual dimorphism in placental morphology and function has increasingly been acknowledged, and differences in adaptation to the intrauterine environment and in perinatal and neonatal outcomes between the sexes has been well described. Sex-specific growth charts are routinely used to evaluate infant growth postnatally and such charts are also available for the evaluation of fetal growth antenatally. Doppler-derived parameters of the feto-placental and utero-placental circulations are commonly used to monitor fetal wellbeing in clinical practice, and longitudinal reference ranges based on serial measurements for these parameters have previously been published. However, whether these parameters are influenced by sex differences have not been adequately scrutinized.

Objectives

The main objective of this thesis was to investigate if significant sex differences exist in the Doppler-derived hemodynamic parameters of feto-placental and utero-placenta circulations in normal pregnancies when the placentation has fully established.

The specific aims were:

1. To explore sexual dimorphism in Doppler-derived parameters of fetal and placental circulation in uncomplicated pregnancies at 22-24 weeks' gestation.
2. To investigate possible sex differences in gestational age-specific serial changes in umbilical vein volume blood flow (Q_{uv}) during the entire second half of normal pregnancy and establish sex-specific longitudinal reference ranges for umbilical vein (UV) diameter, time-averaged maximum velocity (TAMXV), and Q_{uv} (both absolute and normalized for estimated fetal weight).
3. To assess the effect of fetal sex on umbilical artery (UA) Doppler indices, i.e. the pulsatility index (PI), resistance index (RI) and systolic/diastolic (S/D) ratio, during the second half of normal pregnancy and establish sex-specific longitudinal reference ranges for clinical use.

Materials and methods

Data from a total of 520 women with low-risk pregnancies (260 male and 260 female fetuses) were available for analysis from a cross-sectional study performed at 22^{+0}-24^{+0} weeks' gestation (study I). The corresponding numbers for the two longitudinal studies of UV and UA Doppler in low-risk pregnancies examined serially at 4-weekly intervals during 20-40 weeks' gestation,

were 179 (87 male and 92 female fetuses, study II) and 294 (152 male and 142 female fetuses, study III), respectively. Blood flow velocities of the UA, UV and the uterine arteries (UtA) were measured using Doppler ultrasonography. UV and UtA diameters were measured using two-dimensional ultrasonography and power Doppler angiography, respectively. Volume blood flows (Q) of the UV and UtA were calculated as the product of mean velocity and cross-sectional area of the vessel. Maternal hemodynamics was assessed with impedance cardiography (ICG).

Results

At 22^{+0}-24^{+0} weeks of gestation UA PI was significantly higher in female fetuses compared with male fetuses, while the other hemodynamic parameters of feto-placental and utero-placental circulations examined were similar. At no point during the entire course of the second half of pregnancy did we find any significant quantitative differences between the two groups in any of the UV Doppler-derived parameters studied. However, we found a sex-specific difference in the developmental patterns of normalized Q_{uv}. During the same gestational period, we found that the UA Doppler indices were associated with fetal heart rate (HR), and that female fetuses had significantly higher values for these indices during 20^{+0}-36^{+6} weeks' gestation, but not later. When comparing the mean values for fetal HR between the two groups, they were similar from 20^{+0} to 25^{+6} weeks, but a divergent trend was observed thereafter with female fetuses showing increasingly higher HR.

Conclusions

There are significant sex differences in the developmental trajectory of UA Doppler-derived parameters during the second half of physiological pregnancies. Throughout this period female fetuses demonstrate higher values for the UA Doppler indices compared to male fetuses, but these differences are leveled out towards term. For the corresponding UV Doppler-derived parameters no such significant sex differences were found, but there were indications of a deviating pattern of gestational age-dependent temporal changes in $Q_{uv.}$ The sum of these findings reflects temporal sexual dimorphism in placental circulation associated with the maturation of the fetoplacental unit. Sex-specific longitudinal reference ranges for the most commonly used Doppler-derived parameters of both UA and UV were established, believing that it might refine the surveillance of risk pregnancies.

LIST OF ORIGINAL PAPERS

I. Widnes C, Flo K and Acharya G. Exploring sexual dimorphism in placental circulation at 22-24 weeks of gestation: A cross-sectional observational study. *Placenta 2017*; *49: 16-22*.

II. Widnes C, Flo K, Wilsgaard T, Odibo AO and Acharya G. Sexual dimorphism in umbilical vein blood flow during the second half of pregnancy: A longitudinal study. *J Ultrasound Med 2017; 36: 2447-2458.*

III. Widnes C, Flo K, Wilsgaard T, Kiserud T and Acharya G. Sex differences in umbilical artery Doppler indices: A longitudinal study. *Biol Sex Differ 2018; 9: 16.*

1 INTRODUCTION

The use of medically indicated maternal and fetal surveillance by non-invasive techniques during pregnancy is one of the key developments in modern obstetric medicine. Among other things, it serves the purpose of assessing fetal wellbeing. The successful conception induces a period of development and maturation of the feto-placental unit, as well as accompanying maternal adaptations to support the growing conceptus. These changes taking place are both morphological and functional in nature and can be monitored.

The course and outcome of pregnancy are known to be influenced by fetal sex. The knowledge of physiological changes that occur throughout the normal pregnancy, and how they translate into measurable parameters is of paramount importance in understanding, identifying and managing pathological pregnancies. Among the arsenal of techniques used for evaluating maternal and fetal wellbeing, the two-dimensional ultrasonography and Doppler stand out as important clinical methods.

Longitudinal reference ranges for Doppler-derived parameters of utero-placental and feto-placental circulations have previously been established and are constantly under evaluation with the sole aim of increasing their accuracy and precision (sensitivity and specificity) in identifying pregnancies at increased risk of adverse outcome.

This thesis explores some aspects of how fetal sex influences the feto-placental unit and whether this translates into sex differences in the clinically important Doppler-derived parameters of placental circulation.

2 MATERNAL SYSTEMIC CIRCULATION

The maternal adaptation to pregnancy is initiated preconceptionally already during the luteal phase of the menstrual cycle, suggesting a causal relationship with corpus luteum function or changes in ovarian function.[1] Following successful conception these changes are being reinforced in early pregnancy, resulting in (possible) activation of vasodilating substances, with subsequent maternal peripheral vasodilatation, causing significant fall in mean arterial pressure (MAP) and systemic vascular resistance (SVR).[2-7] Consequently, as MAP is directly proportional to cardiac output (CO) and SVR, there is a compensatory increase in heart rate (HR)[7, 8] and stroke volume (SV),[9] and thereby, CO.[10] In parallel to this, the pregnant state causes significant hypervolemia through expanding total volume of circulating blood by up to 50%, reaching a zenith around the middle of third trimester.[2, 11] The relative increase in plasma volume is greater than the corresponding increase in the red cell mass, causing a physiological

hemodilution and a fall in hematocrit.[2, 11, 12] The latter also causes a fall in blood viscosity. The sum of these cardiovascular responses is increased preload and decreased afterload. These adaptations through maternal cardiovascular changes are essential to sustain utero-placental perfusion, and meet the nutritional and supportive demands of the growing feto-placental unit.[13]

2.1 **Cardiac output**

CO is the calculated product of SV and HR, and is defined as the amount of blood pumped into the aorta each minute. As a consequence of the reduced afterload, caused by maternal vasodilatation, the SV increases by 20-30% during pregnancy.[6-9] The early pregnancy also sees an increase in HR by 15-20 beats per minute,[7-9] plateauing in the third trimester and reaching approximately 25% above non-pregnant level.[6] During pregnancy CO increases as much as 30-50%, reaching its maximum in early to mid-third trimester.[4-6, 8, 14] However, there are conflicting results as to how CO develops from this time-point until term, with studies reporting a decrease,[4, 7] steady-state,[6, 9] or an increase towards term.[15] There are also discrepancies in the published literature regarding whether the augmentation in CO is primarily caused by a raise in SV,[6] as a consequence of expanding blood volume, or because of increasing HR.[16] The reasons for these divergent results seem to be use of varied methodologies for data collection and differences in study design.[17-19] In two separate longitudinal studies during second half of pregnancy using impedance cardiography (ICG), our group has found the CO to increase steadily from 20-22 weeks' gestation (range 5.5-6.6 l/min) until 34-37 weeks (range 5.8-7.0 l/min), and thereafter to remain stable until term.[20, 21]

2.2 **Systemic vascular resistance**

The expanding blood volume and increased CO are presumably generated by the reduction in systemic vascular tone and, thereby, fall in SVR.[22] Hence, this fall in SVR seems to be the triggering factor for the succeeding maternal hemodynamic changes, which includes increase in sodium and water retention and CO, all preventing a fall in circulating blood volume.[7] SVR may be defined as the resistance to blood flow offered by the entire vascular tree in the systemic circulation, excluding the pulmonary circulation, and expresses the afterload of the left ventricle. This is sometimes also referred to as total peripheral resistance (TPR). Pregnancy induces maternal changes in circulating blood volume, blood viscosity and vascular tone. All of the aforementioned factors have an impact on SVR.

SVR (dyne s cm^{-5}) is calculated as: pressure (mmHg) x 1333/flow (ml s^{-1}) = (MAP (mmHg) – CVP (mmHg)) x 1333/CO (ml/s) where 1333 is the conversion factor for mmHg to dyne/cm^2.

Since pressure measurements are commonly expressed in mmHg the following simplified formula is used for SVR (dyne s cm^{-5}): 80 (MAP (mmHg) – CVP (mmHg))/CO (l/min).[23]

Several clinical studies show that the fall in SVR is manifested already from 5-6 gestational weeks.[2, 3, 7] Using echocardiography (ECG), Robson et al demonstrated a decrease in SVR from a preconceptional value of 1322 dyne s cm^{-5}, to 1213 dyne s cm^{-5} at 5 weeks' gestation.[3] Along with altered peripheral vascular tone, the SVR is further reduced through the establishment of the physiological low-resistant (high-flow and low-pressure) utero-placental circulation. SVR has a similar developmental pattern to that of MAP with a nadir around 20-28 weeks' gestation, followed by a progressive but modest elevation towards term.[3, 4, 6, 7, 19, 24] Our group has found corresponding values for SVR during second half of pregnancy using ICG, one study showing a range of 957-971 dyne s cm^{-5} from 20-40 weeks,[21] the second study a range of 1112-1179 dyne s cm^{-5} from 22-40 weeks' gestation.[20]

The reason for the pregnancy-associated reduction in peripheral vascular tone and subsequent fall in SVR is not clear. Prostacyclin and thromboxane A_2 have vasodilating and vasoconstricting properties, respectively, of which studies have shown a surge in both maternal and feto-placental tissue during pregnancy.[25] This could possibly be one of the explanations for the observed fall in SVR, as prostacyclin dominates the antagonistic effect of thromboxane A_2, although this causal relationship has been disputed by others.[26]

The essential role of endothelial cells in relaxation of arterial smooth muscle tone was introduced by Furchgott and Zawadsky in 1980.[27] Endothelium-derived relaxing factor (EDRF) is produced and released by the endothelial cells to promote smooth muscle relaxation. Nitric oxide (NO) as an EDRF was later proposed independently by two different groups.[28, 29] In-vivo pregnant sheep-studies have shown that vasodilatation, especially in the uterine vascular bed, is initiated by a surge in NO that is stimulated by endothelial estrogen receptors.[30]

3 PLACENTA

The placenta may be regarded as the interface between the fetal and maternal circulations. However, this structure developing from the fertilized ovum is not just a passive anatomical architecture sustaining the life of the growing fetus but rather a complex organ with a wide diversity of functions. It has proposedly been defined as "the extracorporeal organ that interacts with the endometrium to nourish and protect the fetus and that orchestrates maternal adaptions to pregnancy".[31] As the pregnancy elapses, the placenta serves the functions of organs such as kidneys, gut, lungs, and liver of the fetus, besides playing an essential role in the production of

several hormones and other mediators that induce the important modulation of maternal physiology and metabolism.[32, 33] All these placental functions help to secure a safe and protective environment for the developing fetus.

Following conception, the trophoblast cell lineage starts to differentiate after 4-5 days, eventually surrounding the blastocyst. Around 6-7 days post-conception, the blastocyst is attached to the implantation site in the endometrium by the syncytiotrophoblast, the latter differentiated from the trophoblast, marking the initial creation of the placenta. What remains of the trophoblasts are now referred to as cytotrophoblasts. A few days after implantation, the syncytiotrophoblast quickly proliferates and thereafter invades the maternal endometrium and uterine stroma. The cytotrophoblasts are found in the second layer, never in direct touch with maternal tissue. Eight days after conception, fluid-filled spaces materialize and start to fuse, creating larger lacunae, within the outer syncytiotrophoblast layer. After further development of these layers, trabeculae are formed in between the lacunae and eventually develop into the intervillous space and the villous tree.[34] In order from the embryo towards the endometrium, three distinct and outmost important zones of the placenta can now be distinguished: the early chorionic plate (representing the fetal surface of the placenta), the lacunae with the intervillous space and villous tree, and the primitive basal plate (representing the maternal surface of the placenta).

At about 12-14 days after conception, the protruding trabeculae containing cores of cytotrophoblasts develops into structures called villi that are bathed with maternal blood via spiral arteries in the intervillous space, the latter now called lacunae.[34]

Approximately 18 days following ovulation, the basic placental cellular organization and formation of blood vessels are evident.[35] The fetal and maternal circulations are now two completely different entities where no major mixing of maternal and fetal blood takes place.

Another subset of the cytotrophoblasts migrate further into the endometrial stroma. They differentiate into an initial wave of invading interstitial extravillous trophoblasts, which are succeeded by a “second wave” of endovascular extravillous trophoblasts. In successive order, these trophoblasts penetrate the walls of the spiral arteries, from the outside and inside respectively, where they destruct the smooth muscles and reorganize the structure of the latter.[36-38] This leads to the physiological remodeling of the spiral arteries, normally completed by 18-20 weeks’ gestation,[39] eventually securing the high-flow and low-pressure utero-placental circulation.[34, 40]

4 UTERO-PLACENTAL CIRCULATION

4.1 Uterine arteries

The internal iliac artery most commonly terminates into two main stems, one anterior and one posterior. The uterine artery (UtA) arises from the anterior division of the internal iliac artery bilaterally, and a large degree of anatomical asymmetry between the two sides has been described.[41] It has a characteristic U-shaped course, consisting of a descending and then transversal segment, both running medially, followed by the uterine arch part and the ascending segment coursing along the side of the uterus (Figure 1).

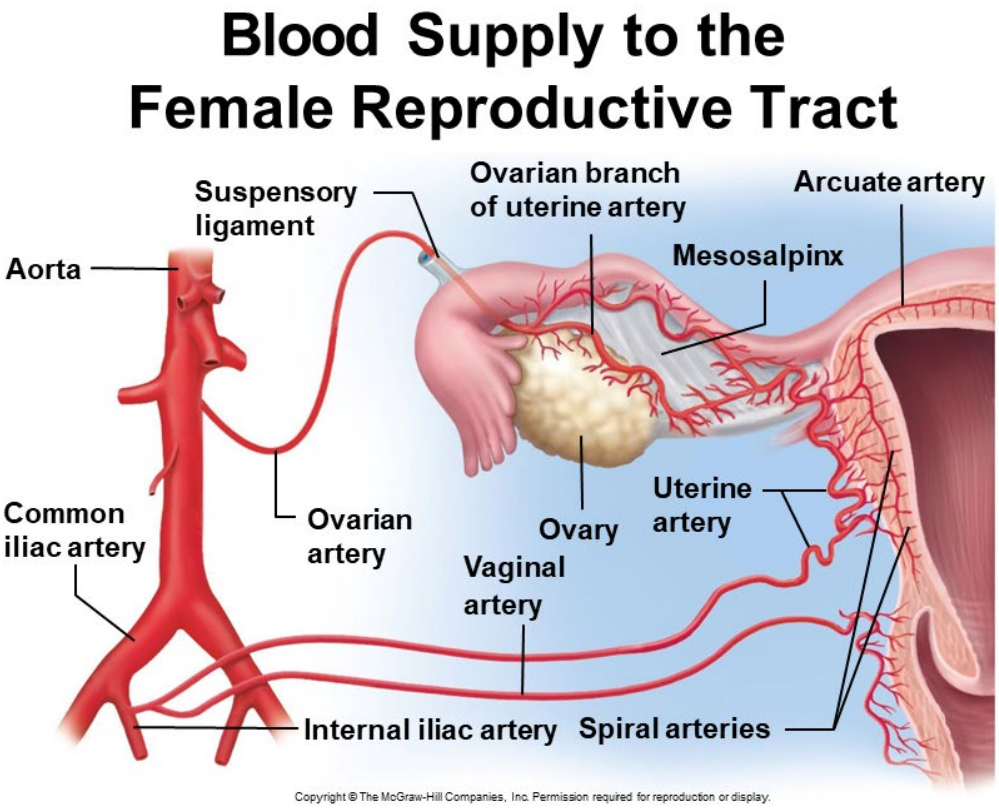

Figure 1
Illustration showing the origin and course of the uterine artery. Reproduced with permission.

The artery divides into the tubal and ovarian terminal branches after it has penetrated into the broad ligament at the superior angle of the uterus, and forms anastomoses with the ovarian artery branches. The ovarian arteries emerge from the abdominal aorta, below the renal arteries. Intramural branches of the UtA, also called arcuate arteries, originate from the ascending segment along the side of the uterus, and form anastomoses with those of the contralateral side, in the midline of the uterus.[42] The arcuate arteries give off radial arteries, which are called spiral arteries when they penetrate into the uterine endometrium. The UtAs also demonstrate

ipsilateral arteriovenous shunts at different branching levels in the uterine wall, thus bypassing the intervillous space.[43, 44] The supply of blood to the pregnant uterus by the ovarian and uterine arteries, with its corresponding anastomoses, has a substantial reserve capacity. This is demonstrated in the case of obliteration of one of the uterine arteries during pregnancy, or even conception following bilateral uterine artery ligation, where successful outcomes of pregnancy may be seen.[45]

Studies in rhesus monkeys showed that the uterine artery provided 91 to 100% of the arterial blood supply to all segments of the reproductive tract (approximately 93% to the uterus) in both non-pregnant and early pregnant state. During late pregnancy, the UtAs only provided 9% and 5% of the blood supply to the ovaries and fallopian tubes, respectively, and the ovarian artery became dominant in providing these segments with blood. Meanwhile, the arterial supply of the uterine arteries to the rest of the uterus (approximately 86%) was not significantly changed.[46] Equivalent studies in humans are scarce but indicate similar values, with the UtAs contributing with roughly 80% of the total uteroplacental blood flow.[47] The relative contribution of arterial blood supply to the reproductive tract is contrasting depending on what species have been studied.[48, 49]

4.2 **Uterine artery Doppler**

The use of UtA Doppler allows a safe and non-invasive evaluation of blood flow through the uterine arteries.[50] During a physiological pregnancy, the corresponding blood flow velocity waveforms through the cardiac cycle are initially characterized by a sharp rise and fall in the measured velocities during systole, followed by an early diastolic notch and low end-diastolic velocities. During the second trimester the diastolic flow demonstrates a gradual increase, and by 20-25 weeks of gestation the diastolic notch normally disappears.[51, 52] The latter is a marker of transiently reduced velocities in early diastole and an expression of vessel elasticity.[53] The progressive disappearance of the early diastolic notch by week 25 reflects the aforementioned physiological remodeling of the spiral arteries.[39] Its persistence might be a normal finding,[54] but generally an indication of increased UtA impedance due to incomplete spiral artery trophoblast invasion and inadequate remodeling of uteroplacental circulation.[55] The presence of an early diastolic notch into the third trimester may be unilateral or bilateral and may be associated with an adverse pregnancy outcome.[54, 56, 57] Both “notching” and the flow velocity waveforms are affected by the implantation site and laterality of the placenta,[58, 59] but a unilateral abnormal waveform does not necessarily indicate an increased risk for pregnancy complications.[60, 61]

From the recorded flow velocity waveforms, the calculations of the Doppler indices, like the pulsatility index (PI),[62] resistance index (RI),[63] and systolic/diastolic (S/D) ratio[64] are made. UtA Doppler indices are widely used in clinical practice as a surrogate measure of uterine artery resistance[58] and in normal pregnancies their values decline with increasing gestational age.[53] Elevated values are regarded as an indication of increased UtA resistance related to inadequate trophoblast invasion and remodeling of the spiral arteries.[65-67] A link between uterine artery Doppler indices and maternal cardiovascular function has also been established.[68, 69] This is shown through a significant association between preexisting manifest or subclinical maternal heart condition and poor placentation, the latter suggested by increased incidence of raised UtA Doppler indices and bilateral notching of UtA waveform during pregnancy, eventually leading to unfavorable pregnancy outcome. The causal mechanisms have yet to be uncovered.

A lot of work has been put into evaluating how UtA Doppler perform in predicting different pregnancy complications like preeclampsia (PE) and intrauterine growth restriction (IUGR), starting nearly four decades ago.[50] There are some conflicting results as to which one of the different Doppler indices to use in the assessment of risk for pregnancy complications,[70, 71] but PI is now the most commonly used. Extensive research unveils low predictive value for pregnancy complications with the use of UtA Doppler indices alone, especially in low-risk pregnancies.[71-73] A review from 2002 on studies of one-stage second-trimester UtA Doppler screening in unselected populations indicates that the finding of abnormally elevated PI will pinpoint 40% of the pregnancies later developing PE,[74] while another large meta-analysis reported sensitivity and specificity of corresponding findings in predicting early-onset PE to be 47.8% and 92.1%, respectively.[75] However, when combined with maternal characteristics and biochemical markers during first trimester, identification of more than 90% of pregnancies developing early-onset PE will be revealed.[57, 76-79] This may be at a high cost with a large number of false positive tests. The use of UtA Doppler seems to be most valuable in the prediction of the severe early-onset PE when applied on a high-risk population.[71, 80, 81] The conflicting results of evaluation of the UtA Doppler's performance in predicting adverse pregnancy outcomes may largely originate from inconsistency in methodology and criteria for an abnormal test between the different studies.[74, 81]

4.3 **Uterine artery resistance**

As described above, the UtA Doppler indices are commonly used as surrogate measures to express resistance to blood flow in the utero-placental circulation, in which they are considered to be a time-efficient, reliable and highly reproducible clinical tool. The more conventional way

of indicating the uterine artery resistance (R_{UtA}) is through a ratio between the mean pressure and the mean volume blood flow, computed as maternal MAP/uterine artery volume blood flow (Q_{UtA}). There are several elements influencing the resistance (R) to flow, and these may partly be summed up by the use of Poiseuille's law for steady flow: $R=8L\eta/\pi r^4$, where an increase in blood viscosity (η) or length of a vessel (L) will increase the resistance, as will a reduction in the vessel radius (r). Hence, the total length and diameter of the uterine arteries will affect its resistance to flow. This equation is considered suitable even for the pulsatile flow in the UtA, as it has a steady flow component in the mean arterial flow.

R_{UtA} and the UtA Doppler indices may not be used interchangeably to express the degree of vascular resistance as it has been previously shown that they do not sufficiently correlate.[82] A reduction in vessel diameter will increase the PI, while an increase in MAP may have minimal effect on the UtA PI.[83] Vascular impedance may be expressed as a ratio between pulse pressure and pulse flow. It is defined as an obstruction to pulsatile flow and depicts a different facet of how pressure and flow interact compared to vascular resistance. In a sheep model, pharmacologically induced UtA vasoconstriction caused increased R_{UtA}, while pulsatility expressed through UtA PI, remained largely unchanged.[84] Similar diversity was found in a human study comparing two groups with comparable UtA Doppler-derived indices, demonstrating increased R_{UtA} in one group compared to the other.[85]

4.4 **Uterine artery volume blood flow**

In order to calculate Q_{UtA} using ultrasonography, it is essential to obtain accurate measurements of UtA vessel diameter and mean velocities, as Q_{UtA} is calculated as the product of the cross-sectional area (CSA) of the vessel and its corresponding mean flow velocity. Only then it is possible to also compute R_{UtA} according to MAP/Q_{UtA}. However, the aforementioned measurements are burdened with technical difficulties,[86] explaining some of the reasons for the use of UtA Doppler indices instead of R_{UtA} in the evaluation of blood flow impedance.

The Q_{UtA} increases steadily from early in the first trimester[87] to late pregnancy,[88] in order to support the growing metabolic demands of the fast developing feto-placental unit. This is made possible by augmented maternal CO and the trophoblast-led spiral artery remodeling into low-pressure high-capacitance vessels supplying the intervillous space with nutritious oxygen-rich blood.

With the use of various different techniques, ranging from the application of the Fick principle using N_2O to the use of electromagnetic flow probes and radioisotopes, numerous groups[89-92] have investigated and published studies on the amount of blood flow entering the pregnant

uterus, of which the first was a study in rabbits from 1933.[93] In a publication from 1990, Thaler et al were the first to measure Q_{UtA} with the use of ultrasonography. A transvaginal probe was used to measure Q_{UtA} unilaterally during 6-39 weeks of pregnancy in the same patients, comparing the longitudinal measurements of the pregnant group to the values obtained from a non-pregnant group.[94] They reported a 3.5-fold increase (reaching 342 ml/min) in the volume blood flow near term when comparing the two groups. However, they made the false assumption of equal distribution of blood flow between the two UtAs, by simply doubling the unilaterally measured values to calculate total Q_{UtA}. As previously discussed, the UtA blood flow characteristics are influenced by placental location,[95] and the Q_{UtA} has been shown to be significantly greater on the ipsilateral side when the placenta is not centrally located.[88]

In human studies, the fraction of the maternal CO distributed to the UtA also increases during pregnancy, from 3.5-5.6% in early pregnancy to around 12% near term.[20, 94] While the absolute total Q_{UtA} increases during pregnancy,[20, 88, 94, 96] the total Q_{UtA} normalized for estimated fetal weight (EFW) decreases,[20, 88, 96] the latter being inversely correlated to UtA PI.[96] The absolute total Q_{UtA} is associated with birth weight,[97] and with pregnancy complications like IUGR. When comparing longitudinal total Q_{UtA} changes in pregnancies with appropriate for gestational age (AGA) fetuses and those complicated by IUGR, the absolute total Q_{UtA} is significantly less in the IUGR group.[98] However, when comparing the total Q_{UtA} normalized for EFW, no statistically significant difference was found in the two groups throughout the study period.[98]

5 FETO-PLACENTAL CIRCULATION

5.1 Umbilical cord

The umbilical cord is the crucial lifeline between the mother, placenta and fetus. It consists of three vessels, two arteries and one vein, enveloped in a connective tissue known as "Wharton's jelly". The latter contains myofibroblasts buried in an extracellular matrix. Made up of a mesh of collagen and small fiber bundles, it shields the umbilical vessels from the mechanical stress exerted upon them during pregnancy and delivery.[99-101] The umbilical cord has an impressive tensile strength, where the average mechanical breaking load is reported to be the baby's weight times 2.5.[102, 103] Its size increases with gestational age, the mean length for boys and girls at term reaching 60.1 cm and 57.7 cm, respectively, thereby showing a significant sex-dependent association.[104] The umbilical vein (UV) supplies the fetus with oxygen-rich nutritious blood, while the umbilical arteries (UA) return deoxygenated blood loaded with metabolic waste-products back to the placenta.

5.2 Umbilical vein volume blood flow

The umbilical circulation is a closed circuit, and hence the umbilical vein volume blood flow (Q_{uv}) may be used to reflect placental perfusion as it gives a reasonable estimate of the blood volume passing through the two UAs to the placenta. It may be used as a surrogate measure of nutrients and oxygen delivery to the placenta. Over the years, the techniques for its measurements have seen an extensive development, from invasive, resource-demanding procedures,[105, 106] to safer and less interfering methods suitable for use in human pregnancies.[107]

The use of ultrasonography in estimating Q_{uv} was first introduced four decades ago in a system combining B-mode scanning with pulsed Doppler.[108, 109] Qualitatively, the UV flow velocity waveform is normally found to be continuous without pulsations during second and third trimester,[110] while a pulsatile pattern is typically observed in the first trimester.[111] A pulsatile UV flow pattern during the second half of pregnancy is regarded as pathological, reflecting increased placental vascular resistance and systemic venous pressure associated with IUGR or congestive heart failure.[112-114]

When the UV flow has been assessed quantitatively, reduced Q_{uv} has been shown to be an early finding in IUGR fetuses, even when the UA Doppler indices are normal.[115-117] It has also been proven valuable in monitoring fetal anemia,[118] and in twin-to-twin transfusion syndrome.[119]

Regardless of whether the measurements where done in the free loop or the intra-abdominal potion of the UV, absolute mean Q_{uv} shows a considerable increase throughout the last half of pregnancy, ranging from 53- to approximately 100 ml/min at 22 gestational weeks, to 245-529 ml/min at 38 weeks.[120-122] When the mean Q_{uv} is normalized to EFW, the values display a slow and steady decreasing trend during the second half of pregnancy.[120-123]

In theory, as the UV is a single vessel, the Q_{uv} would be expected to be similar, irrespective of the site of measurement. However, Figueras et al have reported significant differences in the measured values for Q_{uv}, depending on whether they were recorded at the free loop or the intra-abdominal portion of the UV.[124] In a comparative longitudinal study, the calculated average Q_{uv} obtained from the two sites were found to be similar, but due to inadequate agreement between the individual pair of measurements they should not to be used interchangeably.[125] It has also been shown that the UV flow velocity profiles vary along the course of the umbilical cord.[126]

The measurements of Q_{uv} using ultrasonography is burdened with technical and methodical difficulties mainly related to the accuracy in the measurement of the correct vessel diameter and the corresponding mean blood velocity.[86] However, when used clinically, the accuracy and reproducibility are acceptable,[106, 121, 124] and measurement of Q_{uv} has been validated in experimental settings.[124, 127, 128] Nonetheless, different results reported by different investigators

emphasize the need for adherence to coherent techniques and methodology (e.g. using a predefined portion of UV for measurement, low angle of Doppler insonation, averaging several repeated measurements of the inner diameter of the vessel) when measuring volume blood flow in the UV.

5.3 Umbilical artery blood flow

The two UAs arise from their respective ipsilateral anterior division of the internal iliac artery, course along each side of the urinary bladder, before they become an integrated part of the umbilical cord, coiling around the UV. They remain unbranched throughout the whole length of the umbilical cord. Just ahead of reaching the placental insertion, they form Hyrtl anastomosis,[129] thereby connecting the two vessels. These anastomoses are believed to have pressure- and flow-equalizing properties,[130, 131] and their existence may explain the frequent finding of near identical flow velocity waveforms in the two UAs,[132] even when the areas supplied by each one of the two vessels are largely different.

The UAs branch out over the placental surface, before penetrating it and repetitively dividing through the depth of the placenta, eventually forming arterioles and capillaries which supply the terminal villi. The impedance to flow in the UAs is mainly determined by the total cross-sectional area at the arteriolar level of the placental vascular bed, the area of which is dictated by structural factors and arteriolar vascular tone. The latter is almost exclusively influenced by locally released vasoactive substances, with no neuronal contribution.[133]

The UA vascular resistance is normally high during first trimester, characterized by flow velocity waveforms with high PI and absent end-diastolic velocities (EDV). As the EDV gradually appears, the PI starts to decrease, and from the beginning of second trimester the UA Doppler signal is present through the entire cardiac cycle.[134] Thereafter, the UA PI steadily decreases with advancing gestational age,[135] throughout the second semester, until term.[136] The flow in the UA is always pulsatile.

The UA Doppler indices, i.e., PI, RI and S/D, are commonly used as surrogate measures for UA vascular impedance. They are important clinical tools in assessing fetal wellbeing in high-risk pregnancies, and in predicting outcome in IUGR fetuses.[137] An increase in the UA PI demonstrates a positive correlation with the magnitude of microvascular lesions in the placental vascular bed, and with the degree of impaired placental function.[138] Correspondingly, the UA PI shows a decline with increasing number of arterioles in the villous vascular tree.[139] When applied in high-risk pregnancies, they have the ability to reduce unnecessary obstetrical interventions and risk of perinatal deaths.[140]

Studies estimating UA volume blood flow (Q_{ua}) with ultrasonography have been performed.[141, 142] The calculation of Q_{ua} requires the measurement of the UA vessel diameter and the corresponding mean flow velocity. However, the estimation of the oscillating diameter in a small-caliber vessel is associated with great inaccuracy.[86] As the calculation of Q_{ua} is given as a product including the radius (diameter/2) squared, the inaccuracy of the former may be further increased. Some studies have even revealed different blood flow patterns in the two UAs,[130] especially when the Hyrtl anastomoses have not developed,[143] which occurs in less than 5% of pregnancies. This necessitates individual measurements in both UAs, further accentuating the inaccuracy of the estimated total Q_{ua}. However, the UA absolute velocities are significantly associated with feto-placental volume blood flow and may reflect the latter when evaluating the umbilical circulation.[144]

The Doppler indices have been shown to vary along the length of UA, depending on the site of measurement.[145] The UA PI is highest in the intra-abdominal portion and progressively decline towards the placental insertion, with statistically significantly different values in the fetal and placental ends, respectively.[146] The reproducibility of the UA PI, expressed by the intra-observer coefficient of variation (CV) and the inter-observer CV, has been assessed and reported to be reasonably good.[136, 147]

6 SEXUAL DIMORPHISM IN PLACENTA

6.1 Structural

Evidence for structural differences in the human placenta related to fetal sex is scarce. When searching for relevant published literature on placental morphology, it yields few results. This could be due to under-investigation, or publication bias.

In general, male fetuses have larger placentas than females.[148-150] The birth weight/placental weight (BW/PW) ratio does not explicitly describe placental structure, but in severe placental dysfunction, and in normal pregnancies alike,[148, 149] it is higher in male fetuses compared to female fetuses, reflecting smaller placentas in males relative to birth weight.[151] This has been linked to the assumption that BW/PW ratio may be used a proxy for placental efficiency,[152] and consequently, male placentas are more efficient than the female placentas.[148, 153]

Khong et al demonstrated a decreased male/female ratio in the occurrence of manual removal of the placenta, used as a surrogate for placenta accreta, and suggested this as evidence for a trend for deeper placentation in pregnancies carrying a female fetus.[154] In a study of histopathology in the setting of maternal obesity, certain placental pathologies were found more frequently in female placentas, chronic villitis and fetal thrombosis being more prevalent than in male placentas.[155] Another histopathologic study of 262 pregnancies with impaired placental function (severe PE and/or IUGR), revealed significant sex-related differences in placental gross pathology.[156] Male placentas demonstrated higher occurrence of velamentous insertion of the umbilical cord and chronic deciduitis, while villous infarction was more frequent in female placentas. Umbilical cord anomalies, like knots, nuchal cords and umbilical cord prolapse, are also more common in pregnancies with a male fetus.[157-159] When comparing placental capillary density between asthmatic and non-asthmatic pregnancies, a significantly lower capillary volume was observed in asthmatic pregnancies, and the reduction was linked to male sex.[160]

Maternal overweight/obesity is regarded as a condition inducing low-grade inflammation, associated with adverse pregnancy outcome. Mandò et al studied placental morphometric characteristics in uncomplicated pregnancies related to pre-pregnancy maternal body mass index (BMI) and its influence on placental development.[161] When they compared placental adaptation in overweight pregnant women ($25 \leq BMI < 30$) to that in normal-weight pregnant women ($18 \leq BMI < 25$), they found heavier, thicker, and less efficient placentas (lower BW/PW ratio) in the overweight group. However, this adaptive chance was sex-specific as significant differences were present only in female offspring.

Even though rodent placentas differ to some extent from human placentas in structure,[162] they are commonly used in experimental settings, and fundamental sex-specific structural differences have been found.[163] When evaluating the effect of maternal hypoxia on placental morphology in mice, female hypoxic placentas were found to have reduced labyrinth blood spaces.[164] Undoubtedly, evidence for sex-related difference in placental morphology exists.

6.2 **Genetic**

The placenta is a highly active organ, executing metabolic, respiratory, excretory and endocrine functions to sustain fetal life. In order to orchestrate both fetal and maternal physiology during pregnancy, it expresses a wide pattern of genes. It has until recently been considered to be an asexual organ. Comparative studies of sexual dimorphism in the level of gene expression between male and female placentas from normal pregnancies in humans have been executed.[165-167] They do, unsurprisingly, not only show divergence in the expression of genes located on the sex chromosomes, but also of autosomal genes. Genes linked to immune response were found to be more upregulated in female placentas compared to male placentas,[165] with a possible difference in how the fetus respond to infections and other inflammatory states. The same pattern was seen in the sexually dimorphic expression of genes taking part in placental development, sustainment of pregnancy and maternal immune tolerance to the growing feto-placental unit.[167] Another microarray study of various placental cells revealed sex-bias in the gene expression related to a wide range of cellular functions, in particular gene transcripts involved in promoting a pro-inflammatory milieu and graft-versus-host-disease. These were significantly more abundant in male placentas.[166] This leads to the assumption of at least subtle differences in the physiology of male and female fetuses. Empirical evidence supports the notion that these differences are due to sexual dimorphism in gene regulation, rather than in gene architecture.[168]

In pregnancies complicated by asthma, with or without the use of inhaled glucocorticoids, a microarray study has identified sex-differences in stress-adaptive responses, with 59 gene alterations found in female placentas, compared to only six in male placentas.[169] These genes were tightly linked to pathways involving cellular growth, inflammation and immune response, and the presence of maternal asthma was associated with reduced growth in female fetuses only.[170] However, the normally growing male fetuses had worse outcome in case of secondary asthma exacerbations, showing a trade-off in favor of continued growth at the expense of increased risk of an adverse outcome.[169, 170]

Epigenetics is defined as “the structural adaption of chromosomal regions so as to register, signal, or perpetuate altered activity state.”[171] The epigenome, being the overall epigenetic modifications in a cell, is affected by the sex of the placenta and its environment.[172] The evidence for differences in the sex-specific gene expression and adaption to the same environment is growing.

6.3 **Endocrine**

The human placenta is an important endocrine organ during pregnancy, and one of its principal functions is to synthesize hormones and mediators critical to the achievement of a favorable pregnancy outcome. These hormones take part in the establishment and maintenance of pregnancy, fetal-maternal interaction, placental and fetal development and growth, as well as in parturition. The placental tissue mainly responsible for this function is the syncytiotrophoblast layer.

Human chorionic gonadotropin (hCG) is abundantly produced in the placenta from the time of implantation. It has a role in early-pregnancy sustainment of the progesterone-producing corpus luteum, in placental and endometrial angiogenesis, maternal immunotolerance, trophoblast invasion and in myometrial relaxation.[32] The level of maternal serum hCG is significantly higher with female fetuses compared to male fetuses during third trimester. Female pregnancies show increasing values at this gestational age, while the opposite trend is seen with male pregnancies.[173] Later studies have revealed a significantly higher level of maternal serum hCG in female pregnancies already as early as three weeks post-fertilization, a difference being maintained until delivery, strengthening the assumption of its sexually dimorphic placental expression.[174] A study of sex-differences in steroid profile from umbilical cord-sampled blood indicated that the level of production of four unknown steroids was sex-dependent.[175] Human placental lactogen (HPL), synthesized by the placental syncytiotrophoblast, is another hormone specific to the placenta showing sex-related differences in maternal serum samples, with significantly higher levels in pregnancies carrying a female, compared to a male fetus.[176] Human placental lactogen is known to be able to regulate maternal metabolism and influence fetal growth.

6.4 **Immune response**

The fetal-placental immune system has a critical immunomodulatory role, particularly in the maternal-placental interface. During pregnancy, its immunological competency secures

placental implantation and vital adaptive responses to stressors threatening the feto-placental unit. The actions of the immune system are mediated by specific signaling molecules.

Male sex is known to be an independent risk factor increasing the likelihood of premature birth,[177, 178] and the reason for this is not fully understood. However, in a histological study of premature deliveries of less than 32 gestational weeks, severe lesions of chronic inflammation were found to be more abundant in the maternal-placental interface of placentas from male compared with female fetuses.[179] The findings were suggestive of a sex-biased and more pronounced immune response towards the invading placental tissue (interstitial trophoblasts) of male placentas, orchestrated by maternal immunological processes. The same pattern was seen in another study examining sex-differences in placental lesions and positive placental membrane microbiological cultures, where maternal immune reaction was found to occur more frequently in the placentas of male newborns compared to females.[180] The males were also more prone to demonstrate infected placentas.[180]

Cytokines are examples of small cell signaling molecules, and in the immune system they are acting as immunomodulating agents. They comprise groups like tumor necrosis factors (TNF) and interleukins (IL). It has been shown that when maternal asthma is present, the mRNA levels of the pro-inflammatory cytokines TNF-α, IL-1β, IL-5, IL-6 and IL-8 are significantly higher in female compared with male placentas.[181] The difference was negatively correlated with umbilical cord cortisol concentration, but in female placentas only.[181, 182] This sex-biased placental expression of mRNA in the presence of an adverse maternal environment, indicates a sex-dependent immunological response, reflecting different strategies of survival. An inflammatory state may also be induced through stimulation of the immune system with different antigens. In a comparative in vitro study of unstimulated fetal blood and fetal blood stimulated with a bacterial antigen (Escherichiae coli K12-LCD25 lipopolysaccharide (LPS)), the levels of IL-1β and IL-6 were significantly more abundant in LPS-stimulated blood samples from males, while there were no sex-differences at baseline, in the unstimulated samples.[183] Similar studies on placental and chorion trophoblasts reveal increased production of the pro-inflammatory TNF-α and reduced synthesis of the anti-inflammatory IL-10 with male fetuses compared with female fetuses.[184] Lastly, even PE may be regarded as a condition of excessive inflammation. Accordingly, recent studies have shown sex-bias in inflammatory response, demonstrating significantly increased concentrations of the pro-inflammatory TNF-α, IL-6 an IL-8 in preeclamptic male placentas, compared to their female counterparts.[185] All this supports the aforementioned concept of differences in immune response related to fetal sex, with male fetuses provoking a more pro-inflammatory status.

6.5 **Hemodynamics**

The hemodynamics of the placental circulation is unambiguously related to fetal wellbeing and growth, as previously stated. As described above, numerous placental differences related to sex have been described. However, sex differences in placental hemodynamics have not been fully elucidated.

In a study using electronic fetal HR monitoring in normal term labor by applying computerized cardiotocography (CTG), female fetuses demonstrated significantly faster heart rates than their male counterparts, even when considering possible confounding variables.[186] However, the described differences were not related to differences in clinical outcome. Another large study using analyzed data of 423033 deliveries, examined the correlation between fetal sex and fetal distress during labor. This study revealed male sex as an independent risk factor for fetal distress.[187] Fetal distress during labor was defined by the attending obstetrician as pathological CTG and/or fetal scalp sampling, and was associated with increased risk of operative delivery. The same pattern of increased incidence of fetal distress during active labor in male fetuses have also been reported by other groups.[157, 188] Porter et al recorded intrapartum CTG during the last 30 minutes prior to delivery in normal pregnancies.[189] Deliveries with signs of acidemia (arterial cord pH<7.20, 5-minute Apgar<7, or admission to neonatal intensive care unit) were excluded. After adjusting for confounding factors, there were significant fetal sex differences in the CTG recordings, with males being at higher risk of demonstrating prolonged decelerations and repetitive decelerations.[189]

Several studies have been conducted examining the antenatal baseline fetal HR dynamics related to sex, using computerized CTG. Two of these studies have revealed significant sex differences in fetal HR variability,[188, 190, 191] while another study showed more complex HR patterns in female fetuses compared to male,[192] possibly due to sex related differences in the rate of maturation of the cardiovascular and autonomous nervous system.[190] In two large retrospective cross-sectional studies of gestational age-related antepartum mean fetal HR and short-term variability (STV) in normal pregnancies, significant sex differences in the studied parameters were shown. From about 34 weeks of gestation female fetuses displayed higher mean baseline fetal HR and lower average STV than male fetuses.[193, 194] Yet another study revealed near identical results, with the aforementioned sex differences been demonstrated already from 24-30 gestational weeks.[188, 190] These findings of sex related differences, although small in their magnitude, have recently been confirmed by Bhide and Acharya in a similar large study of 9259 cases.[195]

First trimester Doppler ultrasonographic blood flow studies of possible sex differences in the ductus venosus (DV) unveil conflicting results.[196-198] However, during 28-34 weeks of gestation, a study of placental circulation and fetal cardiac function demonstrated increased preload and significantly lower UA PI in male fetuses.[199] Correspondingly, Prior at al demonstrated sex differences in third trimester fetal hemodynamics. Immediately preceding active labor, they reported significantly lower middle cerebral artery (MCA) PI, MCA peak systolic velocity (PSV) and normalized Q_{uv} in male fetuses set against their female counterpart.[200] In a longitudinal study of low-risk pregnancies published recently, Acharya et al demonstrated significant differences in cerebro-placental ratio (CPR) and umbilico-cerebral ratio (UCR) between male and female fetuses during the second half of pregnancy.[201] Although all these studies are not directly comparable, they add up to a growing testimony of sexual dimorphism in fetal and placental hemodynamics.

6.6 **Implications for the neonate**

Fetal development related to growth and adaption to the intrauterine environment differ in a sex specific manner.[159] Male sex is known to be an independent determinant for adverse outcomes in pregnancy and delivery,[157] often referred to as "the male disadvantage".[202] This includes adverse effects of male sex on the incidence of intrapartum fetal distress,[187, 203] premature birth,[177, 204] neonatal outcome[205] and early neonatal death.[159, 206] Male fetal sex is also reported to be associated with increased frequency of failure of progression in labor, true umbilical cord knots and cord prolapse.[207] A large systematic review and meta-analysis including more than 30 million births, showed a 10% increased risk of stillbirth in males, irrespective of whether the cut-off was placed at 20 or 28 gestational weeks.[208]

Studies investigating possible sex differences in early neonatal vascular hemodynamics are scarce. A small pilot study of sex differences in cerebral blood flow following chorioamnionitis in healthy term infants (52 participants, consisting of 17 controls and 35 histologically proven chorioamnionitis), between 24 and 72 hours postnatally, showed interesting results.[209] Doppler ultrasonography was performed in MCA, anterior cerebral (ACA) and basilar arteries, measuring time-averaged maximum velocity (TAMX) RI. The male infants with histologically proven chorioamnionitis demonstrated a significantly increased MCA TAMX, and a correspondingly decreased mean MCA and ACA vascular resistance than their female counterparts.[209] Stark et al studied possible sex differences in basal microvascular blood flow and vasoactive stimuli responsiveness during the first 5 days (24, 72 and 120 hours) postnatally in extreme premature infants (24-28 weeks).[210] Following a healthy pregnancy, male infants

were found to have significantly increased microvascular blood flow and being more responsive to vasodilating stimuli than females. These sex differences were no longer present at 72 hours of age. Similarly, during the immediate neonatal period, sexual dimorphism in microvascular function and regulation of vascular tone in premature infants has been found following PE[211] and antenatal betamethasone exposure (within 72 hours of birth),[212] respectively.

The above described sexual dimorphism in neonatal hemodynamics could be an expression of sex related differences in adaptation to the transitional circulation from intrauterine to extrauterine conditions and the regulation of vascular resistance. This may lead to the known excess hemodynamic instability, morbidity and neonatal deaths in premature male infants.[213]

Figure 2 gives a brief overview of some of the sex differences existing during the prenatal, perinatal and postpartum periods.

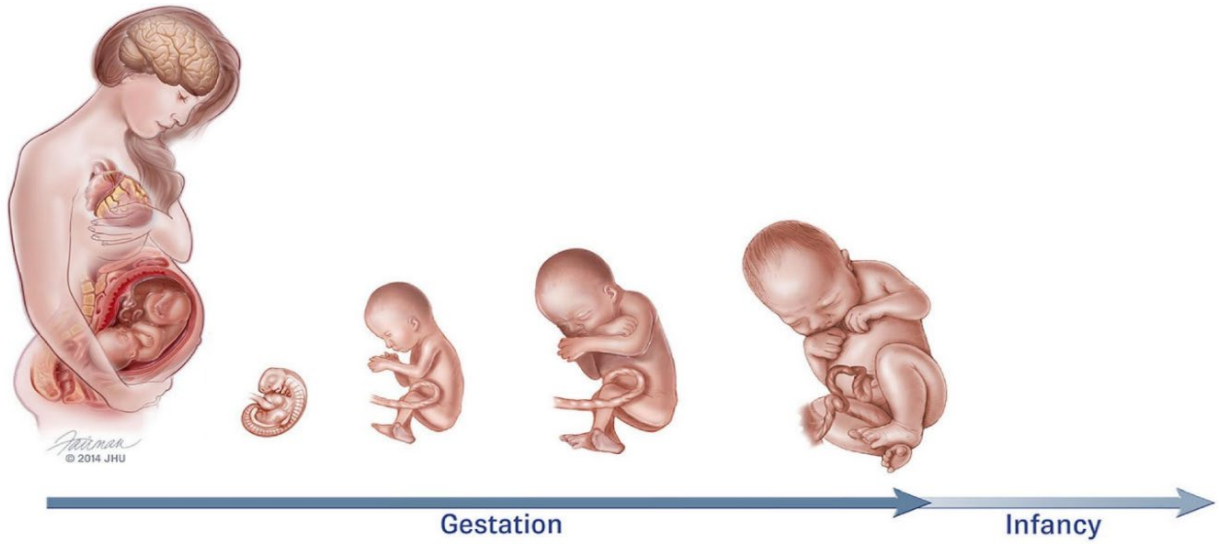

Figure 2
Graphic description of sex differences, according to gestational length. The sex differences presented are the best supported and documented in pregnant women, fetuses and neonates. Even if some differences may not emerge until later in development, they originate in the prenatal or perinatal period. Some of the observed differences affect both the mother and fetus, or may be manifested during both the prenatal and perinatal periods, depending on the time of exposure or delivery.
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7 BIO-SAFETY OF ULTRASONOGRAPHY

The bio-safety of ultrasonography continues to be of some concern among obstetricians and ultrasonographers.[214, 215] However, over the years, since medical ultrasonography was first introduced, an increasing amount of research widely indicates that its use during pregnancy is safe.[216] Harmful effects to the fetus or the mother related to obstetric ultrasound have not been found. Nevertheless, the absence of any evidence of deleterious effects of ultrasonography is not equivalent to the guarantee of its safety. A precautionary approach must therefore be applied and, as a consequence of this, a wide set of guidelines for the safe use of ultrasonography has been issued by several professional bodies.[217-220] With the development of more advanced, high resolution ultrasound systems, the acoustic output has increased.[221] The current upper limit for energy output from the equipment used in diagnostic ultrasound, was set in 1993 by the United States Food and Drug Administration (FDA) to 720 mW/cm^2, augmented from the previous upper limit set in 1976 to 94 mW/cm^2. These new limits have not been properly tested for safety, nor have the potential biological effects of these increased intensities been evaluated in epidemiological studies.[222] The magnitude of the energy output increases progressively from B-mode and M-mode through color Doppler to pulsed wave Doppler mode. In the latter, the beam is focused on a small area and held in a fixed position, potentially further reinforcing any unfavorable bio-effects.

There are two mechanisms through which ultrasonography can affect fetal tissue: thermal and mechanical (non-thermal). As the ultrasound waves are absorbed, their energy is converted into heat. The level of conversion is highest in dense tissue with a high absorption coefficient, like mineralized bone, and is low where there is little absorption, like fluids. Most modern ultrasound devices display risk indicators expressed as ratios. The thermal index (TI) is the ratio of total acoustic power required to cause a maximum temperature increase of 1°C. A TI of 1 indicates a power causing a temperature increase of 1°C. The mechanical index (MI) is an estimate of the maximum amplitude of the pressure pulse in tissue. It gives an indication as to the relative risk of mechanical (non-thermal) effects, like cavitation (caused by rapid formation and collapse of gas bubbles), radiation force and acoustic streaming. As the fetus contains no defined compartments of gas (like inflated lungs or intestines), the risk of mechanical damage to the fetus is negligible. To our knowledge there are no reported studies on cavitational effects in the fetus.[223] In the international academic community it is recommended to display the energy output on screen and always keep the TI<1.9 and the MI<1.5, and to apply the ALARA (as low

as reasonably possible) principle,[217] encouraging self-regulation of acoustic exposure by the ultrasonographer.

8 HYPOTHESIS AND AIMS OF THE THESIS

Our main hypothesis was the following:

In normal human pregnancies, significant sex differences exist in the feto-placental and utero-placental circulation and the magnitude of these differences is associated with gestational age.

The specific aims of this thesis were:

4. To explore sexual dimorphism in Doppler-derived parameters of fetal and placental circulation in uncomplicated pregnancies at 22-24 weeks' gestation.
5. To investigate possible sex differences in gestational age-specific serial changes in Q_{uv} in the abdominal portion of the UV during the entire second half of normal pregnancy and establish sex-specific longitudinal reference ranges for UV diameter, blood flow velocity, and Q_{uv} (both absolute and normalized for EFW).
6. To assess the effect of fetal sex on UA Doppler indices, i.e. the PI, RI and S/D ratio, in the free loop of the UA during the second half of normal pregnancy and establish sex-specific longitudinal reference ranges for clinical use.

9 MATERIALS AND METHODS

9.1 Ethical approval

All study protocols were approved by the Regional Committee for Medical Research Ethics-North Norway (REK Nord 52/2005; date of approval: 27.09.2005 and REK Nord 2010/586; date of approval: 27.09.2010 (Paper I). REK Nord 74/2001; date of approval: 27.11.2001 and 52/2005 (Paper II). REK Nord 74/2001, 52/2005 and 105/2008; date of approval: 16.12.2008 (Paper III).)

9.2 Study design

This thesis investigates the effect of fetal sex on placental circulation based on the data from three prospective studies investigating placental blood flow in the second half of singleton low-risk pregnancies, conducted at the Department of Obstetrics and Gynecology, University Hospital of North Norway, Tromsø, Norway. One of the studies had a cross-sectional design and was performed at 22-24 weeks of gestation (paper I) whereas the other two (paper II and III) were longitudinal studies, where the examinations were performed at approximately 4-weekly intervals from 19 weeks to term.

9.3 Study population

The study participants were recruited from the population of pregnant women ≥18 years of age attending the antenatal clinic for routine ultrasound screening between 17-20 weeks of gestation, at the Department of Obstetrics and Gynecology, University Hospital of North Norway, Tromsø, Norway. The gestational age was confirmed by ultrasound measurement of the fetal biparietal diameter (BPD) before 20 weeks. Women who consented to participate were considered for inclusion if they had no complications in the current pregnancy prior to recruitment. The presence of multiple pregnancy, major fetal structural or chromosomal abnormalities, or any maternal systemic diseases that may affect the course and outcome of the current pregnancy, were reasons for not being included in any of the studies. Smoking or a previous history of IUGR, preeclampsia, preterm labor, gestational diabetes or placental abruption were exclusion criteria in the longitudinal studies. Data from a total of 520 women (260 male and 260 female fetuses) were available for analysis in the cross-sectional study (study I), while the corresponding numbers for the two longitudinal studies were 179 (87 male and 92 female fetuses, study II) and 294 (152 male and 142 female fetuses, study III), respectively.

9.4 Methods

All measurements were performed at daytime between 08.00 AM and 15.00 AM under standardized conditions, with a room temperature maintained at around 22˚C. The participants had a period of rest of minimum 10 minutes in the supine semi-recumbent position prior to being examined. The latter position was chosen in order to avoid compression of the inferior vena cava by the gravid uterus.

9.5 Impedance cardiography

For measurement of maternal hemodynamics, such as SV, HR and MAP during study I, we used ICG (Phillips Medical Systems, Androver, MA, USA) along with a connected sphygmomanometer cuff attached around the left arm. The height and weight of the participants were entered into the software, and the central venous pressure (CVP) and pulmonary arterial occlusion pressure (PAOP) were preset to 4 and 8 mmHg, respectively. Two pairs of sensors were placed on each side of the lower part of the thorax, in the mid-axillary line, while the last two pairs of sensors were placed on each side of the neck, corresponding to the sternocleidomastoid muscle. Each of the four sensors consists of an outer sensor transmitting current and an inner sensor measuring impedance. The current transmitted through the thorax seeks the path of least resistance, which is the blood-filled aorta. With each cardiac cycle the blood's volume and velocity in the aorta will fluctuate and the impedance will be measured as it changes accordingly. The changes in the impedance are integrated with echocardiography (ECG) and blood pressure (BP) measurements in order to provide the hemodynamic parameters. The SV, HR and BP were directly measured, and CO and SVR were calculated by the software of the ICG machine, and displayed continuously on the screen. MAP was calculated as: diastolic blood pressure (DBP) – (systolic blood pressure (SBP) – DBP)/3, CO as: SV x HR, and SVR as: (MAP – CVP)/CO.

9.6 Ultrasonography

For all examinations, either an Acuson Sequoia 512 ultrasound system fitted with a 2-6-MHz curvilinear transducer (Mountain View, CA, USA) or a Vivid 7 Dimension ultrasound system equipped with a 4MS sector transducer with frequencies of 1.5-4.3 MHz (GE Vingmed Ultrasound AS, Horten, Norway) was used. A total of five experienced clinicians performed the examinations (two operators in study I, one in study II and three in study III), and the ALARA principle[219] was employed. The sex of the fetus was neither acknowledged nor

recorded prenatally during ultrasonography, and at all time the mechanical and thermal indices were kept below 1.9 and 1.5, respectively.

9.7 **Fetal weight estimation**

During each examination EFW was computed from the measurements of the biparietal diameter (BPD), abdominal circumference (AC) and femur length (FL), according to the Hadlock 2 formula: Log_{10} weight = 1.335 – 0.0034 AC x FL + 0.0316 BPD + 0.0457 AC + 0.1623 FL.[224]

9.8 **Measurement of uterine artery blood flow**

The UtA blood flow velocity waveforms were obtained from both the right and the left UtA, immediately downstream from the apparent crossing of the external iliac artery, using color-directed pulsed-wave Doppler. The angle of insonation was kept close to 0˚, and always less than 30˚. To ensure sampling of the maximum velocities from the entire blood vessel, a large Doppler sample gate was used. The blood flow velocities, i.e. peak systolic velocity (PSV), end-diastolic velocity (EDV), time-averaged maximum velocity (TAMX) and time-averaged intensity weighted mean velocity (TAV), were measured using the maximum velocity envelope, and the averaged value of three consecutive cardiac cycles were recorded. The presence of notching was noted, which was defined as a decline in the maximum flow velocity below the maximum diastolic velocity, immediately following the systolic wave.[56] The PI was calculated as: (PSV – EDV)/TAMXV, and the RI as: (PSV – EDV)/PSV, and the mean UtA PI and RI were the respective averages of the two sides. The UtA diameters were measured in the same portion of the vessel from where the blood velocity measurements were obtained, using power Doppler angiography (Figure 3).

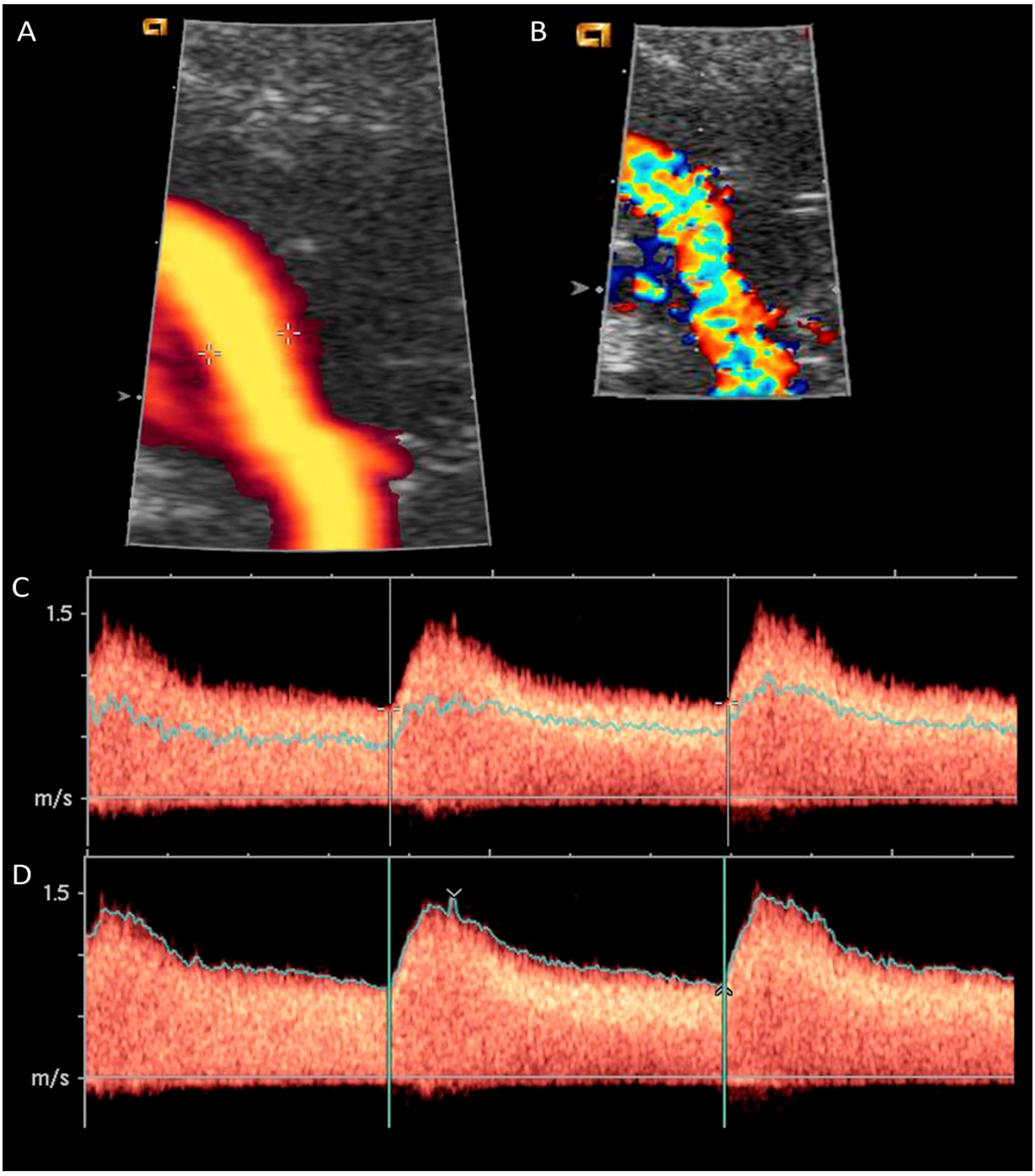

Figure 3

A. Uterine artery diameter measurement using power Doppler angiography.
B. Color Doppler image showing the uterine artery with Doppler gate placed just distal to the apparent cross-over with iliac vessels.
C. Pulsed-wave Doppler waveforms demonstrating the measurement of time-averaged intensity-weighted mean velocity in the uterine artery.
D. Measurement of the uterine artery blood flow velocities using the maximum velocity envelope.

The scale of Doppler intensity was set at maximum and the gain was optimized to avoid possible overestimation of the UtA diameter. The Q_{UtA} (ml/min) was calculated as the product of the

cross-sectional area (cm^2) of the vessel and the TAV (cm/s) x 60. The total Q_{UtA} was calculated as the sum of volume blood flow in the right and left UtA. UtA resistance (R_{UtA}) was computed as MAP/Q_{UtA}.

9.9 **Measurement of umbilical vein blood flow**

UV diameter was measured using two-dimensional ultrasonography in an insonation perpendicular to the vessel and Doppler ultrasonography was performed to measure the UV velocities in an insonation aligned with the vessel (Figure 4).

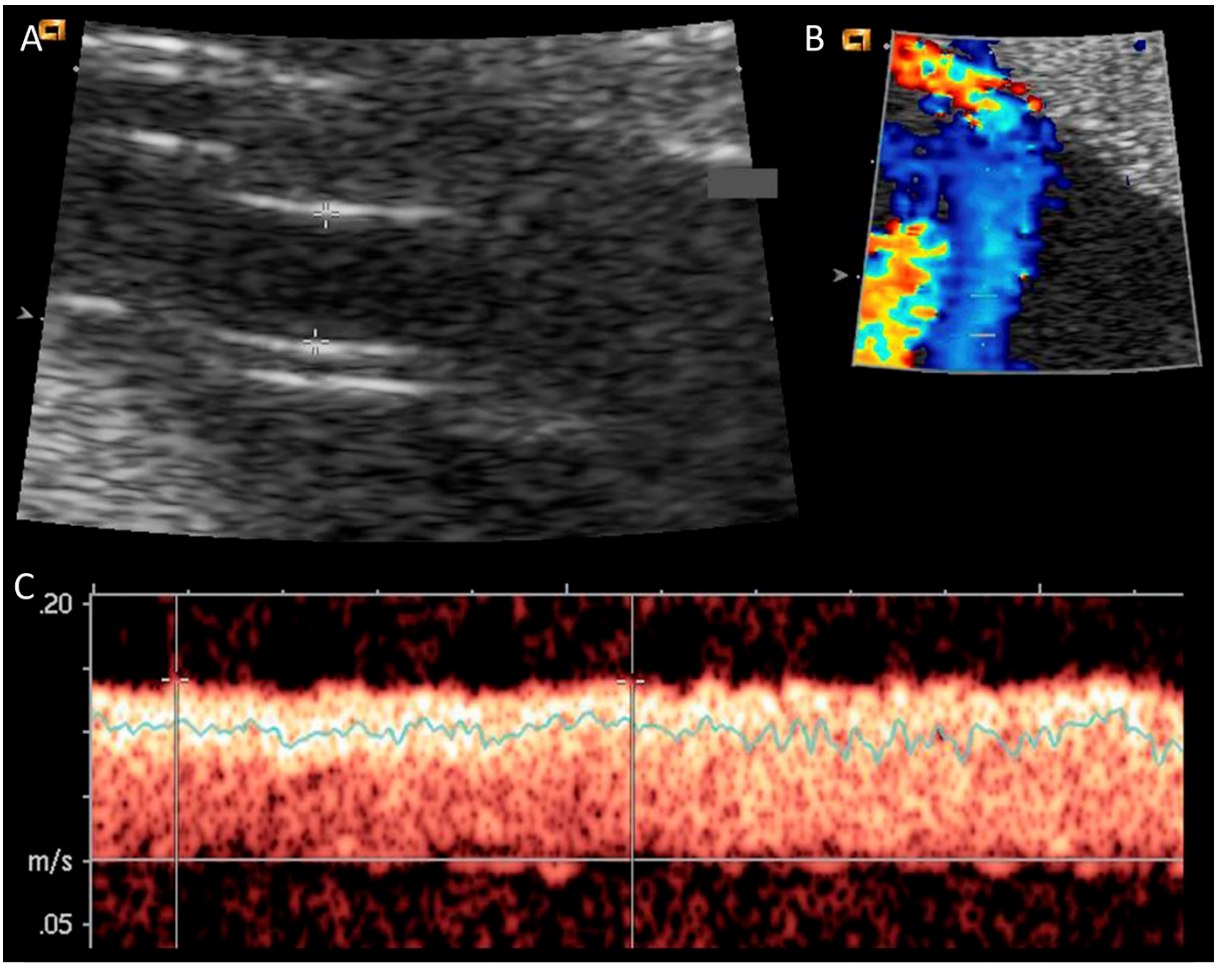

Figure 4

A. Measurement of the umbilical vein diameter.

B. Color Doppler image showing the umbilical vein and how the Doppler gate is placed to measure the flow velocity.

C. Pulsed-wave Doppler waveforms demonstrating the measurement of time-averaged intensity-weighted mean velocity in the umbilical vein.

In Study I, all UV measurements were done in a randomly selected free-floating loop of the umbilical cord, while the intra-abdominal portion of the UV vessel was examined in Study II.

Color Doppler was used to visualize the direction of blood flow and optimize the angle of insonation, which was kept close to 0˚, and always <30˚. Blood flow velocities were recorded using pulsed-wave Doppler with a wide sample volume (gate size of 5-12 mm depending on the gestational age). The high-pass filter was set at low. The UV velocities were recorded in the absence of fetal movements for 4-6 seconds with a sweep speed of 50-100 mm/s and the time-averaged maximum velocity (V_{max}) was measured by manually tracing the velocity envelope over two seconds. The mean velocity (V_{mean}) was calculated as 0.5 x V_{max} (cm/s) assuming a parabolic velocity profile in the intra-abdominal straight portion of the UV[120] (Study II) or automatically computed as TAV (cm/s) by the software of the ultrasound system (Study I). The inner diameter of the UV was measured in the straight portion of a frozen, zoomed B-mode ultrasound image, in the same portion of the vessel from where the blood velocity measurements were obtained. An average of three measurements was recorded. In Study I, the Q_{uv} (ml/min) was calculated as the product of the cross-sectional area (CSA) of the vessel and the TAV x 60, where CSA = π x (UV diameter/2)^2. In Study II, the Q_{uv} (ml/min) was calculated as the product of V_{mean} (cm/s) and CSA x 60. Normalized Q_{uv} (ml/min/kg) was calculated as Q_{uv}/EFW (kg).

9.10 **Umbilical artery Doppler velocimetry**

Blood flow velocity waveforms of the UA were obtained from the free-floating loop of the umbilical cord using pulsed-wave Doppler optimizing the insonation with simultaneous use of color Doppler. The angle of insonation was always kept <15˚ and angle correction was used if the angle was not zero. To ensure Doppler recording of the spatial maximum blood velocity, an expanded sample gate of 5-12 mm was used depending on gestational age. The high-pass filter was set at low. The blood flow velocities (i.e. PSV, EDV, and TAMXV) and fetal HR were measured online using the maximum velocity envelope recorded over the cardiac cycle. An average of three consecutive cycles were used for statistical analysis. The PI and RI were automatically computed by the software of the ultrasound system. The Doppler indices were calculated from the recorded velocities as follows: PI=(PSV–EDV)/TAMXV,[62] RI=(PSV–EDV)/PSV,[63] and S/D ratio=PSV/EDV.[64]

9.11 **Middle cerebral artery Doppler velocimetry**

The middle cerebral artery (MCA) was imaged using color Doppler and blood velocity waveforms were obtained using pulsed-wave Doppler, placing the Doppler gate at the proximal third of the distance from its origin at the circle of Willis. The insonation angle was kept close

to 0˚, and always <15˚. The blood flow velocities (PSV, EDV and TAMXV) were measured, and the PI and RI were automatically computed by the software of the ultrasound system, all according to the description and formulas given above. The average value from three consecutive cycles was used. The CPR was calculated as MCA PI/UA PI.

9.12 **Pregnancy outcomes**

All women had a regular antenatal follow-up according to local guidelines. Following delivery, the information on the course and outcome of pregnancy, including any maternal or fetal complications, gestational age at delivery, mode of delivery, birth weight, placental weight, neonatal sex, Apgar scores, umbilical cord blood acid-base status, and neonatal outcome, including transfers to neonatal intensive care unit, was obtained from the electronic medical records. On the second day post-partum, a pediatrician routinely examined all neonates prior to discharge.

9.13 **Statistical analysis**

Statistical Software for Social Sciences for windows, version 22 (IBM SPSS Statistics, Chicago, IL, USA) was used for the analysis of the cross-sectional data. Data were checked for normality using Shapiro-Wilk test and parametric tests were used for comparing groups only after verifying normal distribution. For the variables where the latter was not the case, logarithmic or power transformations were utilized as appropriate to best meet the criteria of normal distribution. Comparison between the two groups was performed using independent samples t-test for the continuous variables and chi-square test for categorical variables. Association between parametric variables was tested using Pearson correlation. Statistical analysis of the longitudinal data in Study II and Study III were performed with Statistical Analysis Software version 9.3 (SAS Institute Inc., Cary, NC, USA). All numerical variables not being normally distributed were transformed to achieve normal distribution. The best transformation for each variable was determined using the Box-Cox regression. Fractional polynominals were used to obtain best-fitting curves in relation to gestational age for each variable, accommodating for nonlinear associations. We used multilevel modeling to construct gestational age-specific reference percentiles from each fitted model according to Royston and Altman.[225] The comparison of UA Doppler indices between male and female fetuses was performed for each gestational week by including a cross-product term between sex and age in the above-mentioned multilevel models. The level of statistical significance was set at a two-tailed p-value of <0.05.

10 SUMMARY OF RESULTS

Paper I

The function of the placenta and its circulation plays a crucial role in maintaining offspring health both in short- and long-term. Doppler-derived parameters describing placental circulation, in particular the UA PI, are commonly used in clinical settings to assess placental function, and thereby, fetal wellbeing. Recent studies suggest that there are sex differences in feto-placental blood flow[197, 199, 200] but yet this has been scarcely investigated and mostly not taken into account while assessing and monitoring fetal wellbeing.

In a prospective cross-sectional study of 520 healthy pregnant women at 22^{+0}-24^{+0} weeks of gestation, we examined blood flow velocities of the MCA, UA, UV and UtA using Doppler ultrasonography. Based on the mean blood flow velocities and measured diameters of the UV and UtA, the Q_{uv} and Q_{UtA} were calculated.

We found statistically significant (p=0.008) sex-specific differences in UA PI, with female fetuses having higher PI (1.19) compared with male fetuses (1.15) at 22^{+0}-24^{+0} weeks. There were no statistically significant differences neither in maternal baseline characteristics nor in MCA PI, CPR, $Q_{uv,}$ Q_{uv} normalized for EFW, UtA PI, Q_{UtA}, or R_{UtA} between the two groups. Neonatal outcomes, expressed as 5-min Apgar score, UA pH, UA base excess, presence of meconium stained liquor, mode of delivery and admission to neonatal intensive care unit, were the same for the two sexes. The mean birth weight and placental weight of female infants (3504 g and 610 g) were significantly (p=0.0005 and p=0.039) lower than that of male infants (3642 g and 634 g) at delivery. The birth weight/placental weight ratios were similar.

At 22-24 weeks of gestation, we have demonstrated sex differences in UA PI, a surrogate for placental vascular resistance, which could indicate sexual dimorphism in placental circulation and possible differences in placental function.

Paper II

The importance of sex-specific data analysis has increasingly been brought to attention over the last few years. The oxygenated nutrient-rich blood is provided to the fetus through a single umbilical vein. As umbilical circulation is a closed circuit, the Q_{uv} may be used a proxy for placental perfusion and function. Reference values for UV blood flow velocities, diameter and Q_{uv} have been published previously, but these have not been scrutinized for fetal sex differences.

In a prospective longitudinal study of 179 singleton low-risk pregnant women and their fetuses (87 male and 92 female), UV diameter and V_{max} were serially measured by ultrasonography at the intra-abdominal portion of the UV during 19 to 41 weeks' gestation (a total of 746 observations). Q_{uv} was calculated and normalized for EFW.

We constructed sex-specific reference intervals based on longitudinal data but found no statistically significant differences between the two groups in any of the UV parameters examined. However, the Q_{uv} normalized for EFW appeared to have differences in the temporal development pattern when comparing male and female fetuses. From 20 to 24 weeks, and again from 32 weeks onward, male fetuses had lower blood flow. The opposite trend was seen from 24 to 32 weeks, with slightly lower blood flow in female fetuses. There were no differences in maternal baseline characteristics, neonatal outcomes, fetal weight, placental weight or birth weight/placental weight ratio between the two groups.

Paper III

Longitudinal reference ranges for UA Doppler indices calculated from serial measurements have been published previously, but these do not take into account possible sex differences. However, sexual dimorphism has been described in both placental size and function. In this prospective longitudinal study, we aimed to examine whether sex of the fetus may have an impact on the clinically important UA Doppler waveform, and to consequently establish sex-specific reference ranges for the UA Doppler indices.

We examined 294 singleton low-risk pregnancies and their fetuses (152 male and 142 female), with a total of 1261 observations, at approximately 4-weekly intervals during 19-40 weeks of gestation. Color-directed pulsed-wave Doppler ultrasonography were used to obtain UA Doppler indices from a free loop of the umbilical cord. Sex-specific reference ranges for the fetal HR, UA PI, UA RI and UA S/D ratio were then calculated for the last half of pregnancy.

The UA Doppler indices and the fetal HR were significantly associated with gestational age ($P<0.0001$). There was an association between UA Doppler indices and fetal HR ($P<0.0001$). Female fetuses had significantly ($P<0.05$) higher values for UA PI (range 2.1-4.2%), RI (range 1.7-3.3%) and S/D ratio (range 4.0-8.1%) from 20^{+0} weeks to 32^{+6}, 36^{+6} and 35^{+6} weeks, respectively, but these differences then faded towards term. When comparing the mean values for fetal HR between the two groups, they were similar from 20^{+0} to 25^{+6} weeks, but a divergent trend was observed thereafter with female fetuses showing increasingly higher HR (range 0.7-2.2%). We found no sex differences in maternal baseline characteristics, neonatal outcomes, fetal weight, placental weight or birth weight/placental weight ratio between the two groups.

11 DISCUSSION

11.1 Main findings

Doppler ultrasonography of the placental circulation is widely used clinically to assess placental function and fetal wellbeing. We found significant sex differences in some important Doppler-derived parameters during the second half of pregnancy. At 22^{+0}-24^{+0} gestational weeks, female fetuses had significantly higher UA PI than male fetuses, while no such differences were found in any of the other examined Doppler-derived parameters of feto-placental and utero-placental circulation. Even if we found no significant quantitative sex differences in any of the UV Doppler-derived parameters studied during any point in the entire second half of pregnancy, we found a sex-specific qualitative difference in the developmental patterns of normalized Q_{uv}. During the same gestational period, UA Doppler indices (UA PI, RI and S/D ratio) were found to be associated with fetal HR, and female fetuses had significantly higher values for the aforementioned indices during 20^{+0}-36^{+6} gestational weeks, but not later. Mean fetal HR were similar between the two groups from 20^{+0}-25^{+6} weeks of gestation, but a significant divergent trend was observed thereafter with female fetuses showing increasingly higher HR.

11.2 Interpretation of results

11.2.1 *Sex differences in umbilical artery Doppler indices*

We found significantly higher UA PI among female fetuses compared to males, both in the cross-sectional study (Paper I), and in the longitudinal study (Paper III). The UA Doppler indices, in particular UA PI, are widely implemented as a surrogate measure for placental vascular resistance. It has been demonstrated that the UA PI decreases with advancing gestational age[136] and increasing number of small arterioles in the placental vascular bed.[139] Increased PI in the UA has been shown to correlate with morphologic alterations in the placenta (reduced vascularity) and impaired placental function.[138]

The resistance at the arteriolar level of the microcirculation is the main determinant of resistance to blood flow in a vascular bed. However, this association is not uniform,[226] as previously reported in sheep experiments.[82, 227] Although injected microspheres caused embolization with subsequent reduction in vascular cross-section and increased PI, similar effect on the cross-sectional area caused by Ang II did not increase the PI. The latter could even decrease the PI while vascular resistance increased. This may be caused by differences in vessel properties, consequently influencing the wave reflection, and thereby the arterial waveform.[83, 228]

The marginally, but statistically significant, higher UA PI we discovered in female fetuses compared to male could not be translated into reduced placental function or differences in clinical outcomes. The mechanisms responsible for the detected sex differences in UA Doppler indices are not apparent. One explanation could be difference in vascular tone, as reported in a study where male neonates born prematurely at 24-28 weeks demonstrated more peripheral vasodilatation compared to their female counterparts.[210] It has been shown that women pregnant with male fetuses have higher angiotensin (Ang) 1-7 to ANG II ratio in the second trimester.[229] Ang II is vasoconstrictor, while Ang 1-7 mediate vasodilatation, whereby relatively increased vasodilatation of placental vessels could explain the reduced UA PI, RI, and S/D ratio observed in male fetuses.

Published studies exploring sex differences in UA Doppler indices are very limited, and mostly cross-sectional. Our study is in line with a previous cross-sectional study reporting increased UA PI in female fetuses during the transition between second and third trimester (28-34 weeks),[199] and that this divergence had faded off towards term.[200] Our cross-sectional (Paper I) and longitudinal (Paper III) studies that had different but comparable study populations, showed concordant results regarding sex differences in UA PI. When the mean values for each respective gestational age were grouped together, we found near identical longitudinal values of UA PI compared to a much cited previously published report on longitudinal reference ranges for UA Doppler indices.[136] In that study, the effect of neonatal sex on UA PI was indeed analyzed, but no statistically significant differences were found, perhaps due to inadequate sample size with insufficient power to detect such differences.

11.2.2 *Sex differences in umbilical vein blood flow*

Considering well described sexual dimorphism in birth weight and placental weight,[149] as well as certain other aspects of placental function,[162, 169] it is surprising that our longitudinal study scrutinizing sex differences in feto-placental volume blood flow (Paper II) did not show significant differences in quantitative terms. In fact, we found no statistically significant sex differences in any of the UV parameters we examined (UV diameter, TAMXV, absolute Q_{uv} or normalized Q_{uv}). On the other hand, we found a qualitative difference with a biphasic pattern of sequential changes in normalized Q_{uv}, with cross-overs at 24 and 32 gestational weeks, males demonstrating a trend towards lower Q_{uv} normalized for EFW from 32 weeks onward. Prior et al. first reported a difference in fetoplacental blood flow related to sex, finding a significantly lower normalized Q_{uv} in male fetuses at term.[200] Contrary to our study, this was a cross-sectional study, and the participants were examined on admission to labor ward, possibly in latent

stage/early phase of labor. This could have influenced the measurements. Our finding of no significant sex differences in UV blood flow during the second half of pregnancy could also be related to a possible inadequate sample size to demonstrate differences of small magnitude.

11.2.3 *Sex differences in fetal heart rate*

In our study, we found relatively higher fetal HR (range 0.7-2.2 %) among female fetuses, from 26 weeks' gestation and growing more prominent as the length of pregnancy progressed (Study III). This is in concordance with previously published results from other groups who also found higher fetal HR among female fetuses during the second half of pregnancy,[190, 193-195] despite the differences in study design (cross-sectional vs. longitudinal) and methods used for measuring fetal HR (Doppler velocimetry vs. computerized CTG). However, the sex difference in fetal HR reported by all these studies are of small magnitude and are unlikely to be clinically relevant.

A significant inverse correlation between the UA Doppler indices and fetal HR has previously been reported in sheep experiments.[228] The UA Doppler indices have been found to decrease when the HR increases. In our study, we found both relatively increased fetal HR and increased values for the UA Doppler indices in female fetuses. Contrary to what could be expected from the sheep experiments, we found that the relative sex differences in HR increased with advancing pregnancy, while the opposite occurred for the Doppler indices. When the UA Doppler indices were adjusted for fetal HR, the effect size increased through a more prominent quantitative sex difference in the same indices.

Differences in fetal HR related to sex could have its origin in divergent hormone levels and autonomic nervous system maturation between male and female fetuses.[190] The higher heart rate variability, heart rate complexity and more elevated catecholamine levels reported in female fetuses[191, 192, 230] supports this assumption.

11.3 **Strengths and weaknesses**

All our studies had a prospective design and only a limited number of experienced operators performed the measurements under similar conditions. A relatively large number of participants and observations, both for the cross-sectional study (Paper I) and the two longitudinal studies (Paper II and III), assure sufficient power to test our hypothesis. This allowed us to establish robust and valid sex-specific reference charts (Paper II and III). For this purpose, we chose the longitudinal rather than the cross-sectional design, as the former is superior in evaluating the

gestational age associated physiological development pattern. Outcome data was secured for the entire study population, as there were no losses to follow-up.

A major weakness of our study is that for the longitudinal studies we were able to recruit and examine participants only after 18-19 gestational weeks, leaving us in uncertainty as to when in early pregnancy some of the described sex differences emerge. This is due to the fact that first trimester scan is not offered routinely in Norway in low-risk pregnancies. It could also be argued that the non-random recruitment of the study subjects could cause selection-bias. However, all pregnant women in the region attend the same hospital.

Technical aspects related to Doppler velocimetry and precision of Q_{uv} measurements could also be considered as limitations. However, any possible inaccuracy in blood flow measurements would apply to the same extent to both sexes. Furthermore, the methods used to obtain the measured parameters have acceptable accuracy and reproducibility in clinical settings (see the respective sections above). This all sums up to a high degree of internal validity of our results.

We only included presumably uncomplicated pregnancies, and we do not know if our results are transferable to complicated pregnancies. Further, our study population was relatively homogenous both in terms of socioeconomic status as well as ethnicity and may not be representative of a multi-ethnic population with diverse socioeconomic backgrounds. Our assumption is that our results have a high degree of generalizability to normal pregnancies in Nordic and White European populations.

11.4 **Clinical application**

Our study did not find any significant sex differences in placental volume blood flow, leading us to the conclusion that using sex-specific reference intervals of Q_{uv} in clinical practice would not further enhance the prediction of pregnancy complications. Epitomized, our findings bring awareness to a sex-related bias in regard to placental function, *in utero* development and maturation of the feto-placental unit. However, use of sex-specific reference ranges of UA Doppler indices may help to further refine diagnosis, and they could be used in clinical practice for serial surveillance and prediction of high-risk pregnancies. A recent report analyzing a considerably large longitudinal data-set has also demonstrated sex differences in CPR,[201] further reinforcing the possibility of refining antenatal fetal surveillance using sex-specific reference values.

11.5 **Future research**

Future studies should explore possible sex differences in the other circulatory compartments including fetal cerebral and liver circulation. Sex differences in fetal systemic venous blood flow also needs to be investigated. Sex-specific reference charts for several Doppler indices should be constructed using longitudinal design and larger sample size. Such investigations could be performed both in low-risk and high-risk pregnancies and linked to clinical outcomes. They should aim at predicting pregnancy complications, and seek out whether sex-specific reference values perform better in high-risk pregnancies compared to the sex-neutral reference values now in use.

12 **CONCLUSIONS**

There are significant sex differences in the developmental trajectory of UA Doppler-derived parameters during the second half of physiological pregnancy. Throughout this period female fetuses demonstrate higher values for the UA Doppler indices compared to male fetuses, but these differences are leveled out towards term. For the corresponding UV Doppler-derived parameters no such statistically significant sex differences were found, but there were indications of a diverging pattern of gestational age-dependent temporal changes in $Q_{uv.}$ The sum of these findings might reflect temporal sexual dimorphism in placental circulation associated with the maturation of the feto-placental unit.

Sex-specific longitudinal reference ranges for the most commonly used Doppler-derived parameters of both UA and UV have been established, believing that it may refine the surveillance of risk pregnancies.

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APPENDIX

Paper I-III

Paper I

Widnes C, Flo K and Acharya G.

Exploring sexual dimorphism in placental circulation at 22-24 weeks of gestation: A cross-sectional observational study.

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Exploring sexual dimorphism in placental circulation at 22–24 weeks of gestation: A cross-sectional observational study

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ABSTRACT

Introduction: Placental blood flow is closely associated with fetal growth and wellbeing. Recent studies suggest that there are differences in blood flow between male and female fetuses. We hypothesized that sexual dimorphism exists in fetal and placental blood flow at 22–24 weeks of gestation.
Methods: This was a prospective cross-sectional study of 520 healthy pregnant women. Blood flow velocities of the middle cerebral artery (MCA), umbilical artery (UA), umbilical vein (UV) and the uterine arteries (UtA) were measured using Doppler ultrasonography. UV and UtA diameters were measured using two-dimensional ultrasonography and power Doppler angiography. Volume blood flows (Q) of the UV and UtA were calculated. Maternal haemodynamics was assessed with impedance cardiography. UtA resistance (R_{uta}) was computed as MAP/Q_{uta}.
Results: UA PI was significantly ($p = 0.008$) higher in female fetuses (1.19 ± 0.15) compared with male fetuses (1.15 ± 0.14). MCA PI, cerebro-placental ratio (MCA PI/UA PI), Q_{uv}, UtA PI, Q_{uta} and R_{uta} were not significantly different between groups. At delivery, the mean birth weight and placental weight of female infants (3504 g and 610 g) were significantly ($p = 0.0005$ and $p = 0.039$) lower than that of the male infants (3642 g and 634 g).
Discussion: We have demonstrated sexual dimorphism in UA PI, a surrogate for placental vascular resistance, at 22–24 weeks of gestation. Therefore, it would be useful to know when this difference emerges and whether it translates into blood flow differences that may impact upon the fetal growth trajectory.

1. Introduction

There is growing evidence for sex-specific differences in fetal growth and adaption to the intrauterine environment [1]. It has been shown that males are more at risk for various adverse outcomes such as premature birth [2,3], fetal distress during labour [4], poor neonatal outcome [5] and early neonatal death than females [1]. It is often referred to as "the male disadvantage" [6]. In pregnancies complicated by preeclampsia and intrauterine growth retardation (IUGR), perinatal mortality and morbidity are worse for males than for females [7]. Fetal sex also influences placental gene expression and inflammatory response [8,9] resulting in differences in placental function, with the potential of a sex-bias for certain diseases later in life [10,11].

Placental circulation is closely associated with fetal growth and wellbeing [12]. Doppler ultrasonographic measurements of feto-placental and utero-placental blood flow have been used extensively to identify and monitor pregnancies at risk for adverse outcomes, such as preeclampsia and IUGR. One recent study demonstrated differences in middle cerebral artery (MCA) blood flow velocity waveforms and umbilical vein (UV) volume blood flow between male and female fetuses at term [13]. However, the measured differences were not related to fetal outcomes. Studies on ductus venosus Doppler in the first trimester have shown conflicting results regarding sex differences [14–16].

Umbilical artery (UA) blood flow velocity waveforms are used to assess fetal wellbeing in clinical practice, and increased pulsatility index (PI) in the UA has been shown to correlate with morphologic alterations in the placenta (reduced vascularity) and impaired

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placental function [17].

Measurements of the UA and MCA blood flow velocities are used to identify redistribution of blood flow in favour of the brain, i.e. "brain-sparing" [18] in IUGR fetuses. Furthermore, the cerebro-placental ratio (CPR) can be useful in the detection of subtle growth restriction [18]. The UV volume blood flow has been reported to be reduced in fetuses subsequently developing IUGR even before the UA PI is changed [19]. On the maternal side the uterine artery (UtA) PI is increased in pregnancies at risk of preeclampsia and IUGR [20]. Although hemodynamic assessment of fetal and placental circulations are routinely used to make clinical decisions, sex differences in the measured Doppler parameters have been scarcely investigated and are not taken into account.

The objective of this study was to explore sexual dimorphism in fetal and placental circulation in uncomplicated pregnancies at 22–24 weeks of gestation. We tested the null hypothesis that no sex differences exist in the Doppler-derived haemodynamic parameters of feto-placental and utero-placental circulation in normal pregnancy when the placentation has fully established.

2. Methods

2.1. Participants

This is a part of an ongoing prospective cross-sectional study on maternal haemodynamics and feto-placental circulation in normal and complicated pregnancies at the Department of Obstetrics and Gynaecology, University Hospital of North Norway, Tromsø, Norway. All pregnant women ≥18 years of age attending the routine antenatal ultrasound screening at 17–20 weeks of gestation were informed about the study and invited to participate. A total of 584 healthy pregnant women with uncomplicated singleton pregnancy who consented to participate in this study were examined once between 22^{+0} and 24^{+0} weeks of gestation. The gestational age was based on pregnancy dating from second trimester ultrasound biometry of fetal head. The following participants were subsequently excluded due to pregnancy complications: 41 with pre-eclampsia, 20 who delivered preterm, one that had placental abruption and two with IUGR. Thus a total of 520 women were included in the final analysis. The research protocol was approved by the Regional Committee for Medical Research Ethics (ref. no. 5.2005.1386) and an informed written consent was obtained from each participant.

2.2. Measurements

An ultrasound system with a 6-MHz curvilinear transducer (Acuson Sequoia 512, Mountain View, CA, USA) was used for ultrasonography. All participants were examined in the supine semi-recumbent position. Two experienced clinicians (KF and CW) performed all the ultrasonographic examinations and the sex of the fetus was not identified or acknowledged. Only one clinician performed the measurements per patient. Estimated Fetal weight (EFW) was computed based on the fetal biometry using the Hadlock formula [21], and amniotic fluid index (AFI) was measured. Blood flow velocity waveforms were obtained from the UA, UV, MCA and UtA using pulsed-wave Doppler keeping the angle of insonation close to 0°, and always less than 30°. A large sample volume (Doppler gate 5–10 mm) was used to include the entire cross-section of the insonated blood vessels. The blood flow velocities were measured using the maximum velocity envelope recorded over the cardiac cycle. The pulsatility index (PI) was calculated as: (peak systolic velocity − end-diastolic velocity)/time-averaged maximum velocity. Measurements from the UA and UV were obtained from a free-floating loop of the umbilical cord. The UtA measurements were obtained just proximal to the apparent crossing of the external iliac artery seen on color Doppler. The MCA was imaged using color Doppler and measured by placing the Doppler gate at the proximal third of the distance from its origin at the circle of Willis. The average value from three consecutive heart cycles was used. The CPR was calculated as MCA PI/UA PI.

The UV and UtA diameters were measured on the same portion of the vessel from where the blood velocity measurements were obtained, using two-dimensional ultrasonography and power Doppler angiography, respectively. For the latter the scale of Doppler intensity was set at maximum and the gain was optimised to avoid possible overestimation of the UtA diameter. The volume blood flow (Q) of the UV and UtA was calculated as the product of the cross-sectional area (CSA) of the vessel and the time-averaged intensity weighted mean velocity (TAV). The total Q_{uta} was calculated as the sum of volume blood flow in the right and left UtA.

The reproducibility of the Doppler parameters studied has been extensively evaluated and reported previously. We have reported the intra-observer coefficient of variation (CV) for UA PI to be 10.5% (95% CI, 9.9%–11.1%), based on three sets of 513 observations [22], and the mean inter-observer CV between six operator pairs was reported to be 8.4% by Gudmundsson et al. [23]. We have reported the intra-observer CV of 11.6% (95% CI, 4.7–7.3%) for the left Q_{uta} and 13.2% (95% CI, 10.1–15.7%) for the right Q_{uta} [24]. For the Q_{uv}, Barbera et al. evaluated the intra- and inter-observer variations and report to be 10.9% and 12.7%, respectively [25], whereas Figueras et al. have reported the intra-observer intra-class correlation coefficient (ICC) of 0.55 (95% CI, 0.35–0.7) and inter-observer ICC of 0.6 (95% CI, 0.4–0.74), respectively [26].

To measure maternal stroke volume, heart rate and mean arterial blood pressure (MAP) impedance cardiography (ICG) (Phillips Medical Systems, Androver, MA, USA) was used, as described previously [24]. The cardiac output (CO) and the systemic vascular resistance (SVR) were automatically calculated. The body mass index (BMI) was calculated as height/weight^2 using the current weight, and the body surface area (BSA) was computed using the Du Bois formula [27]. UtA resistance (R_{uta}) was computed as MAP/Q_{uta}. The normalized placental volume blood flow was calculated as Q_{UV}/EFW. Following delivery, information on the course and outcome of the pregnancy was recorded from the woman's electronic medical record.

2.3. Statistical analysis

Continuous variables are presented as means ± SDs or median (range) and categorical variables as number (%), as appropriate. Data were checked for normality using Shapiro-Wilk test and parametric tests were used for comparing groups only after verifying normal data distribution. Comparison between the two groups was performed using independent samples t-tests (IBM SPSS Statistics, Version 22) for continuous variables and chi-square tests for categorical variables. Association between parametric variables was tested using Pearson correlation. A two-tailed p-value ≤0.05 was considered significant.

3. Results

The baseline characteristics of the study population, including pregnancy and neonatal outcomes, are listed in Table 1. There were no statistically significant differences between the two groups in maternal characteristics such as age, BMI, parity, previous caesarean section, or previous history of preeclampsia and hypertension. At delivery, the mean birth weight and placental weight of female infants (3504 g and 610 g) were significantly ($p = 0.0005$ and $p = 0.039$) lower than that of the male infants (3642 g and

Table 1
Baseline characteristics for the study population.

Parameter	Female (n = 260)	Male (n = 260)	P-value
Maternal			
Age (years)	29 (range 18–44)	30 (range 18–41)	0.44
Body mass index (Kg/m^2)	25.98 ± 4.05	25.81 ± 3.94	0.63
Nulliparous	139 (53.5)	126 (48.5)	0.25
Previous caesarean section	18 (6.9)	17 (6.5)	0.86
Pre-eclampsia in previous pregnancy	22 (8.5)	13 (5.0)	0.12
Hypertension in current pregnancy	8 (3.1)	10 (3.8)	0.63
Fetal			
Gestational age at birth (days)[a]	281 (range 259–298)	283 (range 261–300)	0.012
Birth weight (g)	3504.25 ± 434.90	3641.75 ± 454.67	0.0005
Placental weight (g)	610.10 ± 128.84	634.17 ± 130.55	0.039
Fetal-placental ratio	5.89 ± 0.93	5.91 ± 1.00	0.865
5- minute Apgar score	10 (5–10)	10 (4–10)	0.343
Umbilical artery pH	7.23 ± 0.09	7.23 ± 0.09	0.735
Umbilical artery base excess (mmol/L)	−4.94 ± 3.50	−4.41 ± 3.24	0.207
Meconium stained liquor	41 (16.6)	39 (16.0)	0.853
Admission to NICU[b]	11 (4.2)	18 (6.9)	0.181
Mode of delivery			
Normal	212 (84.1)	212 (81.3)	0.41
Vacuum/forceps	15 (6.0)	19 (7.5)	0.48
Caesarean section	25 (9.9)	28 (11.1)	0.66

Data are presented as n (%), median (range), or mean ± SD, as appropriate.
[a] 281 days = 40^{+1}, weeks, 283 days = 40^{+3+}· weeks.
[b] NICU, neonatal intensive care unit.

634 g). The fetal weight/placental weight ratios were similar. There was no significant difference in mean gestational age between male and female fetuses at examination (159.98 ± 3.98 vs 159.82 ± 4.31 days; p = 0.650), but there was a significant difference (p = 0.012) in the mean gestational age at birth (283 days for male fetuses and 281 days for females). We did not find any significant differences between the two groups when it came to neonatal wellbeing, represented by 5-min Apgar score, umbilical artery pH, umbilical artery base excess, presence of meconium stained liquor, mode of delivery and admission to neonatal intensive care unit (NICU), counting any admission regardless of the length of the stay.

The results for the utero- and feto-placental blood flow are summarised in Table 2. Placental volume blood flow (Q_{uv}) was similar, but the UA pulsatility index (PI) was significantly (p = 0.008) higher in female fetuses (1.19) compared with male fetuses (1.15), corresponding to the 58th and 46th percentile respectively relative to the whole population studied. The MCA PI (1.83 vs. 1.82) and the CPR were similar (1.56 vs. 1.59). There was no significant difference in the proportion of women with bilateral UtA notching (2.7% vs. 3.1%). The UtA PI, Q_{uta}, R_{uta}, and % of cardiac output distributed to the uterus were not significantly different between groups. Neither were there any differences in Q_{uv} or Q_{uv} normalized by estimated fetal weight. Scatter plots of data distribution of Q_{uv} normalized for EFW and total Q_{uta} at each gestational day during 22^{+0} to 24^{+0} weeks are presented in Fig. 1 and the correlation between UA PI and normalized Q_{uv} is presented in Fig. 2. The variation in the UA PI accounted for only 1% of the variation in placental volumetric blood flow ($r^2 = 0.01$).

4. Discussion

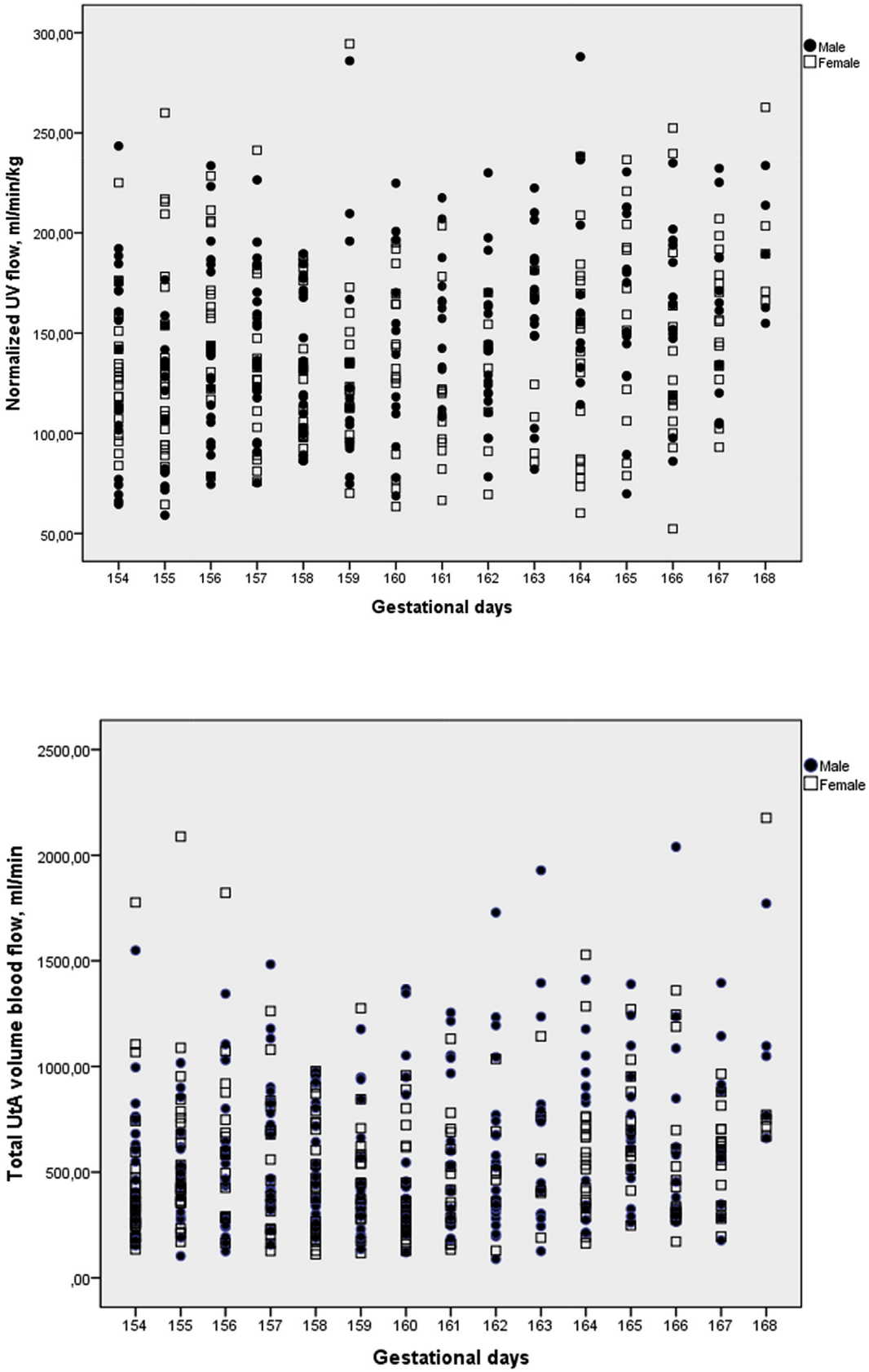
4.1. Main findings

Placenta plays an important role in mediating pregnancy outcomes and its function has an effect on long-term offspring health. Doppler-derived parameters describing placental circulation, especially the UA PI, are widely used to assess placental function in clinical settings. We found sexual dimorphism in UA PI at 22–24

Table 2
Parameters of uteroplacental- and fetoplacental blood flow measured at 22–24 weeks.

Parameter	Female (n = 260)	Male (n = 260)	P-value
Umbilical artery PI	1.19 ± 0.15	1.15 ± 0.14	0.008
Middle Cerebral artery PI	1.83 ± 0.28	1.82 ± 0.25	0.577
Cerebro-placental ratio	1.56 ± 0.30	1.59 ± 0.28	0.167
Mean Uterine artery PI	0.81 ± 0.26	0.81 ± 0.23	0.972
Mean Uterine RI	0.50 ± 0.09	0.51 ± 0.09	0.702
Bilateral UtA notching	7 (2.7)	8 (3.1)	0.793
Uterine artery resistance (mmHg/ml/min)	0.21 ± 0.13	0.19 ± 0.13	0.15
Total Uterine artery blood flow (ml/min)	543.21 ± 339.36	598.58 ± 368.60	0.081
% CO distributed to the uterus[a]	9.05 ± 5.54	9.99 ± 5.97	0.069
Umbilical venous blood flow (ml/min)	80.16 ± 27.49	84.27 ± 28.29	0.112
Umbilical venous blood flow/kg (ml/min/kg)	138.33 ± 43.60	145.99 ± 45.05	0.063
Estimated fetal weight (g)	577.04 ± 64.68	575.77 ± 61.32	0.818
Umbilical artery heart rate (bpm)	145.13 ± 6.82	145.08 ± 7.49	0.934
Maternal heart rate (bpm)	79.16 ± 10.97	78.69 ± 12.57	0.653
Gestational age at examination (days)	159.82 ± 4.31 (154–168)	159.98 ± 3.98 (154–168)	0.650

Data are presented as n (%), mean ± SD or range, as appropriate.
160 days = 22^{+6} weeks.
[a] CO, cardiac output.



154 days=22^{+0} weeks, 168 days=24^{+0} weeks

Fig. 1. Scatter plots of data distribution of UV volume blood flow normalized for EFW (top) and total uterine volume blood flow (bottom). 154days = 22^{+0} weeks, 168 days = 24^{+0} weeks.

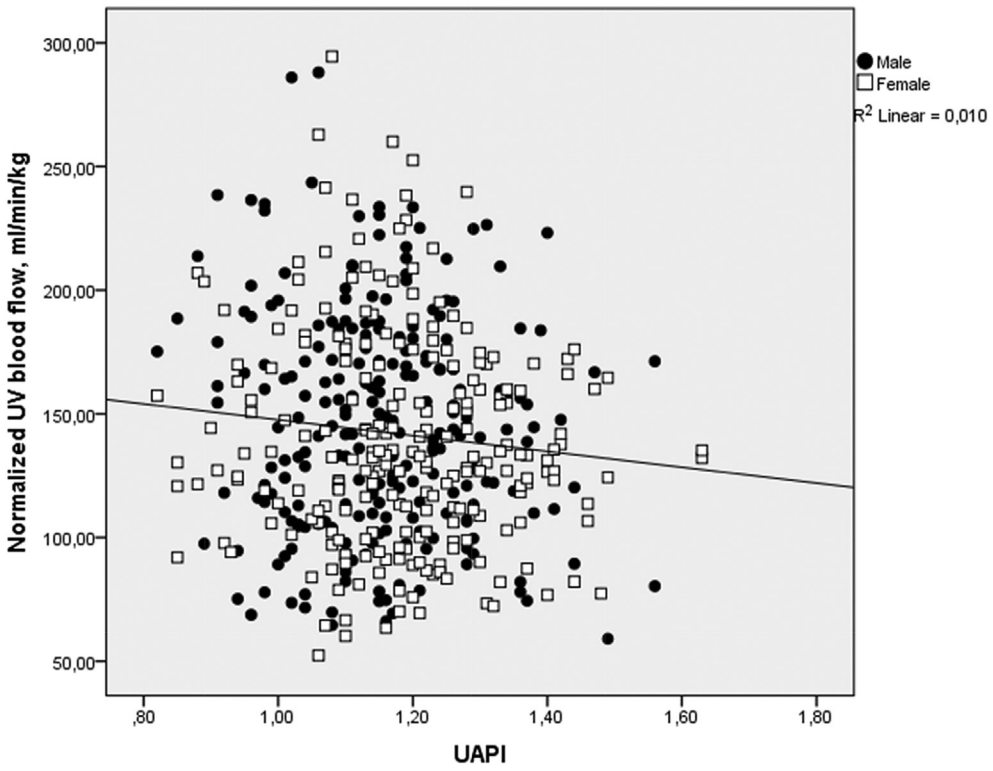


Fig. 2. Correlation plot between UA PI and normalized UV volume blood flow.

weeks of gestation. In female fetuses, the UA PI was significantly higher compared to male fetuses at this stage of pregnancy. There were no differences in the MCA PI, CPR, $Q_{uv,}$ Q_{uv} normalized for fetal weight, UtA PI, the Q_{uta}, the fraction of maternal CO distributed to the uterine arteries or EFW. The male babies had significantly higher birth weight and placental weight compared to females.

4.2. Strengths and limitations

This is a prospective study and two experienced operators performed all measurements under identical conditions. The relatively large number of participants included provides sufficient power to test the null hypothesis. Follow up was complete and we have outcome data on all participants.

Our study does have some limitations. The study is cross-sectional and we have no measurements from the first and third trimester of pregnancy. We chose 22–24 weeks of gestation as a time to explore sexual dimorphism in fetal and placental circulation because at the end of the second trimester placentation is fully established and the vascular remodelling of the uterine arteries into a high flow and low resistance pattern is thought to be complete. There are known technological limitations related to non-invasive measurement of placental blood flow, however these limitations are likely to apply to both male and female fetuses as fetal sex was recorded only after delivery and the mean gestational age at examination was similar between two groups. Observed differences in UA PI are unlikely to have been caused by methodological errors, because gestational age was based on second trimester ultrasound biometry. Some studies have shown that the gestational age of the female fetuses could be potentially underestimated by biometry [28,29], in which case the female fetuses of the same gestation would be expected to have lower UA PI compared to male fetuses reducing the magnitude of observed difference.

As this study included only uncomplicated pregnancies with appropriately grown fetuses, we do not know if the observed differences in UA PI between sexes are present or more pronounced in pregnancies destined to develop complications, such as pre-eclampsia, IUGR, stillbirth, or in pregnancies with pre-existing diabetes. Furthermore, although statistically highly significant, the mean difference in PI between male and female fetuses was small in absolute terms and it might not be clinically significant. However, our finding adds to the growing knowledge that there is a physiological sexual dimorphism in placental function and therefore sex needs to be taken into account when clinically evaluating placental function.

4.3. Interpretation

We found higher UA PI among female fetuses compared to males. UA PI is often used as a surrogate for placental vascular resistance/impedance. However, the UA PI does not always correlate with vascular resistance [30]. Animal experiments have shown that ANG II-induced vasoconstriction with subsequent increase in resistance results in a decrease or unchanged PI [31,32]. Vascular resistance (R) is a ratio between mean pressure (P) and mean flow (Q); i.e. $R = P/Q$. Therefore, placental vascular resistance depends on UA mean arterial pressure and Q_{uv}. We found no difference in Q_{uv} between male and female fetuses, but it is not known whether sex differences in blood pressure exist at 22–24 weeks of gestation. However, there appears to be an inverse correlation between birth weight and systolic BP later in life [33]. According to Poiseuille's law $R = 8L\eta/\pi r^4$, and an increase in blood viscosity (η) or the length of the vessel (L) or a decrease in its radius (r) increase the resistance to flow. The UA PI could be affected by physical properties of umbilical arteries including their elastic properties, length and diameter.

Female fetuses have a slightly shorter umbilical cords compared to male fetuses at 22–24 weeks (33.3–35.3 mm versus 34.6–36.5 mm) [34], but that would be expected to reduce the vascular resistance and thus the PI. The main determinant of resistance in a vascular bed is the resistance at the arteriolar level of the microcirculation. The UA PI is shown to decrease with advancing gestation [22] and with increasing number of small arteries in the placental vascular bed [35]. Therefore, the observed difference in UA PI between male and female fetuses could be explained by a more developed placental vasculature among male fetuses at 22–24 weeks.

Our results for the UA PI, MCA PI and CPR measured at 22–24 weeks of gestation are similar to that reported by other studies (UA PI 1.19 to 1.15 vs. 1.19 to 1.15, MCA PI 1.83 to 1.82 vs. 1.69 to 1.78, CPR 1.56 to 1.59 vs. 1.52 to 1.63) [22,36]. We found comparable values for the UtA PI (0.81 vs. 0.79 to 0.76) [37], R_{uta} (0.21–0.19 vs. 0.26 to 0.23 mmHg/ml/min) [24] and Q_{uta} (543–598 vs. 513–669 ml/min) [38] as previously reported. Bilateral notching was less frequent than previously reported (2.7%–3.1% vs. 9.3%) [39], but in that study women with preeclampsia and IUGR were not excluded. One recently published study found higher UtA PI (represented as a gestational-age-adjusted Z-score, $P < 0.001$) in uncomplicated pregnancies with male fetuses in the second trimester [40], which is different from our results at 22–24 weeks.

Prior et al. found lower MCA PI, lower MCA PSV, lower UV flow velocity and lower normalized Q_{uv} among male fetuses compared to females at term [13]. Reduced Q_{uv} could explain the tendency for lower MCA PI suggesting brain-sparing [41] before clinical evidence of placental insufficiency [42]. We did not find similar sex differences in MCA and UV Doppler at mid gestation, but these differences might develop later in pregnancy. Our finding of similar Q_{uv} among male and female fetuses at 22–24 weeks is also consistent with no sex difference in EFW at this gestation.

Studies have shown that the female placenta responds to adverse environment with more gene alterations than the male placenta [43]. Furthermore, a study on pregnancies complicated by maternal asthma showed that the growth of female fetuses is reduced compared to the male fetuses, but during acute exacerbations of maternal disease the males are more at risk of compromise [43]. Although not significant, we found a tendency towards a higher Q_{UV}/kg estimated weight among male fetuses (Table 2), which is consistent with the observed difference in birth weight. This could indicate that male fetuses prioritize growth, whereas the females grow slower, and this might lead to better adjustment ability to unfavourable conditions among female fetuses.

Steier et al. compared the Q_{uta} between pregnancies with male and female fetuses during the second and third trimesters, and found no difference in the second trimester, but a significantly higher Q_{uta} among pregnancies with female fetus during the third trimester [44]. This supports our finding of no difference in Q_{uta} at mid gestation, and it could indicate that important sex-specific fetal adjustments occur during the second half of gestation. hCG has been shown to be associated with placental growth, and is produced from the time of implantation. Steier et al. did not find significant sex differences during the first and second trimesters, but they found higher hCG values in pregnancies with female fetuses at 35 weeks [45].

In our study, the difference in UA PI at 22–24 weeks of gestation was small, but highly significant. The lower UA PI among males indicates a better placental function and growth potential, suggesting that male fetuses are not disadvantaged at this gestation. However, faster placental growth may be associated with faster placental maturity making male fetuses more vulnerable in late gestation.

5. Conclusion

We have demonstrated sexual dimorphism in UA PI, a surrogate for placental vascular resistance, at 22–24 weeks of gestation. Therefore, it would be useful to know when this difference emerges and whether it translates into blood flow differences that may impact upon the fetal growth trajectory. A high UA PI is known to be associated with placental dysfunction. Therefore, differences in UA PI between male and female fetuses should be taken into account when evaluating fetal wellbeing using Doppler ultrasonography and clinical relevance of this finding should be further investigated. Longitudinal studies are needed to establish sex-specific reference ranges for placental circulation.

Contributors

I declare that I participated in the *study conception and design, recruitment of the participants, collection of data, performing the ultrasound examinations and statistical analysis, interpretation of the results and manuscript writing* and that I have seen and approved the final version. I have no conflict of interest.

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I declare that I participated in the *recruitment of the participants, collection of data, performing the ultrasound examinations, interpretation of the results and manuscript writing* and that I have seen and approved the final version. I have no conflict of interest.

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I declare that I participated in the *study conception and design, interpretation of the results and manuscript writing* and that I have seen and approved the final version. I have no conflict of interest.

Ganesh Acharya

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.placenta.2016.11.005.

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Paper II

Widnes C, Flo K, Wilsgaard T, Odibo AO and Acharya G.
Sexual dimorphism in umbilical vein blood flow during the second half of pregnancy: A longitudinal study.
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Sexual Dimorphism in Umbilical Vein Blood Flow During the Second Half of Pregnancy

A Longitudinal Study

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Objectives—To investigate gestational age–specific serial changes in umbilical vein (UV) volume blood flow during the second half of normal pregnancy and establish sex-specific reference ranges.

Methods—This work was a prospective longitudinal study of singleton low-risk pregnancies. The UV diameter and maximum blood flow velocity were serially measured by sonography at the intra-abdominal portion of the UV over 19 to 41 weeks. Umbilical vein volume blood flow was calculated and normalized for estimated fetal weight.

Results—One hundred seventy-nine women and their fetuses (87 male and 92 female) were included in the final analysis, and a total of 746 observations were used to construct sex-specific reference intervals. We found no statistically significant sex-specific differences in the UV parameters examined. However, the temporal development patterns of normalized UV volume blood flow appeared to differ between male and female fetuses during the second half of pregnancy, with crossovers at 24 and 32 weeks' gestation.

Conclusions—Umbilical vein volume blood flow is similar among male and female fetuses in quantitative terms, but the pattern of gestational age–dependent temporal changes may be different, which may have important physiologic implications with regard to in utero development and maturation of the fetoplacental unit.

Key Words—Doppler sonography; obstetric ultrasound; placental blood flow; sexual dimorphism; umbilical vein

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Abbreviations
UV, umbilical vein

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The importance of sex-specific data analysis in research has been much emphasized.[1] Sex differences are important, as they influence perinatal outcomes.[2–4] A female survival advantage among neonates is well known,[5,6] but total mortality during pregnancy is greater for female fetuses compared with male fetuses.[7,8] There are sex-related differences in the structure and function of the human placenta.[9,10] Girls have smaller placentas[11] and lower birth weight compared with boys. Placental metabolic efficiency expressed in terms of the fetal-to-placental weight ratio[12] is higher in male fetuses,[11] but they may have less reserve capacity.[13]

Sex is determined early in embryonic life, and every cell in all organs including the placenta may have a sexual dimorphism. Therefore, fundamental differences in the functional development of

placenta are to be expected between male and female fetuses. There are sex-dependent differences in the global transcriptomic profile in the placenta, with female placentas expressing more up-regulated autosomal genes and some select transcripts, such as the human chorionic gonadotropin.[14] Genetic as well as epigenetic differences may drive sexually dimorphic responses of the placenta to the intrauterine and external environments, leading to disparities in placental perfusion, metabolism, steroidogenesis, growth, and maturation. However, studies on sex-related differences in placental blood flow are scarce.

Sex-specific fetal weight standards are used while evaluating intrauterine growth,[15] and they are reported to be more accurate[16] and better in identifying small-for-gestational-age fetuses at risk of stillbirth.[17] However, sex differences are not taken into account when evaluating fetoplacental blood flow characteristics.

The oxygenated nutrient-rich blood is supplied to the fetus via a single umbilical vein (UV). As the umbilical circulation is a closed circuit, UV volume blood flow can be used a proxy for placental perfusion and function. Previous research has shown that reduced UV volume blood flow may be the earliest sign of placental insufficiency,[18] and the fraction of fetal cardiac output directed to the placenta is reduced even before the fetal weight becomes abnormal.[19]

In a recent study, Prior et al[20] showed that male fetuses at term had reduced UV volume blood flow compared with female fetuses before the onset of active labor, but this finding was not associated with adverse perinatal outcomes. Both cross-sectional[21] and longitudinal[22] reference standards for UV volume blood flow have been published, but they do not take into account fetal sex differences. Thus, the aim of our study was to test the hypothesis that placental blood flow is different between male and female fetuses by investigating gestational age–specific changes in UV volume blood flow during the second half of normal pregnancy and establish sex-specific longitudinal reference ranges for UV diameter, blood flow velocity, and UV volume blood flow.

Materials and Methods

Data from a total of 183 pregnant women participating in 2 prospective longitudinal observational studies that included investigation of fetoplacental hemodynamics in low-risk pregnancies were used for this study. Women older than 18 years attending their routine sonographic screening at 17 to 20 weeks' gestation were recruited. Gestational age was confirmed by measurement of the biparietal diameter in all cases. The inclusion criteria were singleton pregnancy and no history of hypertensive disorders of pregnancy, intrauterine growth restriction, preterm labor, gestational diabetes, or other maternal disease that may have had any substantial impact on the course and outcome of the current pregnancy. Maternal smoking, multiple pregnancy, and the presence of any chromosomal or major structural fetal anomaly were exclusion criteria. The study protocols were approved by the Regional Committee for Medical Research Ethics–North Norway (REK Nord 74/2001 and 52/2005). All participants gave written informed consent.

Sonographic examinations were performed by 2 experienced clinicians (G.A. and K.F.) using a 6-MHz curvilinear transducer (Acuson Sequoia 512; Siemens Medical Solutions, Mountain View, CA) at approximately 4-week (range, 3–5 weeks) intervals starting from 19 to 22 weeks until delivery. After determining the fetal viability, position, placental location, and amniotic fluid index, biometry was performed to measure the biparietal diameter, head circumference, abdominal circumference, and femur length, and fetal weight was estimated according to formula 2 of Hadlock et al.[23] Doppler sonography was performed to obtain blood flow velocities from the intra-abdominal portion of the UV. Color Doppler imaging was used to visualize the vessel and direction of blood flow and to optimize the insonation angle, which was always kept below 15°. Blood flow velocities were recorded by pulsed wave Doppler imaging with a wide sample volume (gate size of 5–12 mm depending on the gestational age) adjusted to include the entire diameter of the insonated blood vessel and the wall motion filter set at low. The UV velocities were recorded in the absence of fetal movements for 4 to 6 seconds with a sweep speed of 50 to 100 mm/s, and the time-averaged maximum velocity was measured by manually tracing the velocity envelope over 2 seconds. The mean velocity was calculated as 0.5 × time-averaged maximum velocity, assuming a parabolic velocity profile in the intra-abdominal portion of the UV.[22] The inner diameter of the UV was measured at the intra-abdominal straight portion in a zoomed B-mode sonogram. An average of 3 measurements was recorded. The ALARA (as low as reasonably achievable) principle was observed during sonography. The

mechanical and thermal indices were displayed on the screen and were always kept below 1.9 and 1.5, respectively. The UV blood flow recording was successful in 694 of 746 (93.03%) observations.

The UV volume blood flow was calculated as the product of the mean velocity and cross-sectional area of the UV, where cross-sectional area = π (UV diameter/2)^2; ie, UV volume blood flow (milliliters per minute) = 0.785 × mean velocity (centimeters per second) × [diameter (centimeters)]^2 × 60. Normalized UV volume blood flow (milliliters per minute per kilogram) was calculated as UV volume blood flow (milliliters per minute)/estimated fetal weight (kilograms).

All included participants received standard care. The sex of the fetus was not examined and not recorded antenatally. The information on the course and outcome of pregnancy, including any complications, gestation at delivery, mode of delivery, birth weight, placental weight, sex of the neonate, Apgar score, umbilical cord blood gases, acid-base status, and neonatal outcome, was obtained from the medical records. All neonates were examined by a pediatrician routinely before discharge.

Data analysis was performed with SAS 9.3 software (SAS Institute Inc, Cary, NC). All variables that were not normally distributed were transformed (logarithmic or power transformation) to achieve a normal distribution of data. The best transformation for each variable was chosen by Box-Cox regression. Regression curves were fitted by fractional polynomials. The best-fitting curves in relation to gestational age were chosen, accommodating nonlinear associations and a repeated-measures design. The association of the UV diameter, time-averaged maximum velocity, UV volume blood flow, and normalized UV volume blood flow (dependent variables) with the gestational age (independent variable) was investigated by multilevel modeling. Gestational age–specific reference percentiles were constructed from each fitted model as described by Royston and Altman.[24] Differences between groups (male and female fetuses) with regard to the UV diameter, velocities, and blood flow were evaluated by an independent-samples t test for each gestational week separately. A χ^2 test was used for categorical variables. Statistical significance was set at 2-tailed $P < .05$. The number of study participants required to establish normal reference intervals was estimated to be approximately 180 based on the assumption that about 15 observations per gestational week (ie, a total of 330 observations between 19 and 41 weeks) for each sex would be sufficient to calculate reference intervals with adequate precision.

Table 1. Baseline Characteristics of the Study Population

Characteristic	Female (n = 92)	Male (n = 87)	*P*
Maternal			
Age, y	29 (21–43)	30 (18–40)	.658
Body mass index at booking, kg/m^2	25.18 ± 4.04	25.16 ± 3.77	.968
Nulliparous	46 (50.0)	46 (52.9)	.701
Fetal			
Gestational age at birth, d[a]	280 (238–297)	281 (255–297)	.950
Birth weight, g	3614.55 ± 461.86	3660.05 ± 516.37	.535
Placental weight, g	637.57 ± 137.41	665.98 ± 147.24	.185
Fetal-placental ratio	5.84 ± 1.08	5.66 ± 1.00	.260
5-min Apgar score	10 (4–10)	10 (0–10)	.160
Umbilical artery pH	7.26 ± 0.09	7.24 ± 0.08	.251
Umbilical artery base excess, mmol/L	–4.30 ± 3.65	–4.73 ± 3.19	.516
Meconium-stained liquor	17 (18.8)	18 (20.9)	.681
Admission to NICU	5 (5.4)	5 (5.7)	.928
Mode of delivery			
Normal	72 (78.3)	68 (78.2)	.987
Vacuum/forceps	6 (6.5)	6 (6.9)	.920
Cesarean	14 (15.2)	13 (14.9)	.959

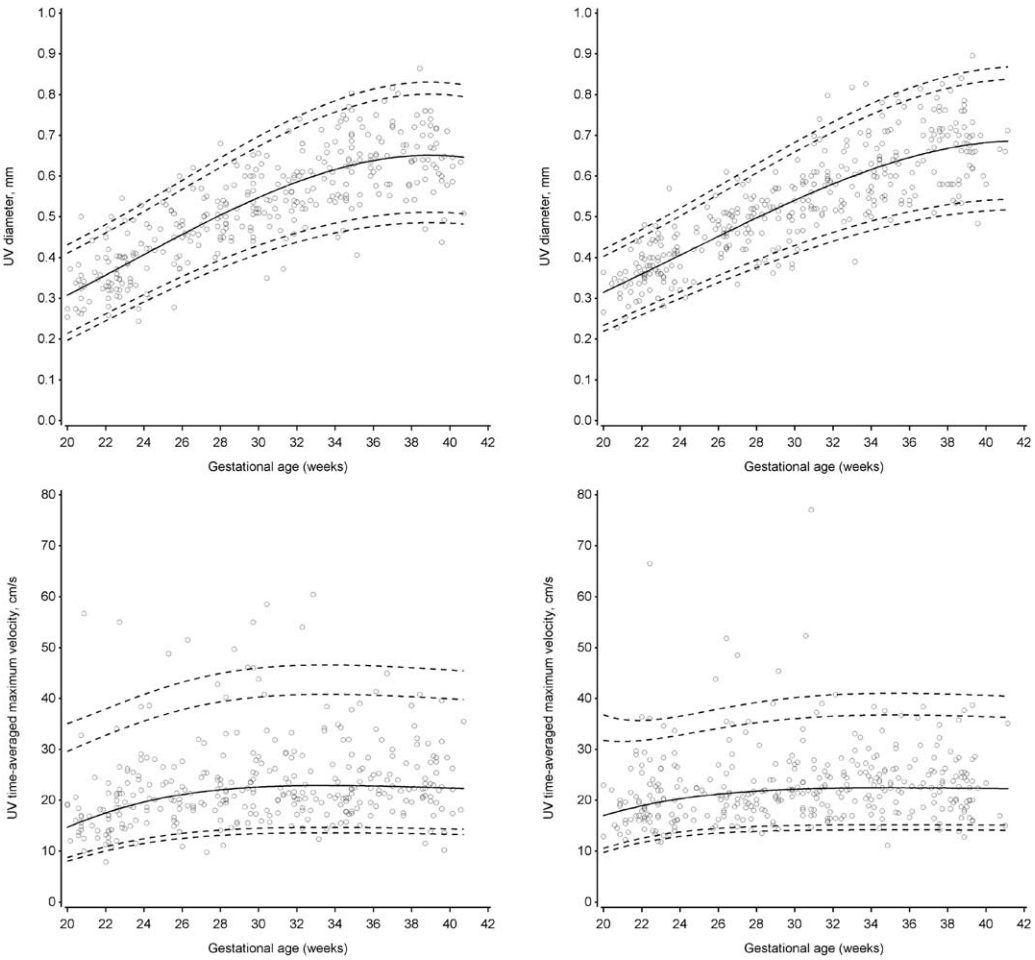
Data are presented as median (range), mean ± SD, and number (percent), as appropriate. NICU indicates neonatal intensive care unit. *P* values were calculated by an independent-samples t test for continuous variables and a χ^2 test for categorical variables.
[a]280 days = 40 weeks; 281 days = 40 weeks 1 day.

Results

Of a total of 183 participants, 4 were excluded (because of a diagnosis of a fetal anomaly or smoking during pregnancy), and complete data were available for analysis for 179 pregnancies. There were 87 male and 92 female fetuses. The baseline demographic and clinical characteristics of the study population, including pregnancy outcomes, are presented in Table 1. There were no significant differences between groups with regard to maternal characteristics and perinatal outcomes.

A total of 746 observations (356 for male fetuses and 390 for female fetuses) were used to construct sex-specific reference intervals of the UV diameter, time-averaged maximum velocity, UV volume blood flow, and normalized UV volume blood flow for each gestational week during the second half of pregnancy. The UV diameter, time-averaged maximum velocity, UV volume blood flow, and normalized UV volume blood flow were significantly associated with gestational age ($P < .00001$). Sex-specific reference ranges for the UV diameter, time-averaged maximum velocity, UV volume blood flow, and normalized UV volume blood flow in the second half of pregnancy are presented in Figures 1 and 2, and their respective 2.5th, 5th, 10th, 25th, 50th, 75th, 90th, 95th, and 97.5th percentiles for each gestational week from 19 to 41 weeks are presented in Tables 2–9. The gestational age–related sex differences in the

Figure 1. Umbilical vein diameter and blood flow velocity: sex-specific reference ranges for UV diameter and time-averaged maximum velocity at the intra-abdominal section (left, male; right, female). The solid lines represent the means, and the interrupted lines represent 2.5th, 5th, 95th, and 97.5th percentiles.



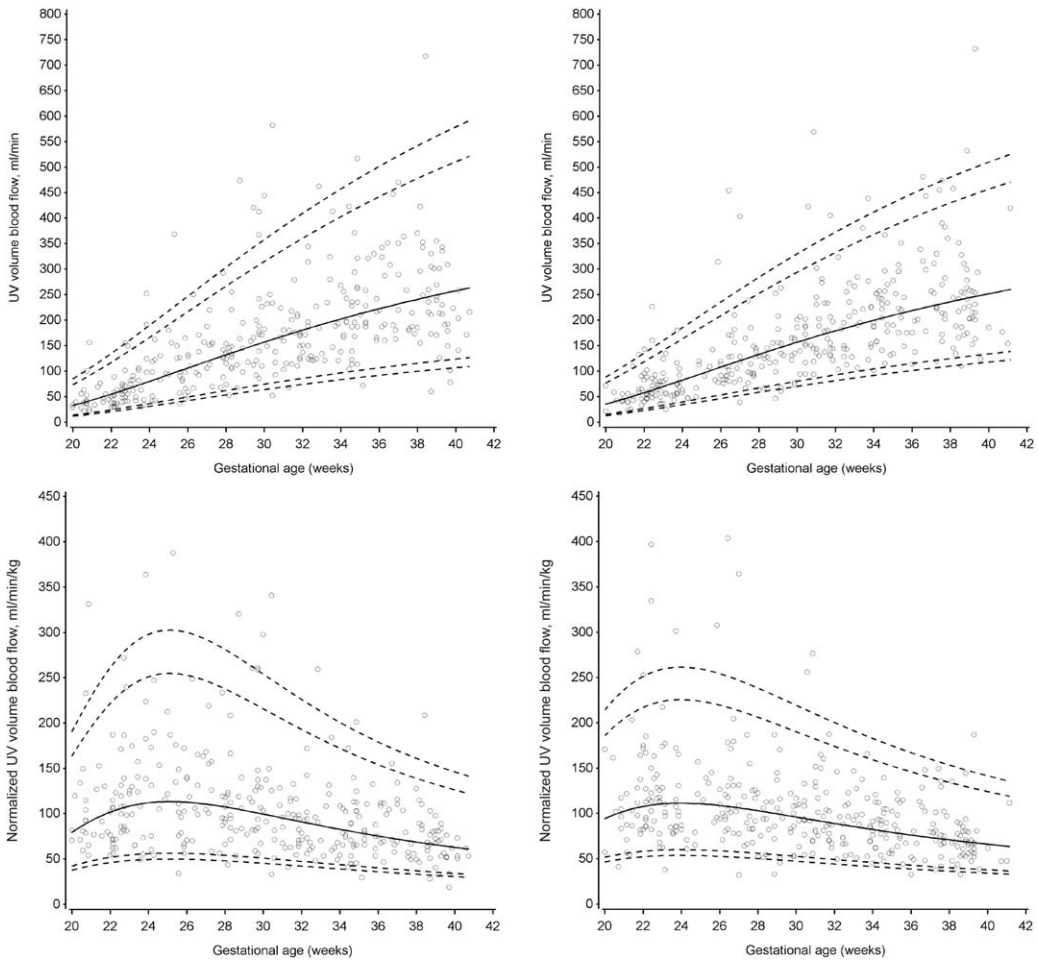
time-averaged maximum velocity and normalized UV volume blood flow during the second half of pregnancy are shown in Figures 3 and 4, respectively.

We found no statistically significant differences in the UV diameter, time-averaged maximum velocity, and absolute or normalized UV volume blood flow between male and female fetuses neither at each gestational week nor when the observations were grouped (<24 weeks, 24–32 weeks, and >32 weeks). The female fetuses started (at mid gestation) with a slightly higher time-averaged maximum velocity compared with male fetuses, but the values equalized at 26 weeks, with male fetuses having slightly higher UV velocities thereafter until term (Figure 4), but the differences were not statistically significant. Although there were no statistically significant differences in quantitative terms, the UV volume blood flow normalized for estimated fetal weight showed an interesting temporal trend (a biphasic pattern), with male fetuses having lower blood flow compared with female fetuses from 20 to 24 weeks, slightly higher flow from 24 to 32 weeks, and then lower flow again from 32 weeks onward (Figure 4).

Discussion

Sexual dimorphism in placental weight[11] and certain aspects of placental function have been described,[10,25] but studies on sex differences in placental blood flow and perfusion are scarce. Longitudinal studies evaluating sexual dimorphism in gestational age–related serial

Figure 2. Umbilical vein volume blood flow: sex-specific reference ranges for UV absolute volume blood flow and volume blood flow normalized for estimated fetal weight measured at the intra-abdominal portion (left, male; right, female). The solid lines represent the means, and the interrupted lines represent the 2.5th, 5th, 95th, and 97.5th percentiles.



changes in fetoplacental blood flow are lacking. To our knowledge, a study that determined sex-specific reference ranges for UV volume blood flow has not been reported previously. We found no statistically significant differences in UV velocities and blood flow during the second half of normal pregnancy. Despite this finding, a biphasic pattern of serial changes in normalized UV volume blood flow was observed, with crossovers at 24 and 32 weeks' gestation. A sex-related difference in fetoplacental blood flow in human pregnancy was first reported recently by Prior et al,[20] who found significantly lower normalized UV volume blood flow in male fetuses compared with female fetuses at term (56 versus 61 mL/min/kg; $P = .02$). However, it was a cross-sectional study, and the blood flow measurements were performed on admission to a labor ward; therefore, the women might have been in the latent/early phase of labor.

The main strength of our study was its longitudinal design and reasonable number of observations in each gestational week, which allowed us to construct valid reference charts. The study limitations were related to technical issues associated with accurate estimation of UV volume blood flow. However, volume blood flow measurements using modern ultrasound systems have been shown to be reasonably accurate in experimental settings,[26,27] and measurements of UV volume blood flow have acceptable accuracy and reproducibility in clinical settings.[28,29] Furthermore, substantial effort to improve accuracy was made by choosing a fixed and easily identifiable site (ie, the intra-abdominal portion of the UV) for blood flow measurement, measuring the UV diameter 3 times and averaging the values and keeping the Doppler insonation angle as low as possible ($<15°$) while recording UV velocities. Another limitation was the lack of observations before 19 weeks' gestation.

The placenta sustains fetal life and also acts as a barrier that protects the fetus from environmental hazards. Poor placental perfusion is associated with placental dysfunction, which can have serious life-long consequences. Edwards et al[30] reported higher rates of severe placental dysfunction in male fetuses, evidenced by absent or reversed end-diastolic flow in the umbilical artery in a cohort of growth-restricted fetuses. The UV blood flow is reduced in placental insufficiency even before the UA Doppler indices become abnormal.[18] Therefore, using sex-specific reference ranges might help improve

Figure 3. Sex differences in UV blood flow velocity: gestational age–related sex differences in UV time-averaged maximum velocity during the second half of pregnancy. Red lines represent female, and blue lines represent male. The interrupted lines represent the corresponding 95% confidence intervals.

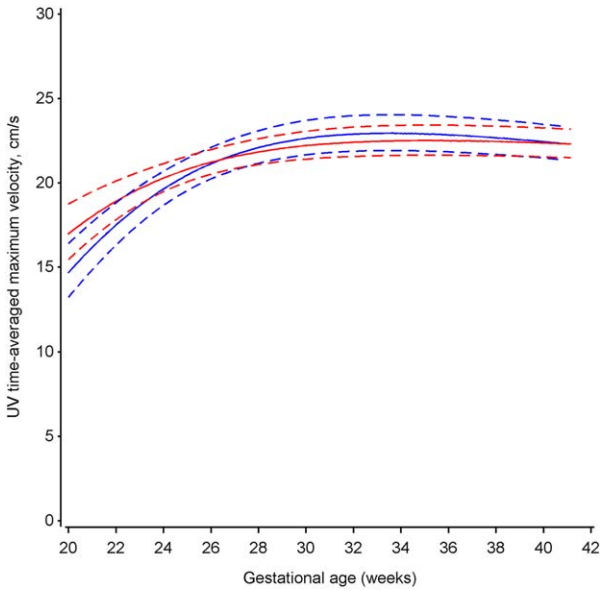


Figure 4. Sex differences in UV volume blood flow: gestational age-related sex differences in UV volume blood flow normalized for estimated fetal weight during the second half of pregnancy. Red lines represent female, and blue lines represent male. The interrupted lines represent the corresponding 95% confidence intervals.

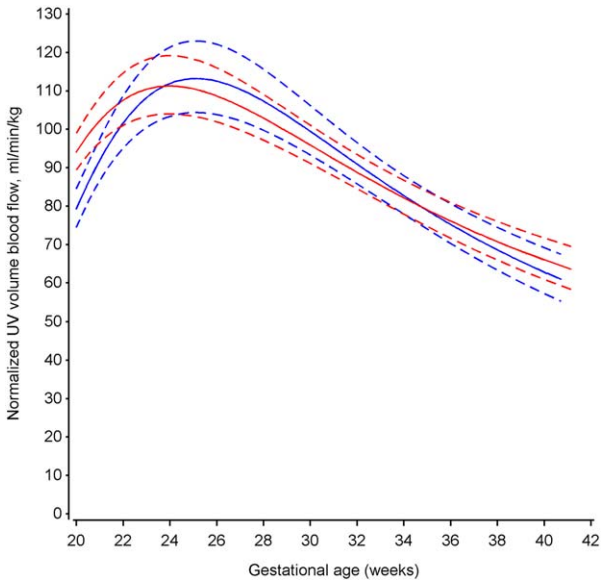


Table 2. Umbilical Vein Diameter (Male), cm

Gestation, wk	2.5th Percentile	5th Percentile	10th Percentile	25th Percentile	Mean	75th Percentile	90th Percentile	95th Percentile	97.5th Percentile
19	0.18	0.19	0.21	0.25	0.29	0.33	0.37	0.39	0.41
20	0.20	0.21	0.23	0.27	0.31	0.35	0.39	0.41	0.43
21	0.22	0.24	0.26	0.30	0.34	0.38	0.42	0.44	0.46
22	0.24	0.26	0.28	0.32	0.36	0.40	0.44	0.46	0.48
23	0.27	0.29	0.31	0.34	0.38	0.42	0.46	0.49	0.51
24	0.29	0.31	0.33	0.37	0.41	0.45	0.49	0.51	0.53
25	0.31	0.33	0.35	0.39	0.43	0.48	0.52	0.54	0.56
26	0.33	0.35	0.38	0.41	0.46	0.50	0.54	0.57	0.59
27	0.35	0.37	0.40	0.44	0.48	0.53	0.57	0.59	0.62
28	0.37	0.39	0.42	0.46	0.50	0.55	0.59	0.62	0.64
29	0.39	0.42	0.44	0.48	0.53	0.58	0.62	0.65	0.68
30	0.41	0.43	0.46	0.50	0.55	0.60	0.64	0.67	0.70
31	0.42	0.45	0.47	0.52	0.57	0.62	0.67	0.70	0.72
32	0.44	0.46	0.49	0.53	0.59	0.64	0.69	0.72	0.75
33	0.45	0.47	0.50	0.55	0.60	0.66	0.71	0.74	0.77
34	0.46	0.48	0.51	0.56	0.62	0.67	0.73	0.76	0.78
35	0.47	0.49	0.52	0.57	0.63	0.69	0.74	0.77	0.80
36	0.48	0.50	0.53	0.58	0.64	0.70	0.75	0.78	0.81
37	0.48	0.51	0.54	0.59	0.65	0.71	0.76	0.79	0.82
38	0.48	0.51	0.54	0.59	0.65	0.71	0.77	0.80	0.83
39	0.49	0.51	0.54	0.59	0.65	0.71	0.77	0.80	0.83
40	0.48	0.51	0.54	0.59	0.65	0.71	0.76	0.80	0.83
41	0.47	0.50	0.53	0.58	0.63	0.69	0.75	0.78	0.81

Sex-specific reference values of the UV diameter at the intra-abdominal section for the 2.5th, 5th, 10th, 25th, 50th, 75th, 90th, 95th, and 97.5th percentiles during the second half of pregnancy (male fetuses).

Table 3. Umbilical Vein Diameter (Female), cm

Gestation, wk	2.5th Percentile	5th Percentile	10th Percentile	25th Percentile	Mean	75th Percentile	90th Percentile	95th Percentile	97.5th Percentile
19	0.20	0.22	0.24	0.27	0.30	0.33	0.37	0.39	0.40
20	0.22	0.23	0.25	0.28	0.31	0.35	0.38	0.40	0.42
21	0.24	0.26	0.27	0.31	0.34	0.38	0.41	0.43	0.45
22	0.26	0.27	0.29	0.32	0.36	0.40	0.43	0.45	0.47
23	0.28	0.30	0.31	0.35	0.38	0.42	0.45	0.48	0.49
24	0.30	0.32	0.34	0.37	0.41	0.44	0.48	0.50	0.52
25	0.32	0.34	0.36	0.39	0.43	0.47	0.51	0.53	0.55
26	0.34	0.36	0.38	0.41	0.45	0.49	0.53	0.55	0.57
27	0.36	0.38	0.40	0.43	0.47	0.52	0.56	0.58	0.60
28	0.38	0.39	0.42	0.45	0.50	0.54	0.58	0.61	0.63
29	0.39	0.41	0.44	0.47	0.52	0.57	0.61	0.63	0.66
30	0.41	0.43	0.45	0.49	0.54	0.59	0.63	0.66	0.68
31	0.43	0.45	0.47	0.51	0.56	0.61	0.66	0.68	0.71
32	0.44	0.46	0.49	0.53	0.58	0.63	0.68	0.71	0.74
33	0.45	0.48	0.50	0.55	0.60	0.65	0.70	0.73	0.76
34	0.47	0.49	0.52	0.56	0.62	0.67	0.72	0.75	0.78
35	0.48	0.50	0.53	0.58	0.63	0.69	0.74	0.77	0.80
36	0.49	0.51	0.54	0.59	0.65	0.70	0.76	0.79	0.82
37	0.50	0.52	0.55	0.60	0.66	0.72	0.77	0.80	0.83
38	0.50	0.53	0.56	0.61	0.67	0.73	0.78	0.82	0.84
39	0.51	0.54	0.57	0.62	0.68	0.74	0.79	0.83	0.86
40	0.51	0.54	0.57	0.62	0.68	0.74	0.80	0.83	0.86
41	0.52	0.54	0.57	0.63	0.69	0.75	0.80	0.84	0.87

Sex-specific reference values of the UV diameter at the intra-abdominal section for the 2.5th, 5th, 10th, 25th, 50th, 75th, 90th, 95th, and 97.5th percentiles during the second half of pregnancy (female fetuses).

Table 4. Umbilical Vein Time-Averaged Maximum Velocity (Male), cm/s

Gestation, wk	2.5th Percentile	5th Percentile	10th Percentile	25th Percentile	Mean	75th Percentile	90th Percentile	95th Percentile	97.5th Percentile
19	7.0	7.7	8.6	10.4	13.3	17.5	23.3	28.2	33.9
20	8.0	8.7	9.7	11.7	14.7	19.0	24.8	29.6	35.1
21	9.2	10.0	11.0	13.2	16.4	20.9	26.7	31.5	36.7
22	10.0	10.9	11.9	14.2	17.5	22.1	28.0	32.8	37.9
23	10.8	11.7	12.9	15.2	18.6	23.4	29.4	34.2	39.4
24	11.5	12.4	13.6	16.1	19.6	24.5	30.7	35.6	40.8
25	12.1	13.0	14.3	16.8	20.5	25.5	31.8	36.8	42.1
26	12.5	13.5	14.8	17.4	21.1	26.3	32.7	37.8	43.2
27	12.8	13.8	15.2	17.8	21.7	26.9	33.5	38.7	44.1
28	13.1	14.1	15.5	18.2	22.1	27.4	34.1	39.4	44.9
29	13.3	14.4	15.7	18.5	22.4	27.9	34.7	40.0	45.6
30	13.4	14.5	15.8	18.6	22.6	28.1	34.9	40.3	46.0
31	13.5	14.6	16.0	18.7	22.8	28.3	35.2	40.6	46.3
32	13.6	14.6	16.0	18.8	22.9	28.4	35.3	40.7	46.5
33	13.6	14.7	16.1	18.9	22.9	28.5	35.4	40.8	46.6
34	13.6	14.7	16.1	18.9	22.9	28.5	35.4	40.8	46.6
35	13.6	14.6	16.0	18.8	22.9	28.4	35.4	40.8	46.5
36	13.6	14.6	16.0	18.8	22.8	28.4	35.3	40.7	46.4
37	13.5	14.6	15.9	18.7	22.8	28.3	35.1	40.5	46.3
38	13.4	14.5	15.9	18.6	22.7	28.2	35.0	40.4	46.1
39	13.4	14.4	15.8	18.6	22.6	28.0	34.8	40.2	45.8
40	13.3	14.3	15.7	18.4	22.4	27.8	34.6	39.9	45.6
41	13.1	14.2	15.5	18.2	22.2	27.5	34.2	39.5	45.1

Sex-specific reference values of the UV time-averaged maximum velocity at the intra-abdominal section for the 2.5th, 5th, 10th, 25th, 50th, 75th, 90th, 95th, and 97.5th percentiles during the second half of pregnancy (male fetuses).

Table 5. Umbilical Vein Time-Averaged Maximum Velocity (Female), cm/s

Gestation, wk	2.5th Percentile	5th Percentile	10th Percentile	25th Percentile	Mean	75th Percentile	90th Percentile	95th Percentile	97.5th Percentile
19	8.9	9.7	10.7	12.9	16.1	20.8	27.1	32.3	38.0
20	9.7	10.5	11.6	13.8	17.0	21.5	27.2	31.8	36.8
21	10.9	11.8	12.9	15.0	18.2	22.4	27.6	31.6	35.8
22	11.7	12.5	13.6	15.8	18.9	23.0	28.0	31.8	35.7
23	12.4	13.2	14.3	16.5	19.7	23.7	28.6	32.2	36.0
24	12.9	13.8	14.9	17.1	20.3	24.4	29.2	32.8	36.5
25	13.3	14.2	15.4	17.7	20.9	25.0	29.9	33.6	37.3
26	13.5	14.5	15.6	18.0	21.2	25.4	30.4	34.1	37.9
27	13.7	14.7	15.9	18.2	21.6	25.9	30.9	34.7	38.6
28	13.9	14.8	16.1	18.5	21.8	26.2	31.4	35.3	39.2
29	14.0	14.9	16.2	18.6	22.0	26.5	31.8	35.7	39.7
30	14.1	15.0	16.3	18.7	22.2	26.7	32.1	36.1	40.2
31	14.1	15.1	16.4	18.8	22.3	26.9	32.3	36.3	40.5
32	14.2	15.1	16.4	18.9	22.4	27.0	32.5	36.6	40.8
33	14.2	15.2	16.4	18.9	22.5	27.1	32.6	36.7	40.9
34	14.2	15.2	16.4	19.0	22.5	27.1	32.6	36.7	41.0
35	14.2	15.2	16.5	19.0	22.5	27.1	32.6	36.8	41.0
36	14.2	15.2	16.4	19.0	22.5	27.1	32.6	36.8	41.0
37	14.2	15.2	16.4	19.0	22.5	27.1	32.6	36.7	41.0
38	14.2	15.2	16.4	18.9	22.4	27.1	32.5	36.6	40.9
39	14.2	15.1	16.4	18.9	22.4	27.0	32.5	36.6	40.8
40	14.1	15.1	16.4	18.9	22.4	26.9	32.4	36.4	40.6
41	14.1	15.1	16.4	18.8	22.3	26.9	32.3	36.3	40.5

Sex-specific reference values of the UV time-averaged maximum velocity at the intra-abdominal section for the 2.5th, 5th, 10th, 25th, 50th, 75th, 90th, 95th, and 97.5th percentiles during the second half of pregnancy (female fetuses).

Table 6. Umbilical Vein Volume Blood Flow (Male), mL/min

Gestation, wk	2.5th Percentile	5th Percentile	10th Percentile	25th Percentile	Mean	75th Percentile	90th Percentile	95th Percentile	97.5th Percentile
19	8	10	12	17	25	35	48	57	66
20	11	14	17	23	33	46	62	73	85
21	16	19	23	32	45	62	83	97	112
22	20	24	29	39	55	75	99	117	135
23	26	30	36	49	67	92	120	141	162
24	31	36	43	58	80	108	142	166	190
25	36	43	51	68	93	126	164	191	218
26	42	49	58	78	106	143	186	216	247
27	48	56	66	88	119	160	208	242	275
28	53	62	73	97	132	177	229	267	303
29	59	69	82	108	146	196	253	294	335
30	64	74	88	116	157	210	271	315	358
31	69	80	95	125	169	226	291	338	384
32	74	86	102	134	180	241	311	360	409
33	79	91	108	142	192	256	329	382	434
34	83	96	114	150	202	270	347	403	457
35	88	102	120	158	212	283	365	423	480
36	92	106	126	165	222	296	381	442	501
37	96	111	131	172	232	309	397	460	522
38	100	115	136	179	241	321	412	478	542
39	103	120	141	186	249	332	427	494	561
40	107	124	147	193	259	344	442	513	581
41	114	132	155	204	274	364	468	542	615

Sex-specific reference values of the UV volume blood flow at the intra-abdominal section for the 2.5th, 5fth, 10th, 25th, 50th, 75th, 90th, 95th, and 97.5th percentiles during the second half of pregnancy (male fetuses).

Table 7. Umbilical Vein Volume Blood Flow (Female), mL/min

Gestation, wk	2.5th Percentile	5th Percentile	10th Percentile	25th Percentile	Mean	75th Percentile	90th Percentile	95th Percentile	97.5th Percentile
19	10	12	14	20	28	40	53	63	73
20	13	15	18	25	35	49	65	77	88
21	18	21	26	35	48	65	85	100	114
22	23	26	32	42	58	78	101	118	135
23	28	33	39	52	70	94	121	141	160
24	34	39	47	61	82	110	141	163	185
25	41	47	56	73	97	128	164	189	214
26	46	53	62	81	108	142	181	208	235
27	52	60	70	91	120	158	200	231	260
28	58	67	78	101	133	174	220	252	284
29	64	74	86	110	145	189	238	273	307
30	70	80	93	120	157	204	257	294	330
31	76	86	101	129	168	218	274	313	352
32	82	93	109	139	181	234	293	335	376
33	86	99	114	146	190	245	307	351	393
34	92	104	121	154	200	258	322	368	412
35	96	110	127	161	209	270	337	384	430
36	101	115	133	169	219	281	351	400	448
37	106	120	139	176	228	293	365	415	464
38	110	125	145	183	237	305	379	432	482
39	114	129	149	189	244	313	390	443	495
40	118	134	154	195	252	323	401	457	510
41	122	138	159	201	259	332	413	469	523

Sex-specific reference values of the UV volume blood flow at the intra-abdominal section for the 2.5th, 5fth, 10th, 25th, 50th, 75th, 90th, 95th, and 97.5th percentiles during the second half of pregnancy (female fetuses).

Table 8. Normalized Umbilical Vein Volume Blood Flow (Male), mL/min/kg

Gestation, wk	2.5th Percentile	5th Percentile	10th Percentile	25th Percentile	Mean	75th Percentile	90th Percentile	95th Percentile	97.5th Percentile
19	32	36	41	51	67	88	114	135	156
20	38	42	48	61	79	105	138	164	191
21	43	48	56	71	93	126	167	200	234
22	46	52	60	76	102	138	185	222	261
23	48	54	63	81	108	148	199	240	284
24	49	56	65	83	112	153	208	251	298
25	50	56	65	84	113	155	211	255	303
26	50	56	65	84	113	154	209	253	300
27	49	55	64	82	110	151	204	247	292
28	48	54	62	80	107	146	197	238	281
29	46	52	60	77	103	140	188	226	266
30	45	51	59	75	99	135	180	216	254
31	44	49	56	72	95	128	171	204	240
32	42	47	54	69	91	122	162	193	226
33	41	45	52	66	87	116	153	182	213
34	39	44	50	63	83	110	145	172	201
35	37	42	48	60	79	105	137	163	190
36	36	40	46	58	75	100	130	154	179
37	34	39	44	55	72	95	124	146	170
38	33	37	42	53	69	91	118	139	161
39	32	35	40	51	66	87	112	132	153
40	30	34	39	48	62	82	107	126	145
41	28	31	36	44	58	76	98	115	133

Sex-specific reference values of the UV volume blood flow normalized for estimated fetal weight at the intra-abdominal section for the 2.5th, 5th, 10th, 25th, 50th, 75th, 90th, 95th, and 97.5th percentiles during the second half of pregnancy (male fetuses).

Table 9. Normalized Umbilical Vein Volume Blood Flow (Female), mL/min/kg

Gestation, wk	2.5th Percentile	5th Percentile	10th Percentile	25th Percentile	Mean	75th Percentile	90th Percentile	95th Percentile	97.5th Percentile
19	43	48	54	67	86	113	145	169	194
20	46	52	59	73	94	123	159	186	214
21	50	56	64	80	103	136	176	207	239
22	52	58	66	83	108	142	184	217	251
23	53	59	68	85	110	146	190	224	259
24	54	60	68	86	111	147	191	226	261
25	53	59	68	85	110	146	190	223	259
26	53	59	67	84	109	143	186	220	254
27	51	57	65	82	106	140	181	213	247
28	50	56	64	79	103	135	175	206	238
29	49	54	62	77	99	131	169	198	229
30	47	53	60	74	96	126	162	190	219
31	46	51	58	72	92	121	155	182	210
32	44	49	55	69	88	115	148	173	199
33	43	47	54	67	85	111	143	167	191
34	41	46	52	64	82	107	137	159	183
35	40	44	50	62	79	102	131	152	175
36	39	43	48	60	76	98	126	146	167
37	37	41	47	58	73	95	120	140	160
38	36	40	45	55	70	91	115	134	153
39	35	39	44	54	68	88	111	129	147
40	34	38	42	52	66	85	107	124	142
41	33	37	41	50	64	82	103	120	136

Sex-specific reference values of the UV volume blood flow normalized for estimated fetal weight at the intra-abdominal section for the 2.5th, 5th, 10th, 25th, 50th, 75th, 90th, 95th, and 97.5th percentiles during the second half of pregnancy (female fetuses).

accuracy and precision of the diagnosis of placental insufficiency.

The impact of sex differences on placental pathophysiologic characteristics has become more obvious in recent years. The placenta may respond and adapt differently to pathologic insults in a sex-specific and gestational age–dependent manner. Sexual dimorphism in placental inflammatory, hypoxic, and apoptotic responses and angiogenesis has been observed in preeclamptic placentas, with male fetuses expressing higher levels of tumor necrosis factor α, interleukin 6, interleukin 8, hypoxia-inducible factor 1α, and proapoptotic markers but lower vascular endothelial growth factor compared with female fetuses.[31] Female fetuses born before 72 hours after antenatal betamethasone treatment have greater 11β-hydroxysteroid dehydrogenase activity in the placenta and higher umbilical artery cortisol concentrations compared with male fetuses exposed to a similar treatment.[32] Furthermore, sex-specific alterations in placental genes involved with growth and inflammation have also been observed in cases of maternal hypoxia.[25] Recently, Orzack et al[8] studied the trajectory of the human sex ratio from conception to birth. They found that the sex ratio was unbiased at conception, but a temporal change in the sex ratio was noted, with excess male mortality in the embryonic period (<10 weeks) and third trimester (28–35 weeks), whereas female mortality was higher at 10 to 15 weeks. These observations support the assumption that there is a sex-related bias in placental function, and its growth trajectory during the gestation is influenced by the sex.

A recent study showed that in pregnancies dated by sonography, the male-to-female ratio increased after 40 weeks, reaching 1.69 at 42 weeks.[33] This increase may be because female fetuses are smaller than the male fetuses already at dating, leading to an underestimation of their gestational age, or because male fetuses attain a critical fetal weight earlier than the female fetuses. The sex differences in placental weight are in line with the long-recognized sex differences in fetal growth velocity.[34] Male fetuses have higher absolute placental weight but a greater fetal-to-placental ratio compared with female fetuses, which may suggest higher placental efficiency in male fetuses. However, it can also be interpreted to indicate that male fetuses prioritize body growth at the expense of the placenta, making them more vulnerable to placental dysfunction. As placental metabolism and growth are affected by blood flow, it is plausible that there exists sex-specific dimorphism in placental perfusion and function. However, the differences might not be present throughout the gestation. A previous study reported no sex differences in the human chorionic gonadotropin level in the first and second trimesters, but the maternal serum human chorionic gonadotropin level increased significantly in pregnancies with female fetuses and decreased with male fetuses from the second to third trimesters.[35]

A crossover of normalized UV volume blood flow among male and female fetuses at 24 and 32 weeks is an interesting phenomenon. It supports the assumption that the placental maturation is faster in male fetuses, whereas the pulmonary growth and maturation are faster in female fetuses (leading to a higher proportion of fetal right ventricular cardiac output distributed to the lungs at the expense of the placenta). This assumption could be one of the explanations for higher antenatal loss (miscarriage and stillbirth) rates among female fetuses[7,8] and neonatal mortality rates among male fetuses[5] reported in epidemiologic studies.

In conclusion, there were no statistically significant sex-specific differences in fetoplacental blood flow in quantitative terms during the second half of pregnancy, but the pattern of gestational age–dependent temporal changes in placental volume blood flow may be different between male and female fetuses, with crossovers at 24 and 32 weeks' gestation. These findings may have important physiologic implications with regard to in utero development and maturation of the fetoplacental unit.

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Paper III

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Biology of Sex Differences

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Sex differences in umbilical artery Doppler indices: a longitudinal study

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Abstract

Background: Sexual dimorphism in placental size and function has been described. Whether this influences the clinically important umbilical artery (UA) waveform remains controversial, although a few cross-sectional studies have shown sex differences in UA pulsatility index (PI). Therefore, we tested whether fetal sex influences the UA Doppler indices during the entire second half of pregnancy and aimed to establish sex-specific reference ranges for UA Doppler indices if needed.

Methods: Our main objective was to investigate gestational age-associated changes in UA Doppler indices during the second half of pregnancy and compare the values between male and female fetuses. This was a prospective longitudinal study in women with singleton low-risk pregnancies during 19–40 weeks of gestation. UA Doppler indices were serially obtained at a 4-weekly interval from a free loop of the umbilical cord using color-directed pulsed-wave Doppler ultrasonography. Sex-specific reference intervals were calculated for the fetal heart rate (HR), UA PI, resistance index (RI), and systolic/diastolic ratio (S/D) using multilevel modeling.

Results: Complete data from 294 pregnancies (a total of 1261 observations from 152 male and 142 female fetuses) were available for statistical analysis, and sex-specific reference ranges for the UA Doppler indices and fetal HR were established for the last half of pregnancy. UA Doppler indices were significantly associated with gestational age ($P < 0.0001$) and fetal HR ($P < 0.0001$). Female fetuses had 2–8% higher values for UA Doppler indices than male fetuses during gestational weeks 20^{+0}–36^{+6} ($P < 0.05$), but not later. Female fetuses had higher HR from gestational week 26^{+0} until term ($P < 0.05$).

Conclusions: We have determined gestational age-dependent sex differences in UA Doppler indices and fetal HR during the second half of pregnancy, and correspondingly established new sex-specific reference ranges intended for refining diagnostics and monitoring individual pregnancies.

Keywords: Fetal Doppler, Obstetric ultrasound, Placental blood flow, Sex differences, Umbilical artery, Reference ranges

Background

The importance of conducting longitudinal studies and analyzing data accounting for biological differences related to sex has been highlighted a decade ago [1]. Previous studies have shown sex-specific differences in fetal development regarding growth and adaption to intrauterine environment [2]. Male sex is an independent risk factor for unfavorable perinatal outcomes including fetal distress during labor [3, 4], premature birth [5, 6], adverse neonatal outcome [7], and early neonatal death [2]. This has been referred to as "the male disadvantage" [8] and the female neonatal survival advantage is well recognized [9]. However, the total mortality from conception to birth is greater among female fetuses [10]. The human placenta demonstrates sex-related differences at both structural and functional levels [11, 12]. Both birth weight and placental weight [13] are higher for males compared with females. Sexual dimorphism in the regulation and expression of genes, and signaling pathways [14–17], generate differences in placental function and

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intrauterine environment that may lead to sex differences in health status later in life [12, 18].

Antenatal growth charts show significant differences in biometrics between male and female fetuses [19]. When using two-dimensional ultrasonography to assess fetal growth, these sex-specific growth charts perform better in identifying small for gestational age (SGA) fetuses [20] at increased risk of fetal demise [21].

Umbilical artery (UA) Doppler indices, i.e., pulsatility index (PI), resistance index (RI), and systolic/diastolic ratio (S/D) calculated from blood flow velocities, are used as an important clinical tool for evaluating fetal well-being in high-risk pregnancies and to predict outcome of growth restricted fetuses [22]. Their use in high-risk pregnancies has the potential to reduce obstetric interventions and perinatal deaths [23]. The increased UA PI is a marker of raised placental vascular impedance associated with microvascular lesions [24] and correspondingly reduced placental function. Longitudinal reference ranges for UA Doppler indices calculated from both cross-sectional and serial measurements have previously been published [25, 26]. However, these studies do not take into account possible sex differences.

Doppler ultrasonographic studies exploring fetal sex differences in the ductus venosus during the first trimester have shown antagonistic results [27–29]. Another study performed just prior to active labor, in term pregnancies, demonstrated no differences in UA PI, but statistically significant lower values for the middle cerebral artery (MCA) PI, MCA peak systolic velocity (PSV) and normalized umbilical venous blood flow (Q_{uv}) in male compared with female fetuses [30], but these differences did not translate into differences in perinatal outcome. One recent study of the feto-placental circulation and cardiac function during 28–34 gestational weeks showed higher preload and lower afterload (significantly lower UA PI) in male fetuses [31]. In a cross-sectional study investigating maternal hemodynamics and placental circulation, we recently demonstrated significantly lower UA PI in male compared with that in female fetuses at 22^{+0}–24^{+0} weeks of gestation [32].

Based on such observations, the main objective of our present study was to assess the effect of fetal sex on UA Doppler indices during the entire second half of pregnancy and correspondingly establish sex-specific longitudinal reference ranges for clinical use.

Methods

In this study, we used data from a total of 306 healthy pregnant women with uncomplicated singleton pregnancy participating in three prospective longitudinal observational studies that included investigation of feto-placental hemodynamics. The women, all > 18 years old, were recruited at the time of routine ultrasound screening at 17–20 weeks of gestation at the University Hospital of North Norway. The gestational age was based on the biometry of fetal head performed during this scan. Women with singleton pregnancy with no history of any systemic diseases that may affect the course and outcome of pregnancy were included. A history of preeclampsia, intrauterine growth retardation (IUGR), gestational diabetes or preterm labor before 34 weeks in previous pregnancy, multiple pregnancy, maternal smoking, or presence of any chromosomal or major structural fetal anomaly in the current pregnancy were reasons for exclusion. The study protocols were approved by the Regional Committee for Medical and Health Research Ethics –North Norway (REK Nord 74/2001, 52/2005, and 105/2008), and informed written consent was obtained from each participant.

For Doppler ultrasonography, an Acuson Sequoia 512 ultrasound system with a 6-MHz curvilinear transducer (Mountain View, CA, USA) or a Vivid 7 Dimension ultrasound system equipped with a 4MS sector transducer with frequencies of 1.5–4.3 MHz (GE Vingmed Ultrasound AS, Horten, Norway) was used. Four experienced clinicians performed the examinations at approximately 4-weekly intervals. In two of the studies all measurements were performed by two single operators, and in the third study three different operators did the examinations. The sex of the fetus was neither acknowledged nor recorded prenatally during ultrasonography. Each participant was examined 3–6 times by the same clinician, starting from 19 to 22 gestational weeks until delivery. The estimated fetal weight (EFW) was computed at each visit from measurements of the biparietal diameter (BPD), abdominal circumference (AC), and femur length (FL) based on the Hadlock 2 formula [33]. Blood flow velocity waveforms of the UA were obtained from the free-floating loop of the umbilical cord using pulsed-wave Doppler optimizing the insonation with simultaneous use of color Doppler. The angle of insonation was always kept < 15 degrees, and angle correction was used if the angle was not zero. To ensure Doppler recording of the spatial maximum blood velocity, an expanded sample gate of 5–12 mm was used depending on gestational age. The high-pass filter was set at low. The blood flow velocities (i.e., PSV, end-diastolic velocity (EDV), and time-averaged maximum velocity (TAMXV)), and fetal heart rate (HR) were measured online using the maximum velocity envelope recorded over the cardiac cycle. An average of three consecutive cycles were used for analysis. The ALARA (as low as reasonably achievable) principle [34] was employed. At all times during the ultrasonographic examination the mechanical and thermal indices were kept below 1.9 and 1.5, respectively. We recorded the UA blood flow successfully in 1243 out of 1261 (98.57%) observations. The UA

Doppler indices were calculated from the recorded velocities as follows: PI = (PSV - EDV)/TAMXV [35], RI = (PSV - EDV)/PSV [36], and S/D ratio = PSV/EDV [37].

The reproducibility of the Doppler parameters studied, expressed by the intra-observer coefficient of variation (CV) and the inter-observer CV, has previously been assessed and reported [26, 38, 39].

All women had a regular antenatal follow-up according to local guidelines. Following delivery, the course and outcome of pregnancy, including any maternal or fetal complications, gestation at delivery, mode of delivery, birth weight, placental weight, neonatal sex, Apgar scores, umbilical cord blood acid-base status, and neonatal outcome was obtained from the electronic medical records. On the second day post-partum, a pediatrician routinely examined all neonates prior to discharge.

Statistical Analysis Software version 9.3 (SAS institute Inc., Cary, NC, USA) was used for statistical analysis. Logarithmic or power transformations were performed on all numerical variables that were not normally distributed, in order to best meet the criteria of normal distribution. The best transformation for each variable was determined using the Box-Cox regression. Fractional polynominals were used to achieve best-fitting curves in relation to gestational age for each variable, accommodating for nonlinear associations. We used multilevel modeling to construct gestational age-specific reference percentiles from each fitted model according to Royston and Altman [40]. The comparison between groups was done using independent samples *t* test for continuous variables, while the chi-square test was used for categorical variables. The comparison of UA Doppler indices between male and female fetuses was performed for each gestational week after having checked and adjusted for possible confounding factors (fetal HR, EFW, and placental weight) by including a cross-product term between sex and gestational age in the aforementioned multilevel models. The level of statistical significance was set at a two-tailed p value of < 0.05.

Results

From a total study population of 306 women, 12 were excluded because they had < 3 observations, leaving complete data from 294 pregnancies available for statistical analysis. There were 152 male and 142 female fetuses. The baseline characteristics of the study population, including pregnancy and neonatal outcomes, are presented in Table 1. We observed no statistically significant differences between the two groups in any of the listed baseline variables.

There were 650 and 611 observations for male and female fetuses, respectively. The UA Doppler indices (PI, RI, and S/D ratio) and the fetal HR were significantly associated with gestational age ($P < 0.0001$), and there was also an association between UA Doppler indices and fetal HR ($P < 0.0001$). We found no sex differences in EFW at any gestational week, and there was no confounding effect of EFW on UA Doppler indices.

Table 1 Baseline characteristics of the study population and pregnancy outcomes

	Female ($n = 142$)	Male ($n = 152$)	*P* value
Maternal			
Age (years)	29 (range 20–43)	30 (range 18–40)	0.646
Body mass index at booking (kg/m^2)	24.85 ± 4.00	24.45 ± 3.65	0.374
Nulliparous	70 (49.3%)	76 (50.0%)	0.904
Fetal			
Gestational age at birth (days)[a]	279 (range 238–297)	280 (range 234–297)	0.633
Birth weight (g)	3593 ± 431	3603 ± 533	0.860
Placental weight (g)	631 ± 128	645 ± 142	0.385
Fetal-placental ratio	5.84 ± 1.03	5.74 ± 0.98	0.413
5-min Apgar score	10 (range 2–10)	9 (range 0–10)	0.348
Umbilical artery pH	7.25 ± 0.10	7.25 ± 0.08	0.831
Umbilical artery base excess (mmol/L)	−4.16 ± 3.91	−4.54 ± 3.06	0.472
Meconium stained liquor	29 (20.4%)	25 (16.6%)	0.443
Admission to NICU	8 (5.7%)	11 (7.3%)	0.577
Preterm birth, < 37^{+0} weeks' gestation	1 (0.7%)	6 (3.9%)	0.068
Preeclampsia	3 (2.1%)	6 (3.9%)	0.361
SGA/IUGR	1 (0.7%)	3 (2.0%)	0.348
Mode of delivery			
Normal	114 (80.3%)	126 (82.9%)	0.563
Vacuum/forceps	6 (4.2%)	7 (4.6%)	0.874
Cesarean section	22 (15.5%)	19 (12.5%)	0.459

Data are presented as *n* (%), median (range), or mean ± SD, as appropriate
P values were calculated using independent samples *t* test for continuous variables and chi-square tests for categorical variables
NICU neonatal intensive care unit, *SGA* small for gestational age, *IUGR* intrauterine growth retardation
[a]279 days = 39^{+6} weeks, 280 days = 40^{+0} weeks

Sex-specific reference curves for the UA Doppler indices and the fetal HR for gestational weeks 20–40 are presented in Figs. 1 and 2. The reference values with their respective 2.5th, 5th, 10th, 25th, 50th, 75th, 90th, 95th, and 97.5th percentiles are presented in Tables 2, 3, 4, 5, 6, 7, 8, and 9. The corresponding gestational age-related sex differences in the mean values for UA PI, RI, and S/D ratio, all adjusted for fetal HR, are displayed in Fig. 3, along with the gestational age-specific mean fetal HR for male and female fetuses during the second half of pregnancy.

The results for the gestational age-specific sex differences for fetal HR and, for the adjusted UA Doppler

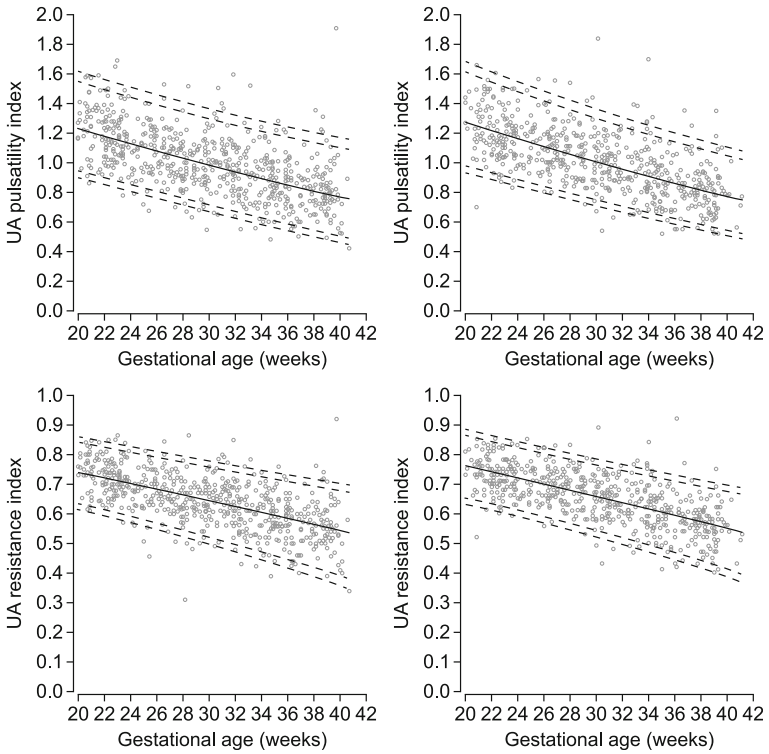

Fig. 1 Umbilical artery pulsatility index and resistance index. Sex-specific reference ranges for umbilical artery (UA) pulsatility index and resistance index (left male, right female). The solid line represents the mean, and the interrupted lines represent 2.5th, 5th, 95th, and 97.5th percentiles

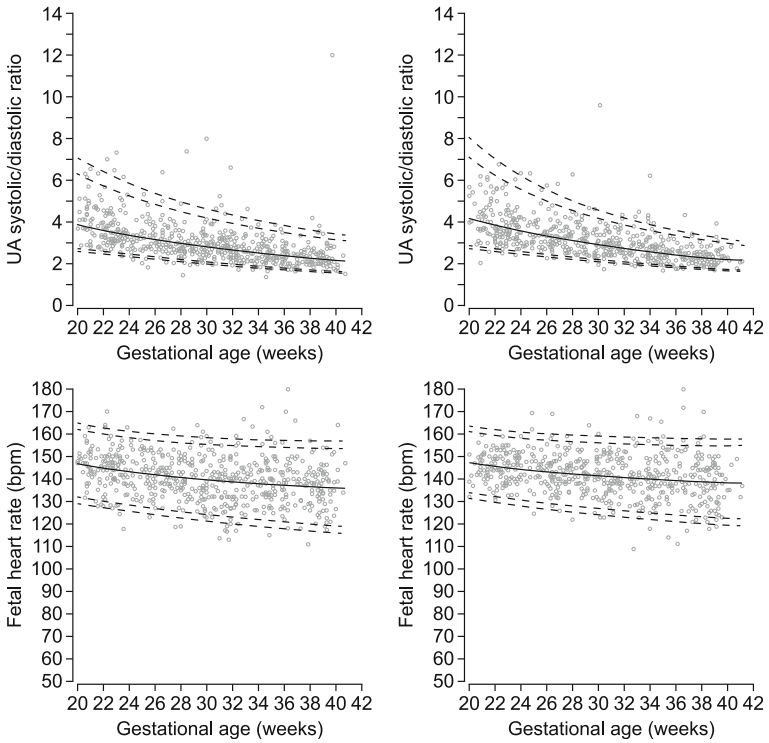

Fig. 2 Umbilical artery systolic/diastolic ratio and heart rate. Sex-specific reference ranges for umbilical artery (UA) systolic/diastolic ratio and fetal heart rate (left male, right female). The solid line represents the mean, and the interrupted lines represent 2.5th, 5th, 95th, and 97.5th percentiles

Table 2 Umbilical artery pulsatility index (male)

Gestation (week)	2.5th percentile	5th percentile	10th percentile	25th percentile	Mean	75th percentile	90th percentile	95th percentile	97.5th percentile
20	0.90	0.95	1.01	1.11	1.23	1.35	1.48	1.55	1.62
21	0.88	0.92	0.98	1.08	1.20	1.33	1.45	1.52	1.59
22	0.85	0.90	0.96	1.06	1.18	1.30	1.42	1.49	1.56
23	0.83	0.88	0.93	1.03	1.15	1.27	1.39	1.47	1.53
24	0.81	0.86	0.91	1.01	1.13	1.25	1.37	1.44	1.51
25	0.79	0.83	0.89	0.98	1.10	1.22	1.34	1.42	1.48
26	0.76	0.81	0.86	0.96	1.08	1.20	1.32	1.39	1.46
27	0.74	0.79	0.84	0.94	1.05	1.17	1.29	1.37	1.43
28	0.72	0.76	0.82	0.91	1.03	1.15	1.27	1.34	1.41
29	0.69	0.74	0.79	0.89	1.00	1.13	1.25	1.32	1.39
30	0.67	0.72	0.77	0.87	0.98	1.10	1.22	1.30	1.36
31	0.65	0.70	0.75	0.84	0.96	1.08	1.20	1.28	1.34
32	0.63	0.67	0.73	0.82	0.94	1.06	1.18	1.25	1.32
33	0.61	0.65	0.70	0.80	0.91	1.04	1.16	1.23	1.30
34	0.58	0.63	0.68	0.78	0.89	1.02	1.14	1.21	1.28
35	0.56	0.61	0.66	0.76	0.87	1.00	1.12	1.19	1.26
36	0.54	0.59	0.64	0.73	0.85	0.98	1.10	1.17	1.24
37	0.52	0.57	0.62	0.71	0.83	0.96	1.08	1.16	1.23
38	0.50	0.54	0.60	0.69	0.81	0.94	1.06	1.14	1.21
39	0.48	0.52	0.58	0.67	0.79	0.92	1.04	1.12	1.19
40	0.46	0.50	0.56	0.65	0.77	0.90	1.02	1.10	1.17

Sex-specific reference values of the umbilical artery pulsatility index (UA PI) for the 2.5th, 5th, 10th, 25th, 50th, 75th, 90th, 95th, and 97.5th percentiles during the second half of pregnancy (male fetuses)

Table 3 Umbilical artery pulsatility index (female)

Gestation (week)	2.5th percentile	5th percentile	10th percentile	25th percentile	Mean	75th percentile	90th percentile	95th percentile	97.5th percentile
20	0.93	0.98	1.04	1.15	1.27	1.41	1.53	1.61	1.68
21	0.91	0.96	1.02	1.12	1.25	1.38	1.50	1.58	1.65
22	0.89	0.94	0.99	1.10	1.22	1.35	1.47	1.54	1.61
23	0.86	0.91	0.97	1.07	1.19	1.32	1.44	1.51	1.58
24	0.84	0.89	0.94	1.04	1.16	1.29	1.41	1.48	1.55
25	0.82	0.86	0.92	1.02	1.13	1.26	1.38	1.45	1.51
26	0.80	0.84	0.90	0.99	1.11	1.23	1.35	1.42	1.48
27	0.77	0.82	0.87	0.97	1.08	1.20	1.32	1.39	1.45
28	0.75	0.80	0.85	0.94	1.06	1.17	1.29	1.36	1.42
29	0.73	0.77	0.83	0.92	1.03	1.15	1.26	1.33	1.39
30	0.71	0.75	0.80	0.90	1.00	1.12	1.23	1.30	1.37
31	0.69	0.73	0.78	0.87	0.98	1.10	1.21	1.28	1.34
32	0.67	0.71	0.76	0.85	0.96	1.07	1.18	1.25	1.31
33	0.64	0.69	0.74	0.83	0.93	1.04	1.15	1.22	1.28
34	0.62	0.67	0.71	0.80	0.91	1.02	1.13	1.20	1.26
35	0.60	0.64	0.69	0.78	0.88	1.00	1.10	1.17	1.23
36	0.58	0.62	0.67	0.76	0.86	0.97	1.08	1.15	1.21
37	0.56	0.60	0.65	0.74	0.84	0.95	1.05	1.12	1.18
38	0.54	0.58	0.63	0.71	0.82	0.92	1.03	1.10	1.16
39	0.52	0.56	0.61	0.69	0.79	0.90	1.01	1.07	1.13
40	0.51	0.54	0.59	0.67	0.77	0.88	0.98	1.05	1.11

Sex-specific reference values of the umbilical artery pulsatility index (UA PI) for the 2.5th, 5th, 10th, 25th, 50th, 75th, 90th, 95th, and 97.5th percentiles during the second half of pregnancy (female fetuses)

Table 4 Umbilical artery resistance index (male)

Gestation (week)	2.5th percentile	5th percentile	10th percentile	25th percentile	Mean	75th percentile	90th percentile	95th percentile	97.5th percentile
20	0.61	0.64	0.66	0.70	0.74	0.78	0.82	0.84	0.86
21	0.60	0.62	0.65	0.69	0.73	0.77	0.81	0.83	0.85
22	0.59	0.61	0.64	0.68	0.72	0.76	0.80	0.82	0.84
23	0.58	0.60	0.63	0.67	0.71	0.76	0.79	0.82	0.84
24	0.57	0.59	0.62	0.66	0.70	0.75	0.78	0.81	0.83
25	0.56	0.58	0.61	0.65	0.69	0.74	0.78	0.80	0.82
26	0.55	0.57	0.60	0.64	0.68	0.73	0.77	0.79	0.81
27	0.53	0.56	0.58	0.63	0.67	0.72	0.76	0.78	0.80
28	0.52	0.55	0.57	0.62	0.66	0.71	0.75	0.77	0.79
29	0.51	0.53	0.56	0.61	0.65	0.70	0.74	0.77	0.79
30	0.50	0.52	0.55	0.60	0.64	0.69	0.73	0.76	0.78
31	0.48	0.51	0.54	0.58	0.63	0.68	0.73	0.75	0.77
32	0.47	0.50	0.53	0.57	0.62	0.67	0.72	0.74	0.76
33	0.46	0.48	0.51	0.56	0.61	0.66	0.71	0.73	0.76
34	0.44	0.47	0.50	0.55	0.60	0.66	0.70	0.73	0.75
35	0.43	0.46	0.49	0.54	0.59	0.65	0.69	0.72	0.74
36	0.42	0.45	0.48	0.53	0.58	0.64	0.68	0.71	0.73
37	0.40	0.43	0.46	0.52	0.57	0.63	0.67	0.70	0.73
38	0.39	0.42	0.45	0.51	0.56	0.62	0.67	0.69	0.72
39	0.37	0.40	0.44	0.49	0.55	0.61	0.66	0.69	0.71
40	0.36	0.39	0.43	0.48	0.54	0.60	0.65	0.68	0.70

Sex-specific reference values of the umbilical artery resistance index (UA RI) for the 2.5th, 5th, 10th, 25th, 50th, 75th, 90th, 95th, and 97.5th percentiles during the second half of pregnancy (male fetuses)

Table 5 Umbilical artery resistance index (female)

Gestation (week)	2.5th percentile	5th percentile	10th percentile	25th percentile	Mean	75th percentile	90th percentile	95th percentile	97.5th percentile
20	0.63	0.65	0.68	0.72	0.76	0.80	0.84	0.86	0.88
21	0.62	0.64	0.67	0.71	0.75	0.79	0.83	0.85	0.87
22	0.61	0.63	0.66	0.70	0.74	0.78	0.82	0.84	0.86
23	0.60	0.62	0.65	0.69	0.73	0.77	0.81	0.83	0.85
24	0.59	0.61	0.64	0.68	0.72	0.76	0.80	0.82	0.84
25	0.58	0.60	0.62	0.67	0.71	0.75	0.79	0.81	0.83
26	0.57	0.59	0.61	0.65	0.70	0.74	0.78	0.80	0.82
27	0.56	0.58	0.60	0.64	0.69	0.73	0.77	0.79	0.81
28	0.54	0.57	0.59	0.63	0.68	0.72	0.76	0.78	0.80
29	0.53	0.56	0.58	0.62	0.67	0.71	0.75	0.77	0.79
30	0.52	0.54	0.57	0.61	0.66	0.70	0.74	0.76	0.78
31	0.51	0.53	0.56	0.60	0.65	0.69	0.73	0.75	0.77
32	0.50	0.52	0.55	0.59	0.64	0.68	0.72	0.74	0.76
33	0.48	0.51	0.53	0.58	0.63	0.67	0.71	0.74	0.76
34	0.47	0.49	0.52	0.57	0.61	0.66	0.70	0.73	0.75
35	0.46	0.48	0.51	0.55	0.60	0.65	0.69	0.72	0.74
36	0.44	0.47	0.50	0.54	0.59	0.64	0.68	0.71	0.73
37	0.43	0.45	0.48	0.53	0.58	0.63	0.67	0.70	0.72
38	0.41	0.44	0.47	0.52	0.57	0.62	0.66	0.69	0.71
39	0.40	0.43	0.46	0.51	0.56	0.61	0.65	0.68	0.70
40	0.38	0.41	0.44	0.49	0.55	0.60	0.65	0.67	0.69

Sex-specific reference values of the umbilical artery resistance index (UA RI) for the 2.5th, 5th, 10th, 25th, 50th, 75th, 90th, 95th, and 97.5th percentiles during the second half of pregnancy (female fetuses)

Table 6 Umbilical artery systolic/diastolic ratio (male)

Gestation (week)	2.5th percentile	5th percentile	10th percentile	25th percentile	Mean	75th percentile	90th percentile	95th percentile	97.5th percentile
20	2.6	2.7	2.9	3.3	3.9	4.6	5.5	6.3	7.1
21	2.5	2.7	2.8	3.2	3.7	4.4	5.3	6.0	6.7
22	2.4	2.6	2.8	3.1	3.6	4.3	5.1	5.7	6.4
23	2.4	2.5	2.7	3.0	3.5	4.1	4.9	5.5	6.1
24	2.3	2.4	2.6	2.9	3.4	4.0	4.7	5.3	5.8
25	2.2	2.4	2.5	2.8	3.3	3.8	4.5	5.0	5.6
26	2.2	2.3	2.5	2.8	3.2	3.7	4.4	4.9	5.4
27	2.1	2.2	2.4	2.7	3.1	3.6	4.2	4.7	5.2
28	2.1	2.2	2.3	2.6	3.0	3.5	4.1	4.5	5.0
29	2.0	2.1	2.3	2.5	2.9	3.4	3.9	4.3	4.8
30	2.0	2.1	2.2	2.5	2.8	3.3	3.8	4.2	4.6
31	1.9	2.0	2.1	2.4	2.7	3.2	3.7	4.1	4.5
32	1.9	2.0	2.1	2.3	2.7	3.1	3.6	3.9	4.3
33	1.8	1.9	2.0	2.3	2.6	3.0	3.5	3.8	4.2
34	1.8	1.9	2.0	2.2	2.5	2.9	3.4	3.7	4.1
35	1.7	1.8	1.9	2.2	2.5	2.8	3.3	3.6	3.9
36	1.7	1.8	1.9	2.1	2.4	2.8	3.2	3.5	3.8
37	1.7	1.8	1.9	2.1	2.3	2.7	3.1	3.4	3.7
38	1.6	1.7	1.8	2.0	2.3	2.6	3.0	3.3	3.6
39	1.6	1.7	1.8	2.0	2.2	2.6	2.9	3.2	3.5
40	1.6	1.6	1.7	1.9	2.2	2.5	2.9	3.1	3.4

Sex-specific reference values of the umbilical artery systolic/diastolic (UA S/D) ratio for the 2.5th, 5th, 10th, 25th, 50th, 75th, 90th, 95th, and 97.5th percentiles during the second half of pregnancy (male fetuses)

Table 7 Umbilical artery systolic/diastolic ratio (female)

Gestation (week)	2.5th percentile	5th percentile	10th percentile	25th percentile	Mean	75th percentile	90th percentile	95th percentile	97.5th percentile
20	2.7	2.9	3.1	3.5	4.2	5.0	6.2	7.1	8.1
21	2.6	2.8	3.0	3.4	4.0	4.8	5.8	6.6	7.5
22	2.6	2.7	2.9	3.3	3.8	4.6	5.5	6.2	7.0
23	2.5	2.6	2.8	3.2	3.7	4.4	5.2	5.9	6.6
24	2.4	2.6	2.7	3.1	3.6	4.2	5.0	5.6	6.2
25	2.4	2.5	2.7	3.0	3.4	4.0	4.7	5.3	5.8
26	2.3	2.4	2.6	2.9	3.3	3.9	4.5	5.0	5.5
27	2.3	2.4	2.5	2.8	3.2	3.7	4.3	4.8	5.3
28	2.2	2.3	2.5	2.7	3.1	3.6	4.2	4.6	5.0
29	2.1	2.3	2.4	2.6	3.0	3.5	4.0	4.4	4.8
30	2.1	2.2	2.3	2.6	2.9	3.3	3.8	4.2	4.6
31	2.0	2.1	2.3	2.5	2.8	3.2	3.7	4.0	4.4
32	2.0	2.1	2.2	2.4	2.7	3.1	3.6	3.9	4.2
33	1.9	2.0	2.2	2.4	2.7	3.0	3.4	3.7	4.0
34	1.9	2.0	2.1	2.3	2.6	2.9	3.3	3.6	3.9
35	1.9	1.9	2.0	2.2	2.5	2.8	3.2	3.5	3.7
36	1.8	1.9	2.0	2.2	2.4	2.8	3.1	3.4	3.6
37	1.8	1.9	2.0	2.1	2.4	2.7	3.0	3.3	3.5
38	1.7	1.8	1.9	2.1	2.3	2.6	2.9	3.2	3.4
39	1.7	1.8	1.9	2.0	2.3	2.5	2.8	3.1	3.3
40	1.7	1.7	1.8	2.0	2.2	2.5	2.8	3.0	3.2

Sex-specific reference values of the umbilical artery systolic/diastolic (UA S/D) ratio for the 2.5th, 5th, 10th, 25th, 50th, 75th, 90th, 95th, and 97.5th percentiles during the second half of pregnancy (female fetuses)

Table 8 Fetal heart rate (male), beats per minute

Gestation (week)	2.5th percentile	5th percentile	10th percentile	25th percentile	Mean	75th percentile	90th percentile	95th percentile	97.5th percentile
20	129	132	135	141	147	153	159	162	165
21	128	131	134	140	146	152	157	161	164
22	127	130	133	139	145	151	156	160	163
23	127	129	133	138	144	150	156	159	162
24	126	129	132	137	143	149	155	158	161
25	125	128	131	136	142	149	154	158	160
26	124	127	130	136	142	148	154	157	160
27	123	126	130	135	141	147	153	156	159
28	123	126	129	134	141	147	153	156	159
29	122	125	128	134	140	146	152	156	159
30	121	124	128	133	140	146	152	155	158
31	121	124	127	133	139	146	152	155	158
32	120	123	127	132	139	145	151	155	158
33	120	123	126	132	138	145	151	155	158
34	119	122	125	131	138	145	151	154	158
35	118	121	125	131	138	144	151	154	157
36	118	121	125	131	137	144	150	154	157
37	117	120	124	130	137	144	150	154	157
38	117	120	124	130	137	144	150	154	157
39	116	120	123	129	136	143	150	154	157
40	116	119	123	129	136	143	150	154	157

Sex-specific reference values of the fetal heart rate (HR) for the 2.5th, 5th, 10th, 25th, 50th, 75th, 90th, 95th, and 97.5th percentiles during the second half of pregnancy (male fetuses)

Table 9 Fetal heart rate (female), beats per minute

Gestation (week)	2.5th percentile	5th percentile	10th percentile	25th percentile	Mean	75th percentile	90th percentile	95th percentile	97.5th percentile
20	131	134	137	142	147	153	158	161	164
21	131	133	136	141	146	152	157	160	163
22	130	132	135	140	146	151	156	159	162
23	129	132	134	139	145	151	156	159	161
24	128	131	134	139	144	150	155	158	161
25	127	130	133	138	144	150	155	158	161
26	127	129	132	138	143	149	154	157	160
27	126	129	132	137	143	149	154	157	160
28	125	128	131	136	142	148	154	157	160
29	125	127	131	136	142	148	153	156	159
30	124	127	130	135	141	147	153	156	159
31	124	126	130	135	141	147	153	156	159
32	123	126	129	135	141	147	152	156	159
33	123	125	129	134	140	147	152	156	159
34	122	125	128	134	140	146	152	156	159
35	122	124	128	133	140	146	152	155	158
36	121	124	127	133	139	146	152	155	158
37	121	124	127	133	139	146	152	155	158
38	120	123	127	132	139	145	151	155	158
39	120	123	126	132	139	145	151	155	158
40	120	123	126	132	138	145	151	155	158

Sex-specific reference values of the fetal heart rate (HR) for the 2.5th, 5th, 10th, 25th, 50th, 75th, 90th, 95th, and 97.5th percentiles during the second half of pregnancy (female fetuses)

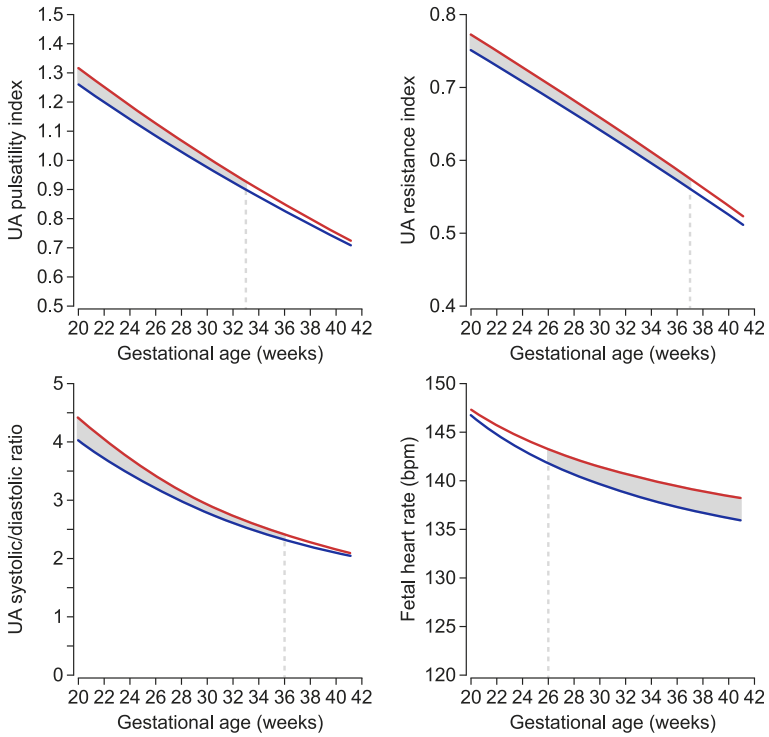

Fig. 3 Sex differences in fetal heart rate and umbilical artery Doppler indices adjusted for fetal heart rate. Gestational age-related sex differences in the mean values for umbilical artery (UA) pulsatility index (top left), resistance index (top right), systolic/diastolic ratio (bottom left), all adjusted for fetal heart rate, and fetal heart rate (bottom right) during the second half of pregnancy. The red line represents female, and the blue line represents male. The shaded area indicates significant differences ($P < 0.05$)

indices, are shown in Table 10. We found significant differences in UA PI, RI, S/D ratio, and HR between male and female fetuses. Female fetuses had significantly higher values for UA PI (range 2.1–4.2%), RI (range 1.7–3.3%), and S/D ratio (range 4.0–8.1%) from 20^{+0} weeks to 32^{+6} and 36^{+6} and 35^{+6} weeks, respectively, but equalized towards term (40 weeks of gestation). For fetal HR, the mean values were similar between male and female fetuses from 20^{+0} to 25^{+6} weeks, but a divergent trend was observed thereafter with the female fetuses showing higher HR (range 0.7–2.2%) compared with male fetuses.

Discussion

The present longitudinal study has demonstrated significant sex differences in UA Doppler indices, female fetuses having significantly more pulsatile waveform than male fetuses during gestational weeks 20^{+0}–36^{+6} but not thereafter. The magnitude of effect ranged between 2.1 and 4.2% for the UA PI. Correspondingly, the study provided sex-specific reference ranges for 20–40 weeks' gestation for the most commonly used indices. As for the fetal HR, the pattern was different; male and female fetuses had similar HR from 20^{+0} to 25^{+6} weeks, but thereafter, the female fetuses had significantly higher HR.

The strength of the study is its longitudinal design and a relatively large sample size (650 observations for male and 611 for female fetuses) providing sufficient power to discover significant sex differences and to construct robust sex-specific reference ranges. The prospective longitudinal design with serial measurements at reasonably spaced intervals during pregnancy is preferable to a cross-sectional design for constructing reference intervals since it better reflects the development during gestation and, in our case, improves the precision of individual participants' observations. The limitations of our study are related to technical issues concerning UA Doppler velocimetry, and the data being collected from three separate studies, with different operators. However, all the measurements were obtained at a free loop of umbilical cord, under fetal quiescence, keeping the angle of insonation as low as possible (always < 15°). The intra-observer CV for UA PI, RI, and S/D ratio were 10.5, 6.8, and 13.0%, respectively [26].

This study confirms the findings of previous cross-sectional studies that report sex differences in UA Doppler indices during the second and third trimester of pregnancy [31, 32] and that these differences tapered off towards term [30]. However, we were not able to establish at what time in gestation these differences emerged.

Table 10 Level of significance for sex differences in umbilical artery Doppler indices and fetal heart rate

Gestation (week)	UA PI[a]	UA RI[a]	UA S/D ratio[a]	Fetal HR
	P value	*P* value	*P* value	*P* value
20	0.00795	0.00282	0.00213	0.58551
21	0.00695	0.00219	0.00171	0.44020
22	0.00621	0.00172	0.00139	0.30634
23	0.00573	0.00139	0.00117	0.19662
24	0.00552	0.00117	0.00103	0.11804
25	0.00564	0.00104	0.00097	0.06888
26	0.00616	0.00102	0.00100	0.04136
27	0.00724	0.00109	0.00113	0.02704
28	0.00915	0.00132	0.00141	0.01991
29	0.01234	0.00178	0.00196	0.01656
30	0.01748	0.00264	0.00295	0.01526
31	0.02550	0.00421	0.00473	0.01518
32	0.03755	0.00699	0.00784	0.01586
33	0.05486	0.01172	0.01309	0.01706
34	0.07849	0.01939	0.02150	0.01864
35	0.10911	0.03110	0.03424	0.02049
36	0.14679	0.04786	0.05239	0.02253
37	0.19098	0.07041	0.07670	0.02470
38	0.24064	0.09901	0.10746	0.02696
39	0.29443	0.13342	0.14444	0.02926
40	0.35092	0.17300	0.18692	0.03159
Overall[b]	0.07560	0.01850	0.01980	0.02560

The results for the gestational age-specific sex differences in mean values for fetal heart rate (HR) and for the adjusted umbilical artery (UA) Doppler indices, organized by gestational week

UA umbilical artery, *PI* pulsatility index, *RI* resistance index, *S/D ratio* systolic/diastolic ratio, *HR* heart rate

[a]Adjusted for fetal heart rate

[b]Overall level of significance for sex differences during 20–40 weeks of gestation

It would have been desirable to have serial measurements starting from early pregnancy.

Our findings add weight to the recognition of sex differences in fetal development and adaption to the intrauterine environment. The male disadvantage in perinatal outcome when it comes to fetal distress during labor [3, 4], premature birth [5, 6], adverse neonatal outcome [7], and early neonatal death [2] is well documented. It is also well documented that there are significant sex differences in growth of estimated fetal weight [19], birth weight, and placental weight [13], and male and female fetuses have significant differences in growth patterns of individual biometric measurements [41]. Such differences in growth dynamics corroborate the findings of Orzack et al. [10] who found that the unbiased male/female ratio at conception had increased at birth due to a higher female mortality during pregnancy. However, male and female mortality during pregnancy had temporal differences causing undulations in the sex ratio. These findings constitute a plethora of details in which our circulatory results add another piece of evidence to sex differentiation being reflected in all organ systems.

With this background, our finding that there was no significant effect of fetal sex on fetal growth (i.e., EFW) is unexpected, as a recent multinational study showed that fetal sex had an effect of 3.5–4.5% on EFW [42]. However, that study had a considerably higher power than our present study. One can therefore speculate that the present finding of no sex effect on fetal weight could be due to chance, or, as shown in the recent WHO study, due to variations in growth patterns. However, the negligible (10 g) difference in birth weight we observed between the sexes (3593 vs. 3603 g) corroborates our intrauterine growth estimates and ensures that the effect on the Doppler indices was due to sex differences. The issue is important because a difference in size could possibly have explained some of the results. It is interesting, however, that a previous study found "no meaningful correlation between fetal weight and impedance indices" [43].

Mechanisms associated with potential male susceptibility are difficult to underpin. Male fetuses appear to prioritize growth to a greater extent than females and continue to grow in spite of unfavorable intrauterine environment [14]. This may put them at higher risk due to lack of reserve. A higher UA PI, as we have observed in females, could result in a reduction in fetal growth velocity and thereby reduce the risk of adverse outcomes. The mechanisms behind the observed differences in UA Doppler indices are not clear. Slightly higher UA Doppler indices cannot be equated to reduced placental function, and these differences were less pronounced close to term. However, it has been shown that male fetuses born at 24–28 weeks of gestation have more peripheral vasodilatation compared to female fetuses [44]. Furthermore, pregnant women carrying male fetuses are reported to have higher angiotensin (Ang) 1–7 to Ang II ratio in the second trimester [45]. As Ang II is a potent vasoconstrictor and Ang 1–7 is a known vasodilator, relative vasodilatation of placental vessels could be responsible for lower UA PI, RI, and S/D ratio observed in male fetuses.

UA Doppler indices, a surrogate for placental impedance [46], have proved valuable in assessing fetal wellbeing and have the potential to save lives [23]. However, these relations are not consistent [47], as shown in sheep experiments [48, 49]. Although PI increases when embolization causes reduction in vascular cross-section, comparable reduction in vascular cross-section due to angiotensin II did not increase the PI and could even decrease the PI while vascular resistance increased. The reason for this may be a difference in vessel geometry that could impact the wave reflection, a major modifier of the arterial waveform [50,

51]. Thus, the exact mechanism behind the sex difference in the UA pulsatility is not certain.

Another significant finding in the present study was the relatively higher HR in female compared with male fetuses after 26^{+0} weeks of gestation, a difference that increased with gestational age (Fig. 3). Higher HR among female fetuses has also been reported previously by others [31, 52]. A plausible cause for having different heart rates in male and female fetuses is differences in hormone levels and rate of maturation of their autonomic nervous system. Higher heart rate variability [53], more complex heart rate patterns [54], and higher catecholamine levels observed in female compared to male fetuses could explain these differences.

In fetal sheep experiments, Morrow et al. demonstrated a significant inverse correlation between the UA Doppler indices (PI, RI, and S/D ratio) and HR [51]. When the HR increased, the UA Doppler indices decreased. We found both higher HR and UA PI, RI, and S/D ratio in female fetuses compared to males, but while the sex differences in HR increased as the pregnancy advanced, the sex differences in the Doppler indices decreased and ceased to exist by term. When we adjusted the gestational age-related sex differences in the mean values for UA PI, RI, and S/D ratio for the fetal HR, the effect size actually increased, i.e., the sexual dimorphism in the UA Doppler indices became more prominent.

Several studies have shown a male preponderance when abnormal UA Doppler waveform is used as a marker of placental dysfunction in pregnancies with IUGR [55, 56]. Increased UA PI correlates with reduced feto-placental perfusion [57] and the degree of microvascular lesions in the placenta [24]. Use of sex-specific reference intervals of UA Doppler indices could potentially improve the identification of pregnancies with placental dysfunction.

Conclusions

We have demonstrated gestational age-dependent sex differences in UA Doppler indices during the second half of physiological pregnancies and therefore established sex-specific reference ranges. Although the sex difference is modest (2–8%), we believe such references are useful for refining prediction and monitoring of risk pregnancies at a time when such parameters easily are added into software applications increasingly used in clinical practice, particularly since individualized diagnostics and management is an issue.

Abbreviations

AC: Abdominal circumference; ALARA: As low as reasonably achievable; BMI: Body mass index; CV: Coefficient of variation; EDV: End-diastolic velocity; EFW: Estimated fetal weight; FL: Femur length; HR: Heart rate; IUGR: Intrauterine growth retardation; MCA: Middle cerebral artery; PI: Pulsatility index; PSV: Peak systolic velocity; Q_{uv}: Umbilical venous blood flow; RI: Resistance index; S/D ratio: Systolic/diastolic ratio; SD: Standard deviation; SGA: Small for gestational age; TAMXV: Time-averaged maximum velocity; UA: Umbilical artery

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Availability of data and materials

The datasets generated and analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

CW, TK, and GA participated in the conception and design of the experiments. KF and GA recruited the patients and performed the ultrasound examinations. CW, KF, and GA participated in the collection of data. CW and TW performed the statistical analysis. CW, TK, and GA made substantial contributions to the interpretation of the data and writing of the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The study protocols were approved by the Regional Committee for Medical and Health Research Ethics–North Norway (REK Nord 74/2001, 52/2005, and 105/2008) and an informed written consent was obtained from each participant.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Publisher's Note

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