From the Department of Woman and Child Health,
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The Human Placenta
An Angiographic Study

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To

Måns, Gustaf, Josefin,
Carl Fredrik, Kristina,
Maria and Disa

So, naturalists observe, a flea
Hath smaller fleas that on him prey
And these have smaller still to bite ´em
And so proceed ad infinitum

Jonathan Swift

Abbreviations

AGA	appropriate for gestational age
BFC	blood flow class
FVW	flow velocity waveform
IUGR	intrauterine growth retardation
PI	pulsatility index
SD	standard deviation
S/D ratio	peak systolic velocity/minimum diastolic velocity
SGA	small for gestational age
SUA	single umbilical artery
UA	umbilical artery

List of papers

This thesis is based upon the following papers which will be referred to in the text by their Roman numerals:

I. Placental morphology in relation to umbilical artery blood velocity waveforms.
Margareta Nordenvall, Ulla Ullberg, Jan Laurin, Göran Lingman, Bengt Sandstedt and Ulf Ulmsten.
European Journal of Obstetrics& Gynecology and Reproductive Biology 1991;40:179-190

II. Scaling properties of the placenta's arterial tree.
Dan Bergman and Ulla Ullberg.
Journal of Theoretical Biology 1998; 193: 731-738

III. The fractal dimension of the placenta's arterial tree is lower in intra-uterine growth retardation with pathologic umbilical blood flow velocity waveforms.
Ulla Ullberg, Dan Bergman and Bengt Sandstedt.
Manuscript

IV. Hyrtl's anastomosis, the only connection between the two umbilical arteries. A study in full term placentas from AGA infants with normal umbilical artery blood flow.
Ulla Ullberg, Bengt Sandstedt and Göran Lingman
Acta Obstetricia et Gynaecologica Scandinavica, 2001; 80, 1-6

V. Hyrtl's anastomosis is normally developed in placentas from small for gestational age infants.
Ulla Ullberg, Göran Lingman, Gunvor Ekman-Ordeberg and Bengt Sandstedt.
In Press, Acta Obstetricia et Gynaecologica Scandinavica

Abstract

The arterial vessels from the umbilical cord spreading over the chorion and its cotyledonary branches constitute the main body of the placental vascular tree. In the present study it was visualized by angiography and related to placental developmental factors. The results were correlated with intrauterine growth and umbilical artery blood flow velocity waveforms (FVW).

The association between FVW and placental morphology was investigated by angiography in 30 pregnancies at risk for intrauterine growth retardation (IUGR). Placentas from fetuses with an end-diastolic zero flow were small and thick with an extrachorial configuration, marginal cord insertion, magistral or mixed allantochorial vessel pattern and few cotyledons. The incidence and the extension of gross lesions were only slightly increased. The results show that placental developmental factors are associated with fetal growth and abnormal FVW.

To further evaluate the arterial pattern, fractal geometry was accomplished and showed to be an applicable model. In 22 placentas from uncomplicated pregnancies with normal FVW, the scaling properties were as good as for constructed perfect fractals. The fractal dimension was in mean 1.864. The results suggest that there is an underlying regular mechanism behind the placental vasculogenesis. Sixteen placentas from SGA fetuses with pathologic FVW showed a significantly decreased fractal dimension (mean 1.755), indicating a lower grade of complexity.

The anatomy of the frequent connection between the two umbilical arteries, Hyrtl's anastomosis, was investigated in 67 placentas from full term infants appropriate for gestational age (AGA) and in 64 placentas from infants small for gestational age (SGA). The anastomosis was constituted by a separate vessel in 112 placentas (85 %), a fenestration in 6 (5%) and a fusion in 3 (2%). In 10 the anastomosis was absent, in five of these due to a single umbilical artery. The anastomosis was wide in placentas in which the relative area supplied by each umbilical artery was asymmetric and thinner with a higher degree of symmetry. Placentas lacking anastomosis were highly symmetric. The occurrence and anatomy of Hyrtl's anastomosis seemed to depend on the demand on shunting between the umbilical arteries, and did not differ between placentas from AGA and SGA infants.

The results show that the placental arterial pattern reveals abnormalities in early placental expansion and development and is linked with impaired fetal growth and pathologic FVW. The structure of Hyrtl's anastomosis is related to the size of the umbilical arteries supply areas proving its role as a shunting mechanism.

Key words: anatomy, angiography, fractal dimension, FVW, Hyrtl's anastomosis, IUGR, placenta, SGA, umbilical artery, vessel pattern.

Contents

Background

The placenta is the largest fetal organ. It has a vital function replacing the lungs, gastrointestinal tract, kidneys and liver during fetal life through maintaining the feto-maternal exchange of oxygen, nutrients and waste products. A well-functioning placenta, the feto-maternal interface, is a prerequisite for the development of a healthy, normal-sized infant. Fetal growth restriction is a substantial cause of perinatal morbidity and mortality (Simchen et al., 2000), but it also results in an enhanced risk of disease in later life (Barker et al., 2002) and even an adverse effect on the next generation (Barker et al., 2000). In fact, aside from developmental and teratogenic processes affecting the fetus alone, all adverse pregnancy outcomes represent failure of the placenta with its uteroplacental and fetoplacental connections (Redline, 1995)

The placenta has always been an object of wonder. For the early Egyptians, the placenta was the seat of the "external soul". Special respect was paid to the royal placenta, a representation of which was figured on a standard, which down to the time of Ptolemy was carried in ceremonial processions in front of the reigning Pharaoh (Boyd and Hamilton, 1970).

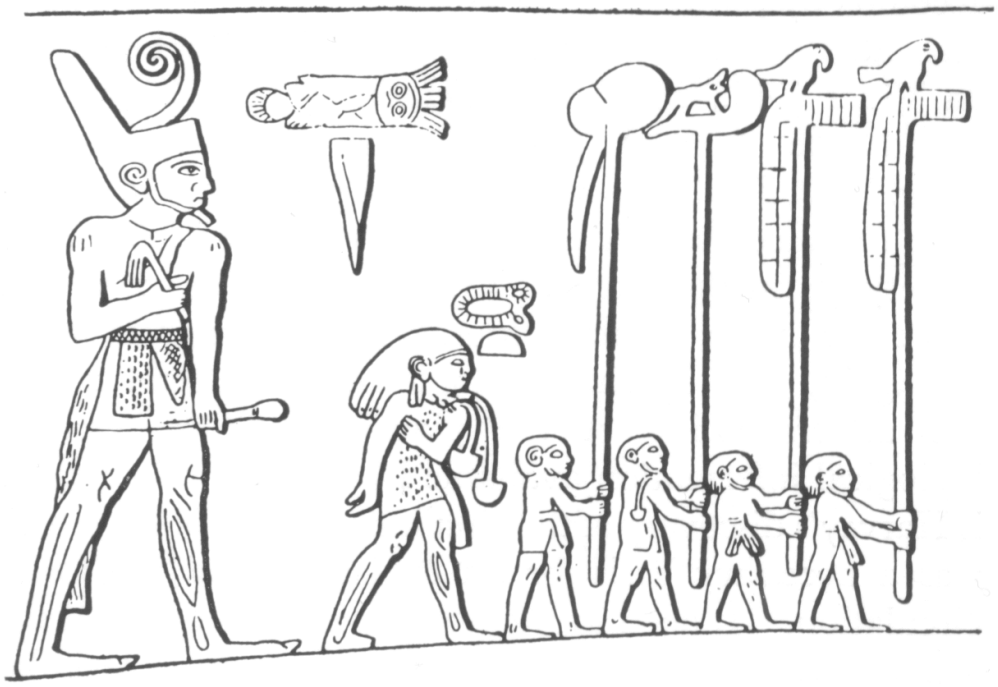


Figure 1. The Pharaoh in a procession as depicted on an Egyptian ceremonial slate of 3400 B.C. The fourth of the standards represents the royal placenta with the umbilical cord.

Awareness of the umbilical cord as a vital link between mother and child is attributed to Aristotle (384-322 B.C.). Galen (circa 130-200) maintained that an artery and a vein running from the uterus into the placenta provided direct communications between the maternal and fetal blood, the arteries supplying "spiritual blood" and the veins supplying "alimentary blood".

On into the seventeenth century many theories flourished about the means and meaning of fetal nutrition (Hallin, 1989): that the embryo is nourished directly by menstrual blood, through the umbilical cord, through pores in its skin, through its mouth by the uterine milk or by the amniotic fluid (Courvee, 1655).

Facts gradually emerged. Harvey's concept in 1628 of the general arterio-venous circulation and Malpighi's description in 1660 of capillaries established a rational anatomic basis for regional circulation as in the placenta. John Mayow in 1668 demonstrated that "nitro-aerial particles", in the next century identified as oxygen, were transferred from the maternal to the fetal circulation.

Etymologically, the word placenta is Latin for a circular cake. As a term for the thick part of the human fetal membranes it was introduced by the anatomist Realdus Columbus in 1559.

Placental development

The fertilized human ovum is transported along the tube while cleaving and enters the uterus as a morula 84 to 96 hours after ovulation. During the next two or three days the morula converts into a blastocyst through storage of intercellular fluid within the space walled off by the trophoblast, and implants in the decidua by mutually supporting processes in the maternal and embryonic tissues. The mural trophoblast represents an early step in the development of the membranes that are to enclose the embryo. From the inner cell mass of the implanted blastocyst, vasculogenesis (de novo formation of blood vessels) will start separately in the intra and extraembryonic mesoderm and later form the fetoplacental vascular unit connected by the umbilical cord. By 8 weeks of post-menstrual gestation a primitive fetoplacental circulation is established. There is not yet any effective oxygen transfer since these villi are immature and the intervillous space is not perfused by oxygenated maternal blood (Kingdom and Kaufmann, 1999). The early

connection of the maternal and foetal vascular system is a cascade of molecular events. Later the number and spacing of the spiral arteries will physiologically mainly govern the development of the placental vascular tree. Between 8 and 12 weeks of gestation the uteroplacental spiral arteries are invaded by the extravillous trophoblast, which is a crucial event to render the vessels unresponsive to vasomotor influence. A failure of this invasion is specific for preeclampsia, but also for a number of growth retarded infants from normotensive pregnancies (Khong et al., 1986).

In the first two months of gestation, the entire chorion is vascularized from the umbilical arteries. Thereafter, the chorionic arteries are progressively restricted to the decidua parietalis and the arterial branches and villi related to the decidua capsularis regress to form the definitive placenta and chorion laeve. Failure of development of these due to abnormal vascularisation in the adjacent endometrium or failure of regression in chorion laeve will result in abnormal placental shape or cord insertion (Krone, 1961, Boyd and Hamilton, 1970, Sandstedt 1974). By the end of the third month almost all of the decidua capsularis is fused with the chorion laeve. Branching angiogenesis continues until 24 weeks. Thereafter non-branching angiogenesis gradually takes over. In the third trimester the gross vessel pattern has reached its final shape (Kingdom, Burrell and Kaufmann 1997). Only vessel dimension but not the dividing pattern or the number of vessels will change.

Connections between the two umbilical arteries at the cord insertion involute progressively throughout gestation. Numerous anastomoses are reported to occur between the third and fourth gestation week (Stieve and Strube, 1933, Patten, 1946) and a 3.5 mm embryo has shown 3 anastomoses (Young, 1972). Term placentas show one or (less frequently) no anastomosis.

The maternofetal diffusion distance through the placental barrier, which will be described below, is reduced from 50 to 100 μm in the second month to between 4 and 5 μm at term.

Anatomy

The anatomy of a normal human third-trimester placenta is shown in figure 2. However, no organ shows such a wide variation in its anatomy, both between species and between individuals, as the placenta. In broad outline, the placenta consists of the two membranes amnion and chorion, the chorionic villi, the intervillous space and the decidual plate. The border between the genetically different fetal and maternal part of the placenta is a thin fibrin layer in the decidua. The “functional border” is

the above mentioned placental barrier between the fetal blood in the villous capillaries and the maternal blood in the intervillous space.

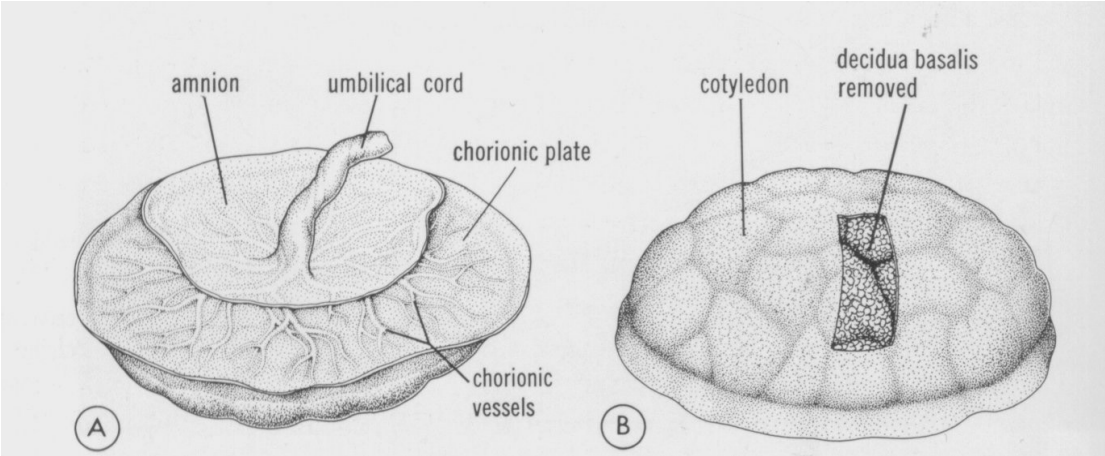


Figure 2. The placenta a) fetal aspect b) maternal aspect post partum. The amnion and decidua basalis are partially removed to show underlying structure.

The two umbilical arteries, which lead blood deficient in oxygen from the fetus to the placenta, and the umbilical vein, which returns oxygenated blood to the fetus, at first spread and divide in the two-dimensional plane between the amnion and the chorion on the fetal side of the placenta, constituting the allantochorial vessels. The allantochorial arteries form either a disperse pattern where they divide dichotomously, in two equal branches, with gradually diminishing diameter or a magistral pattern where the arteries on the contrary run almost to the placental margin before their calibre diminishes, giving off small branches along their course (Shordania, 1929a). A mixed pattern is an intermediate variety that is not uncommon (Bacsich and Smout, 1938).

The allantochorionic arteries of the first to fourth order give rise to a branch, which perpendicularly penetrates the chorionic plate, a primary cotyledonary artery. This vessel, together with the corresponding vein, surrounded by mesenchymal tissue and covered by two layers of trophoblast, forms a stem villus, which bathes in the maternal blood in the intervillous space. Each stem villus gives rise to one functional unit, a cotyledon, formed by its secondary and tertiary branches. A normal placenta contains 15 to 30 cotyledons and each cotyledon has one to five subcotyledons, small replicas of the cotyledons.

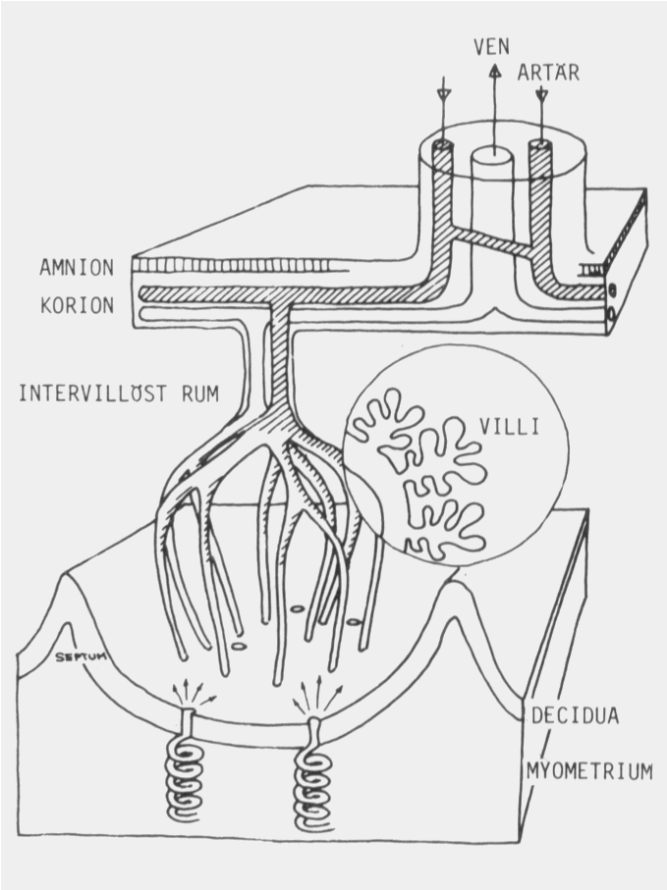


Figure 3. Sketch of a functional unit.

The allantochorial arteries and their branches are end-arteries. In most placentas there is a single anastomosis, first described by Hyrtl (1870), between the umbilical arteries near the cord insertion. The anatomy of Hyrtl's anastomosis is highly variable. There is no additional connection between the branches of the umbilical arteries.

The veins roughly follow the course of the arteries, showing similar branching pattern but seldom with congruent kinks and curves (Arts, 1961, Bhargava and Raja, 1970). Most often the artery passes superficial to the vein at crossings.

The maternal blood is supplied by the spiral arteries, which penetrate the decidua and provide oxygenated blood to the intervillous space. They are derived from the radial arteries, which in turn originate from the arcuate arteries, branches of the uterine arteries. The trophoblast invasion of the spiral arteries mentioned above changes them from small muscular arteries to flaccid tubes with no muscularis or elastic lamina and a diameter at least four times greater than before pregnancy.

Placental pathology

Developmental anomalies

The overall size of the placenta certainly is limiting for the size of the feto-maternal exchange area, but there are also structural developmental anomalies, which are discussed in relation to poor function. The general background for developmental anomalies in single pregnancies is malimplantation of the blastula in an endometrial area with insufficient vascularisation due to for instance uterine inflammation, anomalies or low implantation, which will lead to abnormal placental shape and marginal or velamentous cord insertion. To compensate for this interference with the early expansion of the placenta the concept of "trophotropism", i.e. the migration of the placenta to sites with the best nourishment, has been used (Krone, 1961). Consequently the same anomalies are more usual in twinning and in single umbilical artery.

Configuration. Irregular noncircular or non-ovoid shapes including multilobed placentas reflect abnormalities in implantation (Lewis and Perrin, 1999, Fujikura et al., 1970). A bilobed placenta is shown in fig. 6.

Extrachorial membranes means that the chorion laeve rather than inserting at the disk margin inserts at varying degrees inside that margin.

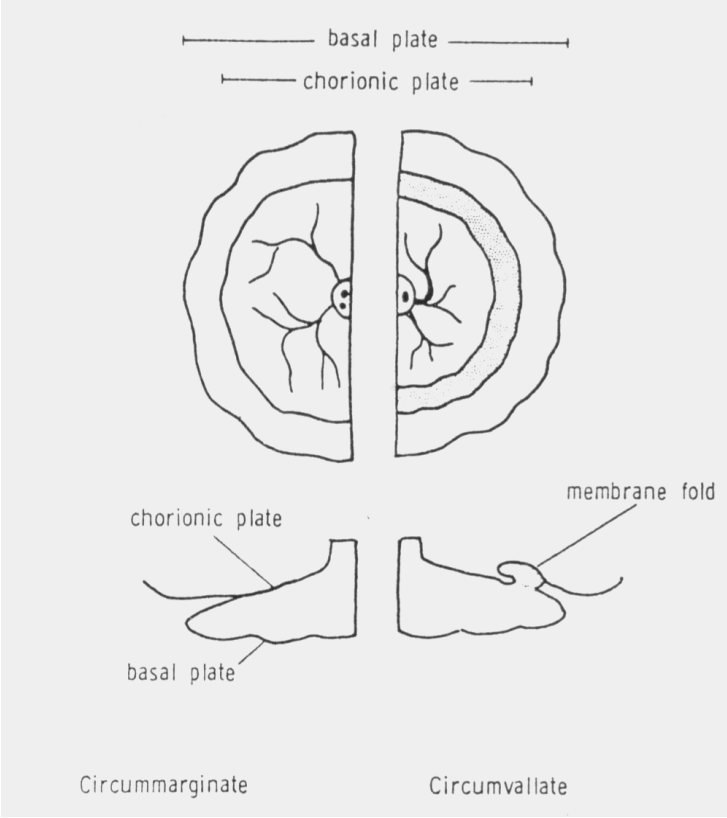


Figure 4. Extrachorial placenta.

Lesions

Infarction is a delimited region of ischemic necrosis caused by disruption of maternal blood supply. In cases of infarcts very early in the gestation, the infarcted tissue might be resorbed, leaving thin, non-vascularized placental areas. At investigation of the post partum placenta, it is not possible to decide whether the cause of a thin area is early infarction or a paucity of cotyledons due to a regionally poor uterine vascular bed. Infarction is strongly associated to preeclampsia.
Villitis is a mononuclear inflammation in the villous mesenchyme, which in most instances is of unknown etiology, but otherwise associated with known maternal viral infections. In pregnancies with placental villitis, this might be the only explanation for growth retardation.

Feto-maternal exchange

The main function of the placenta is as an interface for the exchange of oxygen, nutrients, hormones and waste products between fetus and mother. This transport occurs through the placental barrier, i.e. the syncytiotrophoblast, the cytotrophoblast, the stroma of the chorionic villus and the endothelium of the fetal capillary and includes both simple diffusion and active transport (Ullberg et al., 1967, Ullberg, Lindquist and Sjöstrand 1970, Slanina, Ullberg and Hammarström, 1973). The placenta mass mainly consists of its vessels, providing a surface for exchange and leading blood from the mother and the fetus to circulate in close proximity to each other on the two sides of the barrier. The rate of transplacental exchange primarily depends on the rates of placental blood flow, both maternal and fetal (Reynolds and Redmer, 2001).

There are many different causes of impaired feto-maternal exchange (Bernstein and Divon, 1987 and Ghidini, 1996). Maternal factors can be divided into those due to the composition of the blood and those involving vascular factors. Factors concerning blood composition include suboptimal nutrition, coagulopathies, conditions causing hypoxia and a high hematocrite, the later decreasing uteroplacental circulation through increasing the blood viscosity (Hytten, 1978). Vascular factors can be uterine, as malformation, cicatrisation and low implantation site or general, as in hypertension and collagen vascular disorders. Inadequate invasion of the spiral arteries by the extravillous trophoblast is due to abnormal interaction between fetal placental tissue and maternal cells in the spiral arteries, and constitutes an important cause of decreased feto-maternal exchange (Pijnenborg et al., 1991). Deficient spiral artery invasion is related to pre-eclampsia. Roberts and Lain (2002) describe an emerging view of the etiology of pre-eclampsia, based on interactions of

reduced placental perfusion with diverse maternal factors that alter endothelial function.

Gross anatomical placental factors related to impaired feto-maternal exchange include developmental anomalies and later acquired lesions. To a great extent these developmental anomalies probably are caused by maternal vascular factors as described earlier.

Impaired fetal growth

Small for gestational age (SGA) is defined as fetal weight lower than –2 standard deviations (SD) from the mean for gestational age, corresponding approximately to a weight deviation of –22% (Marsal et al., 1996). Intrauterine growth retardation (IUGR) is present when a fetus is not growing according to its genetic or biologic growth potential. SGA fetuses may be healthy and small with an inherited low intrauterine growth. Genetic aberrations, congenital malformations and metabolic diseases also may cause SGA, which is not synonymous with IUGR, since in these cases it is the potential for growth which is small. On the other hand, approximately one third of IUGR infants have an apparently normal size but have not reached their genetically determined birthweight (Bates et al., 1996).

Methods for identifying the true IUGR infant should include detailed neonatal evaluation, skinfold thickness and ponderal index (Sanderson et al., 1994). Maternal height and weight, parity, ethnicity and the sex of the infant should be taken into account (Wilcox et al., 1993) and even the paternal height (Wilcox et al., 1995).

In clinical practice, SGA during gestation is regarded as suspected IUGR. IUGR both per se and in combination with preterm birth has great socioeconomic consequences.

The condition is related not only to perinatal morbidity and mortality (Simchen et al., 2000) but also to long-term sequels. The latter include permanent effects on organs during periods of rapid growth like increase in obstructive airway disease in fetuses exposed to famine in mid and early gestation (Lopuhaa CE et al., 2000) or increase in early-onset chronic renal failure following low birth weight (Lackland et al., 2000). Further, they include disorders due to adaptive fetal programming like predisposition to depressive disorder (Thompson et al., 2001) or cardiovascular disease (Godfrey and Barker, 2000). Growth restriction in a female fetus may lead to reduced fetal growth and raised blood pressure even in the next generation (Barker et al., 2000, Barker, 2001).

Recently, ultrasonographic assessment of the placental size has been shown to be a tool to detect risk pregnancies at an early stage (Metzenbauer et al., 2002). Placental volume in second trimester, according to Thame et al. (2001), might be a more reliable predictor of size than fetal measurements.

Clinical surveillance of risk pregnancies

Suspected IUGR is a clinical problem demanding fetal surveillance. Velocimetry of the umbilical artery gives a measure of the resistance in the fetal placental arteries, which can predict which growth-restricted fetuses are at greater risk for adverse perinatal outcome (Rochelson et al., 1987, Gudmundsson & Maršál, 1991 Trudinger et al., 1991, Battaglia et al., 1993, Clerici 2001, Schreuder et al. 2002). Velocimetry is superior to cardiotocography and repeated measurements of fetal growth (Almström et al., 1992). Burke et al (1990) state that the small fetus with normal umbilical artery Doppler is at minimal perinatal risk.

Surveillance is important in order to be prepared for complications and for choosing the optimal time for delivery, weighing the implications of intrauterine distress against the hazards of iatrogen prematurity. Possibilities for treatment of restricted fetal growth related to increased placental flow resistance are limited, but evolving (Lin and Santolaya-Forgas, 1999).

Placental aberrations in relation to abnormal FVW

These have hitherto been studied predominantly by morphological methods. The presence of placental infarction has been found to be associated with IUGR and with intrauterine findings of abnormal blood velocity by Laurini, Laurin and Marsal (1994). Karsdorp et al. (1996) found by morphometry that terminal placental villi have a smaller mean profile cross sectional area and diameter and show a more uniform pattern in IUGR compared with those in normal pregnancies. Macara et al. (1995), on the other hand, found no difference in the mean stem villous vessel diameter in IUGR with absent end-diastolic flow.

Kreczy, Fusi and Wigglesworth (1995) found a correlation between abnormal pulsatility index and a lower number of arteries in the tertiary stem villi and interpreted this as being due to an early arrest of placental angiogenesis. In SGA pregnancies, Fok et al. (1990) found a correlation between resistance index and the number of small arteries with thickening and/or degeneration of the vessel wall in addition to partial or complete obliteration of the lumen.

Furthermore, there is a strong association between abnormal FVW, IUGR and pre-eclampsia, which has been shown to be related to failure of the trophoblast to invade normally in the spiral arteries (Pijnenborg et al., 1991, Caniggia et al., 2000).

Earlier investigations have explored the relation between SGA and placental gross anatomy and between SGA, umbilical artery FVW and placental histology. However, the umbilical artery FVW has not previously been related to the placental gross anatomy including allantochorial arterial vessel pattern. Previously used methods for assessment of placental vascular gross anatomy have been semiquantitative and to some extent subjective.

One of the aims of the present study is to test a new approach towards obtaining a reproducible method for evaluating placental gross pathology in relation to pregnancies complicated by an abnormal umbilical artery FVW.

Fractal geometry

Classical geometry, as postulated by Euclides in about 200 B.C., builds on the basic elements of the line and the circle, and is well suited for describing man-made objects. With a few exceptions, like the spherical shape of our planet, nature shows a much higher degree of complexity and, from the perspective of Euclidian geometry, irregularity.

Mandelbrot (1982) was stimulated by these patterns to investigate the morphology of the amorphous. In 1975 he coined the concept of fractals, linguistically derived from the Latin adjective fractus. The corresponding Latin verb frangere means break, to create irregular fragments. A fractal subject has a self-similarity, which can be displayed by observing pictures (of a structure) or temporal registrations (of a course of events) as magnified at different scales. This suggests an underlying regular creating mechanism and makes reconstruction of these complex subjects possible by using an algorithm. The value of the fractal dimension reflects the complexity, the higher being the more complex. The concept of fractal dimension is associated with the classic concept of dimensions, always being between 1 (like a line) and 2 (like a plane).

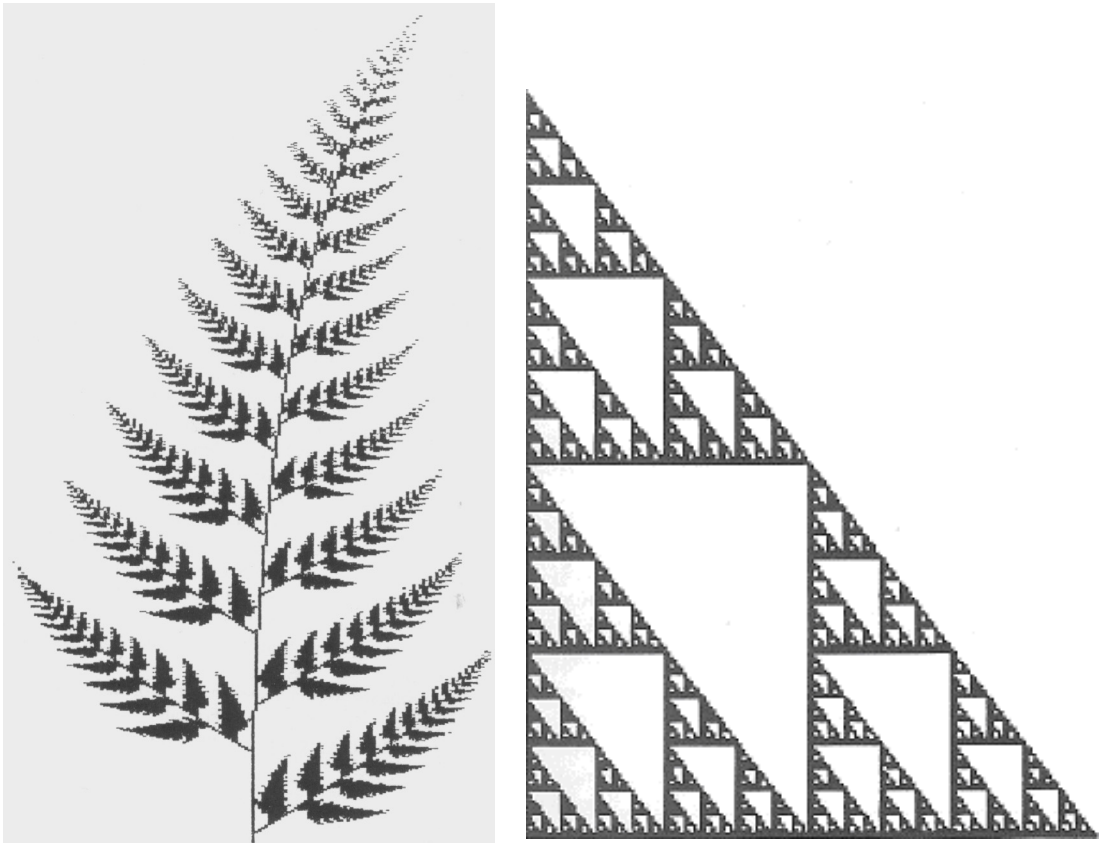


Figure 5. Examples of fractal structures: a) a constructed fern, b) the Sierpinski gasket.

Fractal geometry has been used to study branching structures in organs, such as the parotid ducts (Honda et al. 1991, 1992), skin arteries (Vico et al., 1994), renal arteries (Cross et al., 1993) and bronchial arteries (Boxt et al., 1994). A mathematical computer model based on fractal geometry was used by Umur et al. (2002) to mimic the anatomy of the placental vascular tree to determine the resistance in twin-to-twin shunts. The gross placental arterial pattern seems suitable for application of fractal analysis, but to our knowledge the fractal dimension of genuine placental arteries has not been analysed before.

Hyrtl's anastomosis

Hyrtl's anastomosis is the only connection between the two umbilical arteries which, beyond the anastomosis, are end-arteries. Its existence was discovered by Hebenstreit (1737), for the first time drawn by Albin in 1748 and investigated in detail by Hyrtl (1870). During the 20th century a number of studies have been reported, however, mostly in more or less undefined materials (Bacsich and Smout, 1938, Priman, 1959, Smart 1962, Szpakowski, 1974). Although the assessment of umbilical artery blood flow velocity waveforms (FVW) are recorde in the vicinity of the anastomosis at the cord insertion, its occurrence and varying

anatomy are still not known in relation to pregnancy outcome or placental abnormalities. Young (1972) stated that it had been impossible to link any factor to the presence or absence of the anastomosis. Its function as an equalizer of blood flow resistance between the umbilical arteries has been suggested in relation to discordant umbilical artery FVW (Harper and Murnaghan 1989, Predanic et al. 1998). Hitschold et al. (1992) investigated a case with gross discordant flow, and reported absence of the anastomosis resulting in two completely separate circuits as an explanation. Raio et al. (2001) have been able to assess FVW in the anastomosis and in the umbilical arteries both up- and downstream and thereby confirmed the equalizing function. This was possible only in selected cases with a suitable implantation site and cord insertion.

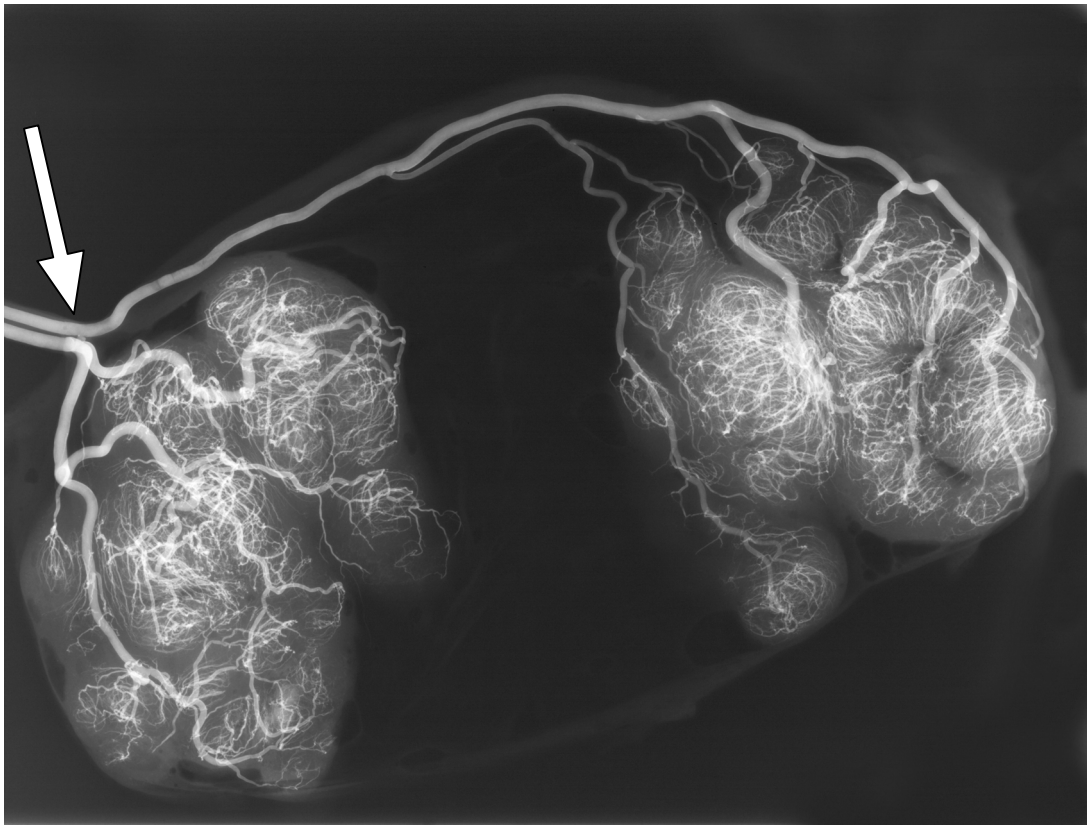


Figure 6. Vessel type anastomosis (arrow), running straight between the umbilical arteries immediately before the marginal cord insertion in a bilobed placenta.

Gross placental vascular architecture often is abnormal in intrauterine growth retardation (Sandstedt, 1974 and 1979). This raises the possibility that the anastomosis also might differ from the usual in small for gestational age (SGA) placentas. Hypothetically the anastomosis is also more important in this condition associated with decreased placental capacity. So, it seemed reasonable to investigate its anatomy and possible role in pregnancies complicated by fetal growth disturbances.

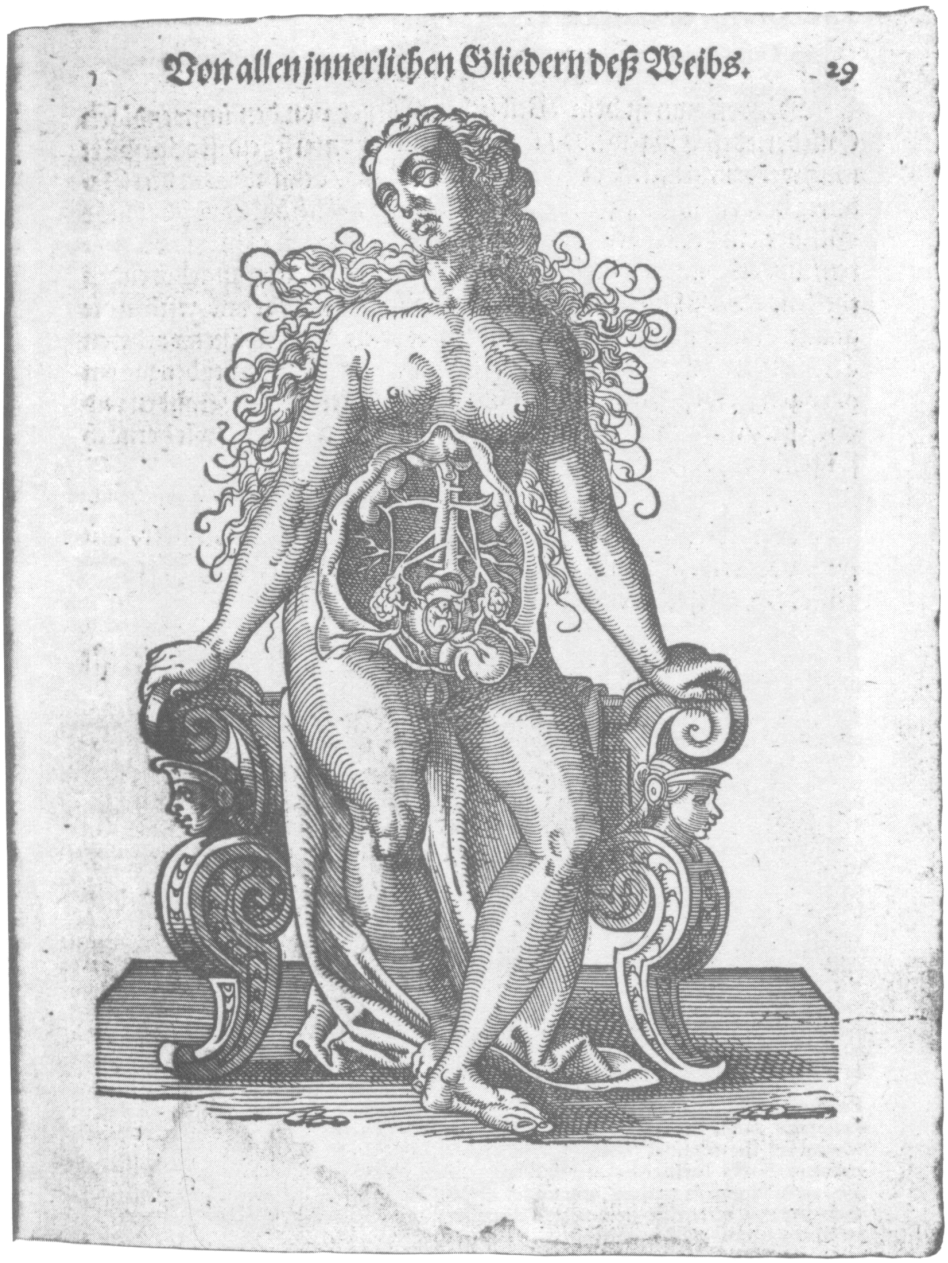


Fig 7. Pregnant woman, woodcut from 1580.

Aims of the investigation

The pathophysiology of IUGR is complex and involves aberrations in placental architecture. Does this also apply to placental vascular anatomy? Our approach to this problem is

1) to study the relationship between umbilical artery FVW and placental gross morphology including the arterial vessel pattern using current methods
2) to elaborate a new method for objective and reproducible evaluation of the placental arterial tree
3) to investigate whether the fractal dimension of the placental arterial pattern differs between placentas with normal and abnormal umbilical artery FVW respectively
4) to investigate the occurrence and appearance of Hyrtl's anastomosis in a normal material
5) to investigate the occurrence and appearance of Hyrtl's anastomosis in placentas from SGA infants.
6) to find a relationship between the structure of Hyrtl's anastomosis and the anatomy of the placental arterial system as visualized by angiography.

Subjects and study design

Developmental abnormalities in relation to FVW (I)

A consecutive series of pregnant women participated in an ultrasound screening program which included two routine examinations. In the first, performed between the 16th and 20th gestational weeks according to the last menstrual period, the gestational age was recalculated on the basis of measurements of fetal biparietal diameter and femur length. In the second, performed after 32 gestational weeks, fetal weight deviation was determined by measuring biparietal diameter, abdominal diameter and femur length. Thirty fetuses which at the second examination were found to be 22% (-2 SD) or more below the expected weight were regarded as being at risk for IUGR and were included in the study. These fetuses were followed by umbilical artery blood flow velocity measurements and divided into three groups according to that.

Fractal analysis (II-III)

The scaling properties of the arterial pattern in healthy human placentas, were derived from 22 placentas from AGA fetuses with normal umbilical artery FVW, BFC 0, as recorded in the 28th gestational week and thereafter every 2nd week until delivery.

To explore the difference between the fractal dimension of the arterial vessel pattern in placentas with impaired function and healthy placentas, 16 placentas from SGA fetuses with severely pathologic FVW, BFC II-III, were studied and compared to 12 of the 22 placentas from study II reinvestigated in exactly the same setting.

Hyrtl's anastomosis (IV-V)

67 placentas from AGA infants and 64 placentas from SGA infants were investigated. 32 of the latter were instrumentally delivered due to threatening asphyxia, judged by CTG registration, which might reflect acute utero-placental insufficiency, and 32 were non-asphyctic and delivered vaginally.

AGA was defined as birth weight according to gestational age within ±2 SD = ±22 % and SGA as ≤-2 SD using the Scandinavian standard curve (Marsal et al. 1996)

Methods

Ultrasound

At 16 weeks of gestation, based on the last menstrual period and/or clinical examination, ultrasound fetometry was routinely performed and gestational age was subsequently based on these results. A second routine fetometry was made at 32 weeks of gestation, when fetal weight deviation was calculated according to gestational age-related curves of fetal weight, as described by Persson and Weldner (1986).

Doppler

Umbilical artery blood flow velocity waveforms (FVW) were in paper I classified according to an early definition based on peak systolic velocity/minimum diastolic velocity (S/D) ratio (Stuart et al., 1980). Normal values for S/D ratios were obtained from a cross sectional study by Gudmundsson and Marsal (1988).
Group 0: normal S/D ratio, within ±2 SD according to gestational age.
Group I: increased S/D ratio, beyond +2 SD according to gestational age.
Group II: diastolic zero flow.

In paper II-IV the FVWs were classified based on pulsatility index (PI) according to Laurin et al. (1987).
PI = (peak systolic velocity – minimum diastolic velocity)/mean velocity.
BFC 0: normal PI, within ±2 SD.
BFC 1: PI ≥ mean + 2 SD.
BFC 2: Not detectable end-diastolic flow.
BFC 3: Absence of a positive flow velocity during the main part of diastole or presence of a reversed flow.

Cardiotocography

The diagnosis of threatening fetal asphyxia in paper V was based on abnormal cardiotocography (CTG) registration. CTG is an electronic recording of fetal heart rate, including its variations with fetal movements and uterine contractions, which reflects acute fetal compromise (FIGO: Guidelines for the use of fetal monitoring, 1986). The use of CTG for detecting asphyxia is generally accepted (Westgren et al., 1986) and the CTG was performed and evaluated as part of the clinical routine. Ingemarsson et al. (1986) have evaluated its value for detecting fetal distress present at admission to the labour ward.

Macromorphology

All placentas were frozen at -20^0 C immediately after parturition. Placental weight was registered after thawing and drainage of blood for 5 minutes. The site of umbilical cord insertion and the configuration were recorded.

The umbilical cord insertion was considered velamentous when it was located in the membranes, marginal when the distance between the cord insertion and the placental margin was less than 3 cm and central in the remaining cases.

Extrachorial placenta implies that the whole of the placental surface is not covered by chorion and includes both the circumvallate and the circummarginate type. Rather than inserting at the disk margin, the chorion laeve inserts at varying degrees inside that margin. When the membranes fold back on themselves before leaving the chorionic plate the term circumvallate placenta applies. In a circummarginate placenta the chorion laeve leaves the surface without folding back. The phenomenon may be partial or include the whole placental circumference. The etiology is unknown.

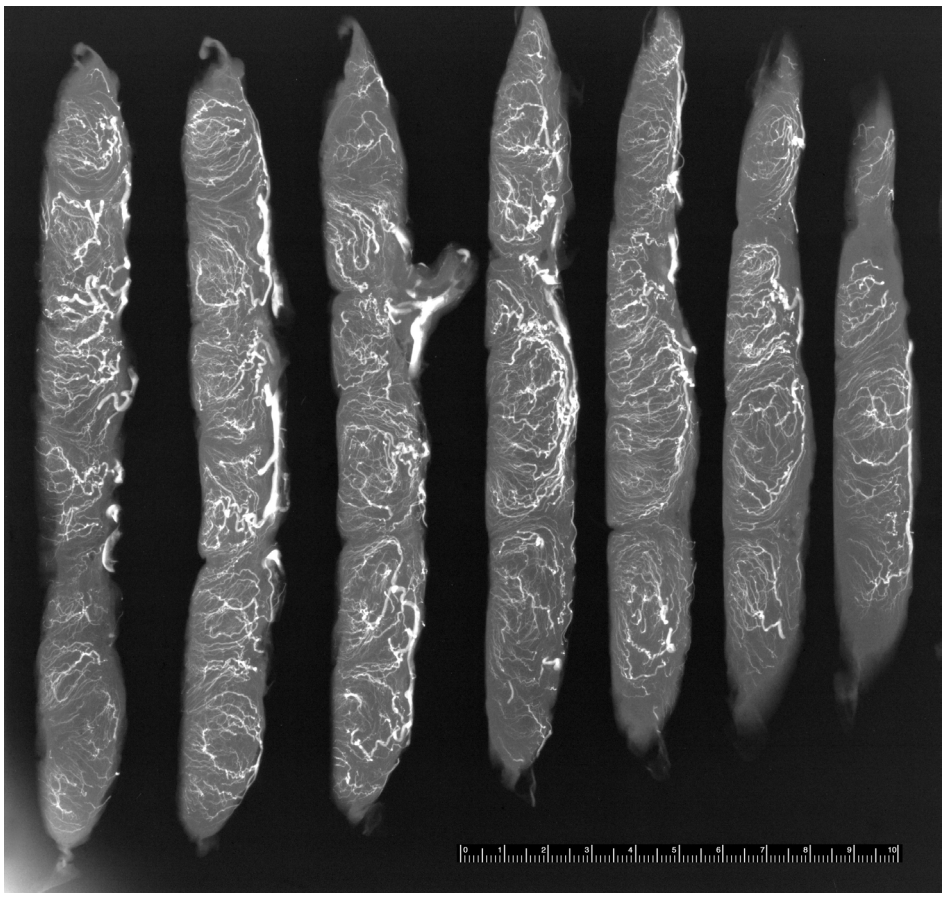


Fig. 8. a) Slices of a placenta with normal configuration (i.e. not extrachorial). The chorion laeve inserts at the edge of the normally tapering placental margin.

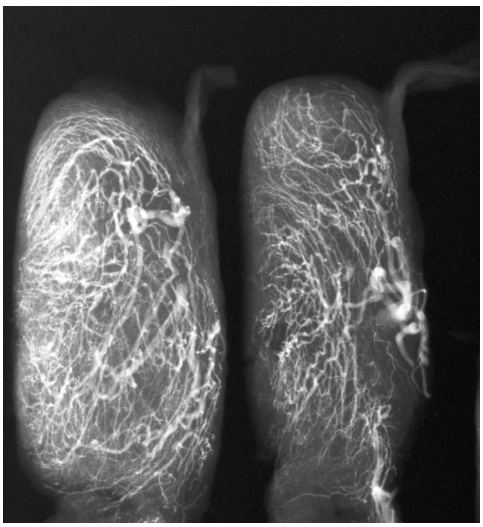


Fig. 9. Slices of a circummarginate placenta (detail). The chorion laeve leaves the placenta surface inside its margin. Note the associated thick, blunt placental edge. See also drawing in fig. 4.

Angiography

At first a plain film was exposed of each placenta before the vessels were filled by contrast medium. Then the arterial tree without previous rinsing was filled with barium x-ray contrast medium through a catheter in one of the umbilical arteries at a constant pressure of 100 mm Hg. In a few placentas without an anastomosis between the umbilical arteries, both arteries were catheterized. The contrast medium used was Mixobar Colon®, Astra Meditec, a colloid water suspension of 1.0 ± 0.1 µm Ba SO_4 particles which was diluted to 0.2 g $BaSO_4$/ml. The contrast medium filled the arterial system down to the tertiary stem villi e.g. small arteries with diameter 0.03-0.05 mm and did not pass over to the capillaries nor to the veins.

At least two angiograms with the umbilical cord in different positions were achieved in frontal projection with the film cassette immediately beneath the placenta. After fixation in 5 % neutral formaldehyde solution for at least 14 days, the placentas were sliced at 1 cm and angiograms were made of the slices.

The arterial vessel pattern was described as magistral, dispersed or mixed in a classic, semiquantitative way according to Shordania (1929a) and Bacsich and Smout (1938), see the introduction section.

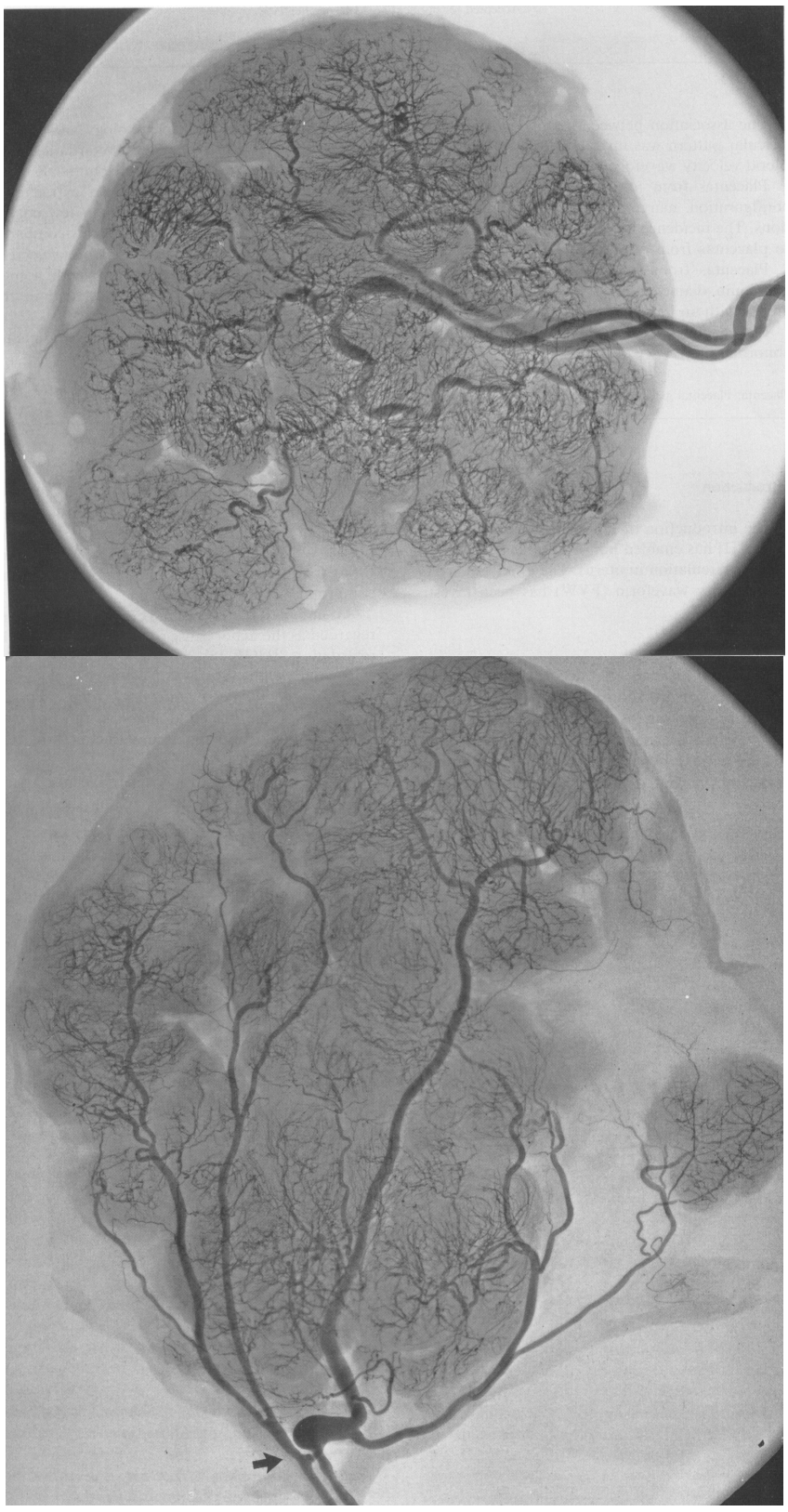


Fig 10. a) dispersed
b) magistral vessel pattern.

The Hyrtl anastomosis was measured and characterized in the angiograms. Its width was measured both in absolute measures and in relation to that of the umbilical arteries it connected, making the comparison between placentas of different sizes feasible.

The occurrence and types of anastomoses were divided into five groups as follows:

1) anastomosis by vessel between the umbilical arteries
2) anastomosis by fenestration of the umbilical arteries, lying adjacent to each other
3) anastomosis by fusion of umbilical arteries
4) lack of anastomosis in spite of two umbilical arteries
5) lack of anastomosis due to single umbilical artery

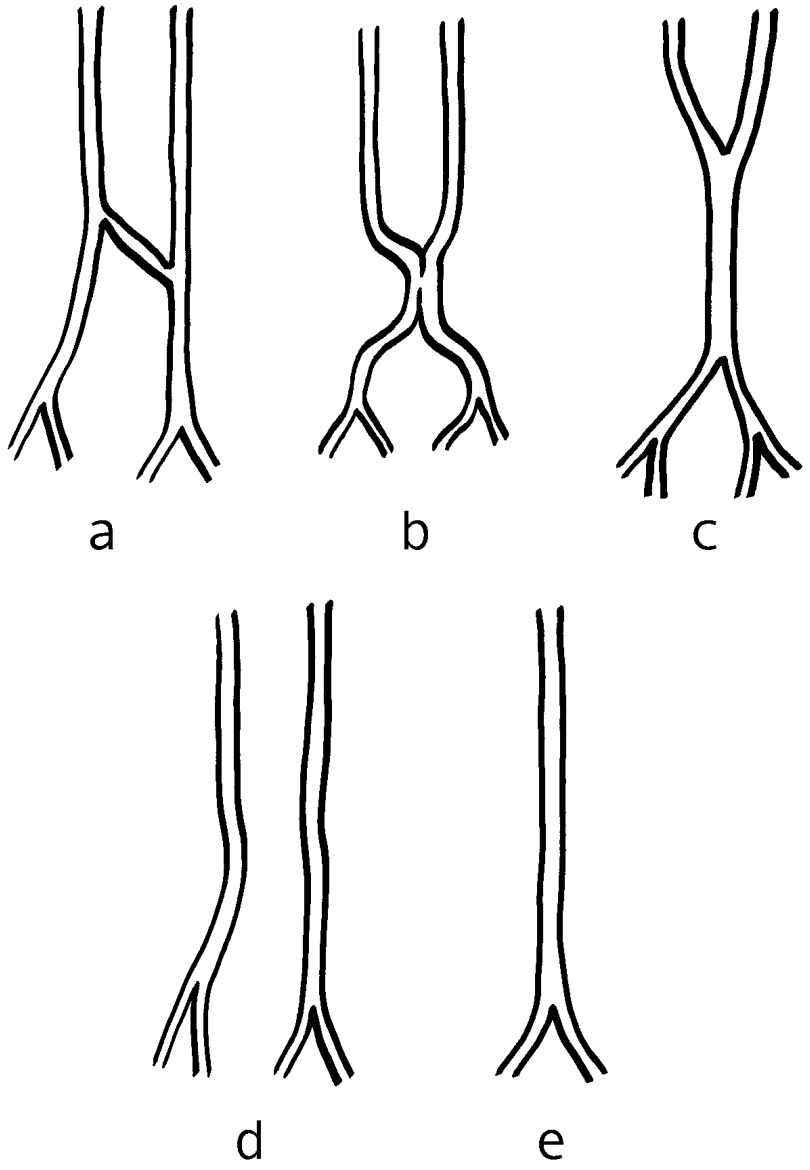


Fig. 11. Schematic representation of the types of anastomosis.

Dissection

Anastomoses that could not be clearly classified by angiography were dissected under a hand lens or a stereo microscope. Furthermore all placentas without any anastomosis identified by angiography were dissected to confirm the lack of it.

Digitizing and area calculation

The angiograms used for area calculations were at first digitized using a Lumiscan 20 scanner from Lumisys, Sunnyvale, California, USA. The overall placental area as well as the relative areas over which each umbilical artery sent its branches were then calculated applying the standard area algorithm in a Sectra Picture Analyzing and Communicating System (PACS), from Sectra, Linköping, Sweden. The area supplied by each artery has been expressed as percentages of the total area.

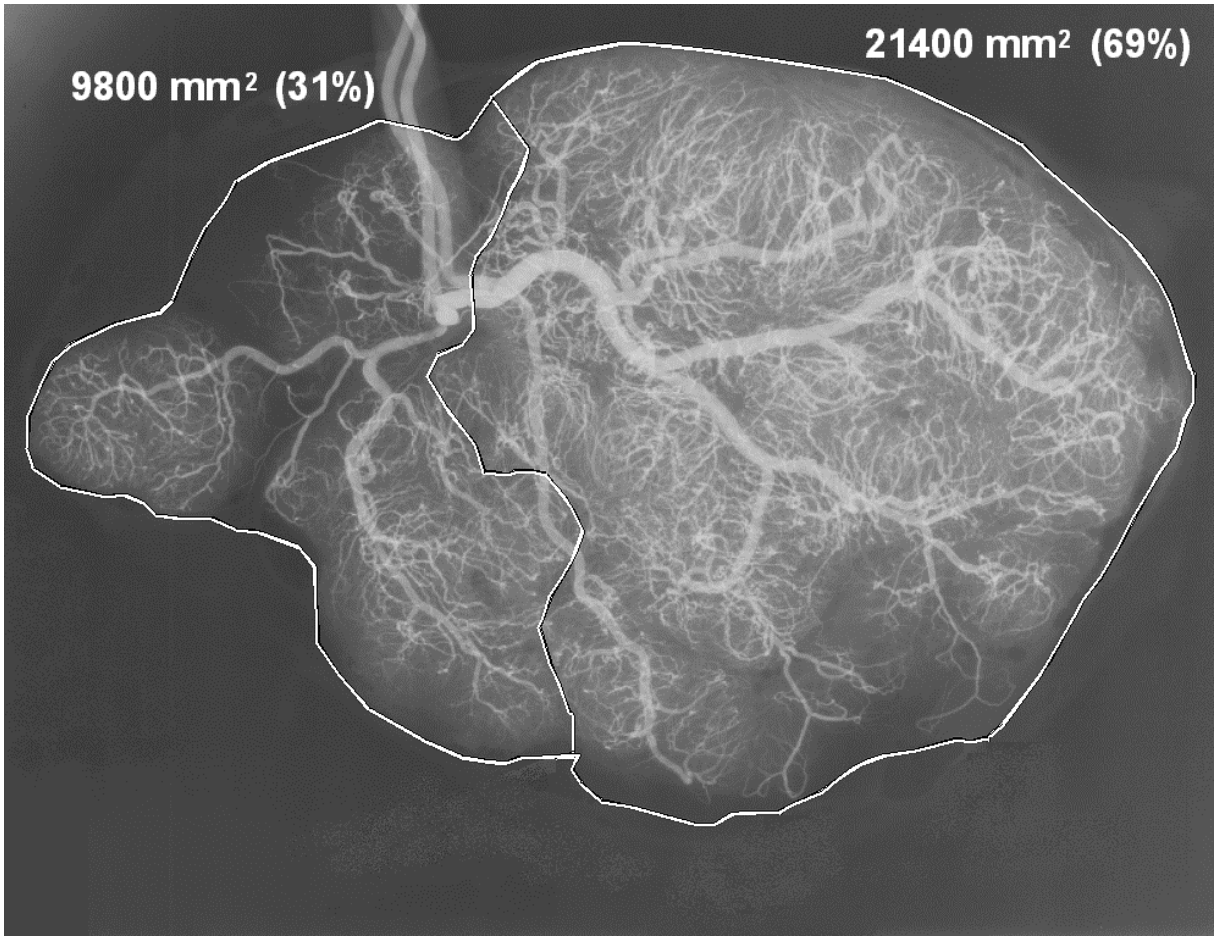


Fig 12. A placenta with asymmetric umbilical artery supply areas, 31/69 %.

In perfect symmetry with each artery supplying an equal area it is expressed as 50% and 50% respectively. In extreme asymmetry the figures move towards 0% and 100 % respectively.

Fractal analysis

The 38 angiograms investigated by fractal analysis were digitized by scanning with a VXR-12 film digitizer from Vidar Systems, Herndon, Virginia, USA. The resolution was 300 x 300 dpi and eight bits were used to represent the gray value of each pixel. Each pixel was thus assigned an intensity value between 0 and 255. The smallest image was 106 x 1593 and the largest was 2631 x 3294 pixels. After scanning, an edge detection algorithm was applied to generate binary images of the edges of the blood vessels. For the image processing the Candela package was used, developed by the Computational vision and active perception (CVAP) laboratory, the Royal Institute of Technology in Stockholm (KTH).

Fig 13. An angiogram after binarization.

Scaling analysis was performed by applying box counting to the images of the edges of the arterial vessel trees.

Box counting is a method for determining the fractal dimension, which is quite perceptible. The image is consequently put under a large number of grids, each with a different box length. The number of boxes with length r which are hit, in our work by a vessel edge, is counted for the box length of each grid and denoted $N(r)$. The number of boxes that are hit can also be considered as the number of boxes needed to cover the arterial tree. A geometrical object scales uniformly over a range of scales, when $N(r)$ varies with r according to a power-law: $N(r) \sim r^{-Do}$. The exponent D_0 is the fractal dimension of the subject. In order to estimate D_0 the relation $\log N(r) = b \log r + a$ was fitted to the data by linear regression, and D_0 was calculated as $-b$.

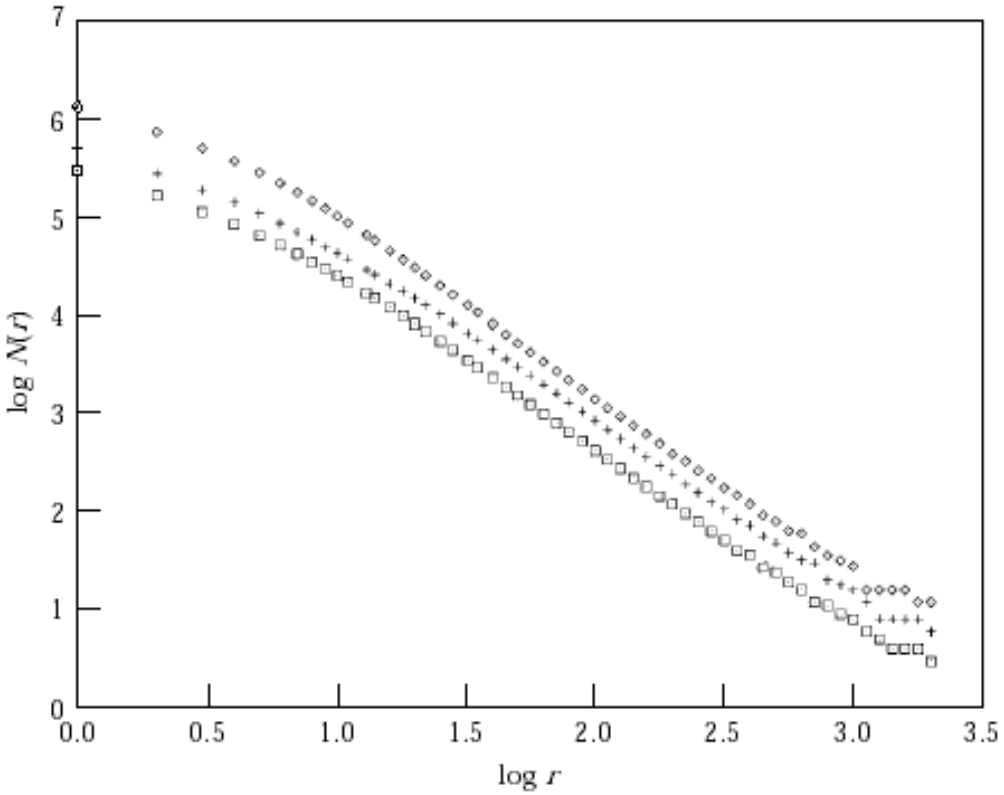


Fig. 14. Log N(r) is plotted as a function of log r for three arterial trees of normal subjects. N(r) is the number of boxes needed to cover the arterial tree. Log r = 0 corresponds to a side length of one pixel, i.e. 85 μm. The data points for two of the trees have been displaced 0.3 and 0.6 units upwards, respectively.

The box sizes were chosen equidistantly in the logarithmic sense with distance 0.05. Only boxes with side lengths ranging from 20 to 200 pixels

(i.e. $1.3 \leq \log r \leq 2.3$) were used. In this interval, the regression line fits the data well.

For box sizes ≥ 1.3 (i.e. ≥ 20 pixels), the number of boxes hitting the solid arterial tree and the number hitting its edges are close. Only a small fraction of the blood vessels have a diameter exceeding 20 pixels. This means that the value of the fractal dimension is not influenced by the edge detection procedure.

The programs for box counting have been written in cooperation with Dan Bergman, KTH, Stockholm.

Histology/Microscopy

The placenta slices were inspected for gross lesions. Specimens were taken from all lesions for microscopic diagnosis. Specimens were also taken randomly from grossly normal tissue to determine the extent of contrast filling and to evaluate villitis.
In addition one vessel-like anastomosis and one of the umbilical arteries were cut for conventional histology. They were also stained immuncytochemically with a monoclonal antibody to desmin (Dako Cytomation Denmark A/S, Glostrup, Denmark) to judge the muscle content.

Statistics

Paper I) Student's *t*-test was used to test differences between groups of continuous variables, normally distributed. Dichotomous variables were tested with Fischer's exact test double sided.

Paper II) Simple linear regression was used to determine the regression coefficient for each subject. The 99% confidence interval of the regression coefficient, the standard deviation of the points from the regression line, and the squared correlation coefficient were also calculated for each subject. The common mean was calculated for the regression coefficients.

Paper III) Student's *t*-test was used to test differences between the values of the fractal dimensions and between the other continuous variables. Chi^2 analysis was used for categorical data.

Paper IV) One- way analysis of variance (ANOVA) was used for statistics of differences between groups.

Paper V) One-way analysis of variance (ANOVA) and chi-square analysis were used for statistics of differences between groups. The analysis of variance was supplemented with post hoc tests to display the location of the calculated overall differences.

Results

The main results are summarized below. For further details, see paper I-V.

Gross arterial pattern in relation to FVW (I, II, III)

In paper I we have addressed the question of the gross arterial vessel pattern in relation to umbilical artery blood flow velocity waveforms. Thirty fetuses at risk for growth retardation were divided into three groups according to umbilical artery FVW. Group 0 had normal systolic/diastolic ratio within ±2 SD, group I showed increased S/D ratio (i.e. decreased diastolic flow velocity) beyond +2 SD and group II showed diastolic zero flow. There were fewer SGA infants but a lower mean Apgar score at 5´ in group II compared to group 0 and I. Compared to group 0, the placentas were larger and thinner in group I and smaller and thicker in group II. All placental developmental abnormalities (extrachorial configuration, marginal cord insertion, mixed or magistral vessel pattern, few cotyledons) were associated with group II. There were no differences in this respect between group 0 and I. The results are summarized in table I. For statistic significance levels, see paper I.

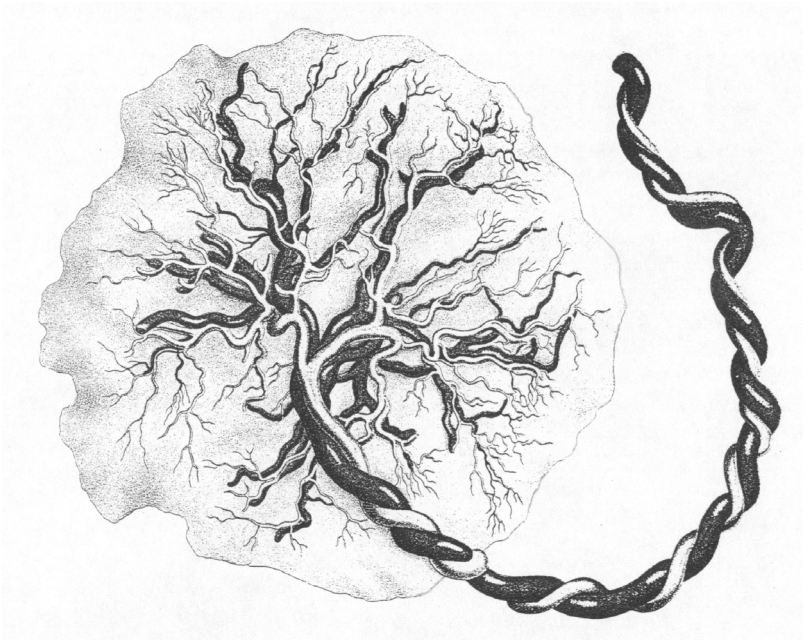


Table I. Summary of maternal, fetal and placental variables in relation to flow velocity waveform (FVW) group in 30 pregnancies at risk for growth retardation.

	Group 0 n = 11	**Group I n = 10**	**Group II n = 9**
UA FVW, systolic (S) and diastolic (D)	S/D within ±2 SD	S/D > +2SD	diastolic zero flow
Normotension/hypertension/preeclampsia	5 / 1 / 5	7 / 1 / 2	3 / 0 / 6
Birth weight g, mean (SD)	2360 (569)	2515 (307)	1675 (800)
BW >-1SD / -1SD>BW>-2SD / SGA	3 / 3 / 5	1 / 4 / 5	2 / 5 / 2
Apgar score 5´ mean (SD)	9.5 (0.7)	9.7 (0.5)	8.2 (2.3)
Placental weight g, mean (SD)	281 (60)	309 (21)	206 (69)
Birth/placental weight ratio mean (SD)	8.1 (1.8)	8.0 (1.2)	7.1 (1.8)
Maximum diameter cm, mean (SD)	18.5 (2.8)	20.2 (2.8)	16.7 (3.3)
Max diameter/max thickness ratio	2.6 (0.3)	2.2 (0.1)	2.8 (0.7)
Normal / extrachorial configuration	10 / 1	10 / 0	6 / 5
Central /marginal cord insertion	9 / 2	8 / 2	2 / 7
Disperse / mixed / magistral vessels	7 / 1 / 3	5 / 2 / 3	0 / 2 / 7
Cotyledons mean (SD)	15.4 (4.2)	16.4 (4.5)	10.4 (5.6)
≥ 15 / < 15 cotyledons	6 / 5	7 / 3	1 / 8
Gross lesions, number of placentas	7	2	9
Villitis	1	2	1
Excessive syncytial knots	6	6	7

The next step was to develop a method for objective and reproducible evaluation of the arterial tree of the placenta (paper II). For that purpose we tried fractal analysis. After devising a method for investigating the placenta's arterial tree by box-counting, which included writing of computer programs, we investigated the scaling properties of 22 angiograms of placentas from uncomplicated pregnancies with normal umbilical artery blood flow velocities. We found that in a one decade wide interval, the arterial trees scaled closely according to a power-law. Perfectly self-similar fractals of the same resolution as the placenta angiograms did not scale more closely according to a power-law. For the Sierpinski gasket, represented with resolution 2600 x 2600 pixels, we determined the standard deviation of the data points from the regression line to 0.0087 and the squared correlation coefficient to 0.99981.

Since we had been able to show that fractal geometry is applicable for investigation of the arterial tree of the normal placenta, we went on to study placentas from pregnancies with pathologic umbilical artery FVWs resulting in SGA infants (paper III). We determined the fractal dimension of angiograms of 16 pathologic subjects and compared these with 12 normal subjects in exactly the same setting. Representative angiograms are shown in figs. 15 and 16.

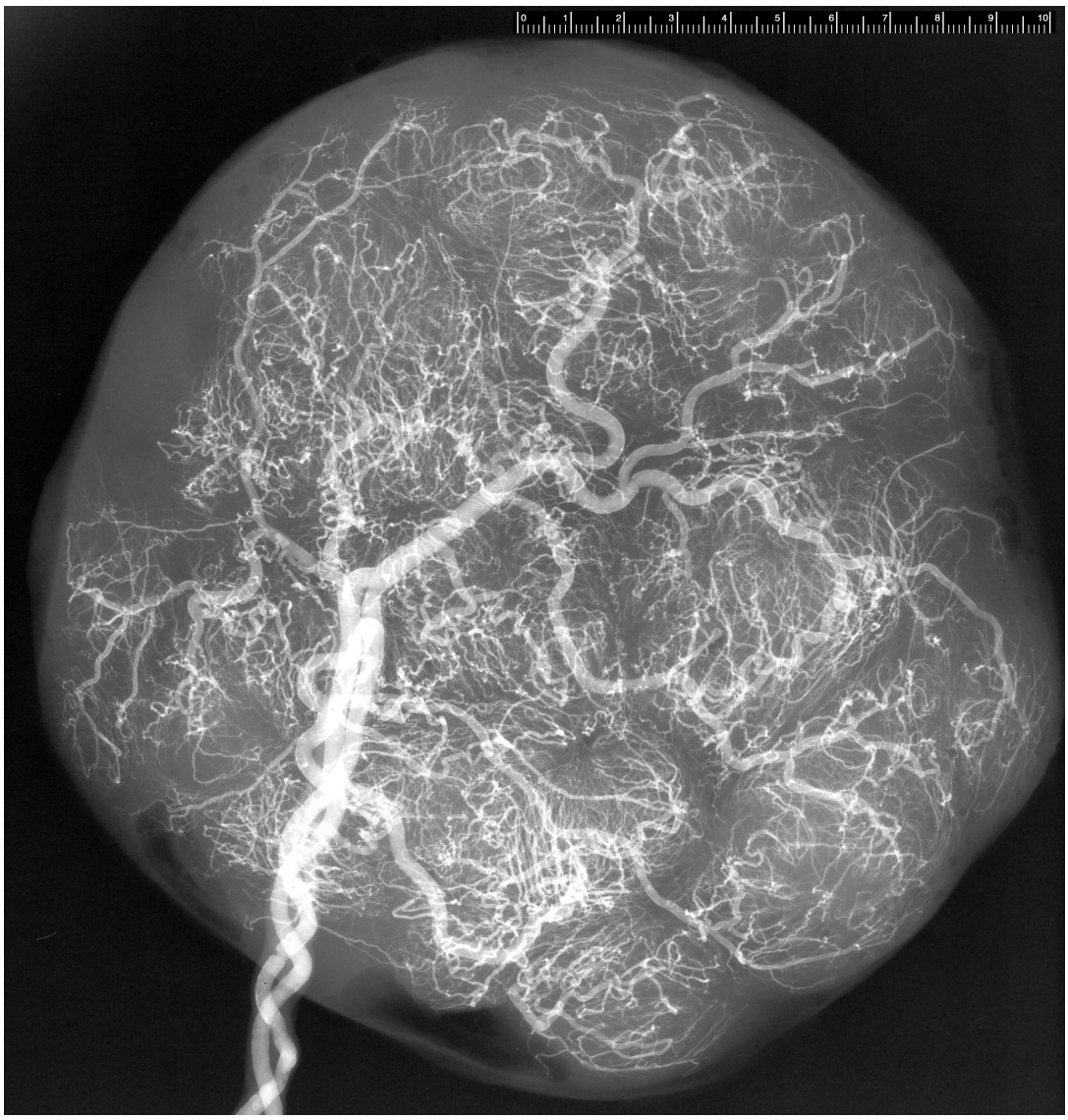


Fig 15. Arterial angiogram of a placenta from a pregnancy with BFC 0 resulting in an AGA infant. The calculated fractal dimension was 1.87.

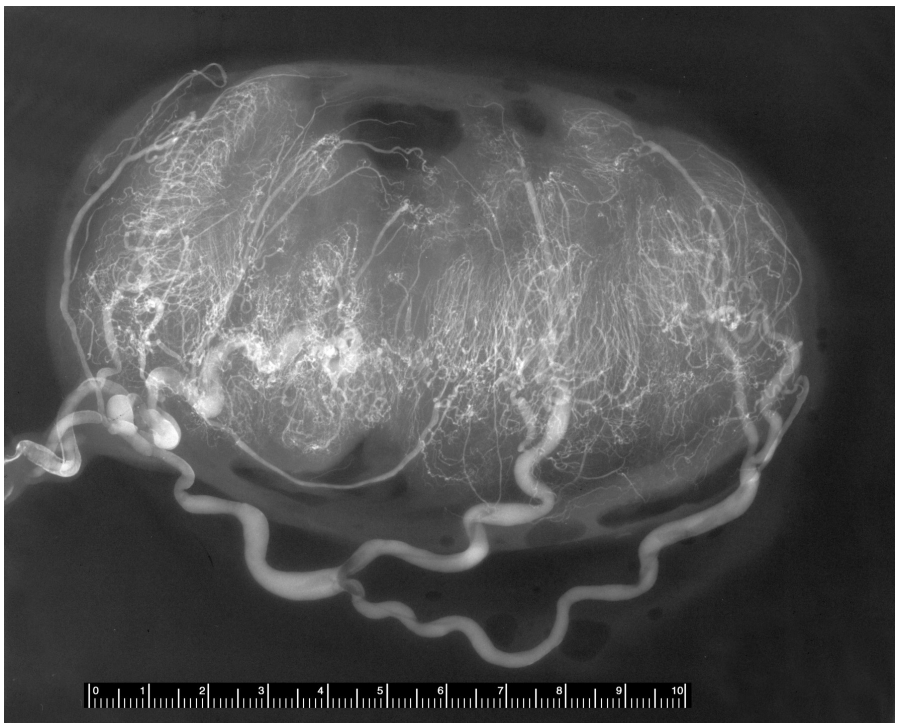


Fig. 16. Angiogram of a placenta with BFC 3. The birth weight deviation was –59% and Apgar score 1-1-0. The fractal dimension was calculated to 1.78.

The mean fractal dimension and a summary of other placental parameters, including different measures of size and semi-quantitative assessments of the arteries, are presented in table II.

Table II. Placental parameters in relation to blood flow class.

	BFC 0 (n=12)	**BFC 2-3 (n=16)**	**Significance of difference**
Fractal dimension, mean	1.864	1.755	$p < 0.001^{\dagger}$
Area, median	301 cm^2	177 cm^2	$p < 0.001^{\dagger}$
Weight, median	436 g	216 g	$p < 0.001^{\dagger}$
Weight to area ratio, median	1.54 g/cm^2	1.30 g/cm^2	$p < 0.001^{\dagger}$
Marg/central cord insertion	1/11	6/10	
Magistral / mixed / dispersed vessel pattern	0 / 8 / 4	6 / 4 / 6	$p < 0.05^{\ddagger}$
Very wide main arteries	0	4	
Wide main arteries	0	4	
Aneurysm in main artery	0	2	
< 15 cotyledons / ≥15 cotyledons	0 / 12	7 / 9	$p < 0.01^{\ddagger}$

†Student's t-test, $^{\ddagger}chi^2$ analysis

The placentas in the pathologic group were in all aspects smaller than the normal ones. They more often had developmental aberrations, and 50% also showed an increased discrepancy in width between the first generation and second generation of allantochorial arteries, two cases even showing a main artery aneurysm. The mean fractal dimension was lower in the pathologic group, displaying a lower grade of complexity of their arterial trees. Figure 17 is a skeleton drawing of the mean fractal dimension of the pathologic and normal group respectively, to give a visual impression of the difference.

Figure 17. Sketch of log N (r) as a function of log r for the mean placenta in the normal and the pathologic flow group respectively. More precipitous slope of the regression line means higher fractal dimension.

The individual fractal dimensions are presented in table III. The squared correlation coefficient is high for all the placentas in both groups.

Table III. The fractal dimension and squared correlation coefficient for 16 placentas with BFC 2-3 and 12 with BFC 0.

Study group (BFC 2-3)	**D_0**	**r^2**
S 1	1.653	0.999607
S 2	1.657	0.999962
S 3	1.714	0.999696
S 4	1.723	0.999965
S 5	1.734	0.999197
S 6	1.739	0.999955
S 7	1.749	0.999938
S 8	1.752	0.999877
S 9	1.755	0.999846
S10	1.782	0.999902
S11	1.784	0.999389
S12	1.786	0.999938
S13	1.790	0.999736
S14	1.800	0.999816
S15	1.825	0.999829
S16	1.839	0.999927
Mean	**1.755**	**0.999786**
Control group (BFC 0)		
C 1	1.828	0.999844
C 2	1.834	0.999911
C 3	1.843	0.999925
C 4	1.856	0.999949
C 5	1.863	0.999971
C 6	1.865	0.999849
C 7	1.869	0.999751
C 8	1.870	0.999856
C 9	1.883	0.999842
C10	1.883	0.999788
C11	1.885	0.999880
C12	1.888	0.999840
Mean	**1.864**	**0.999867**

In figure 18 the individual fractal dimensions are plotted for the two groups respectively. The values are more scattered in the BFC 2-3 than in the BFC 0 group.

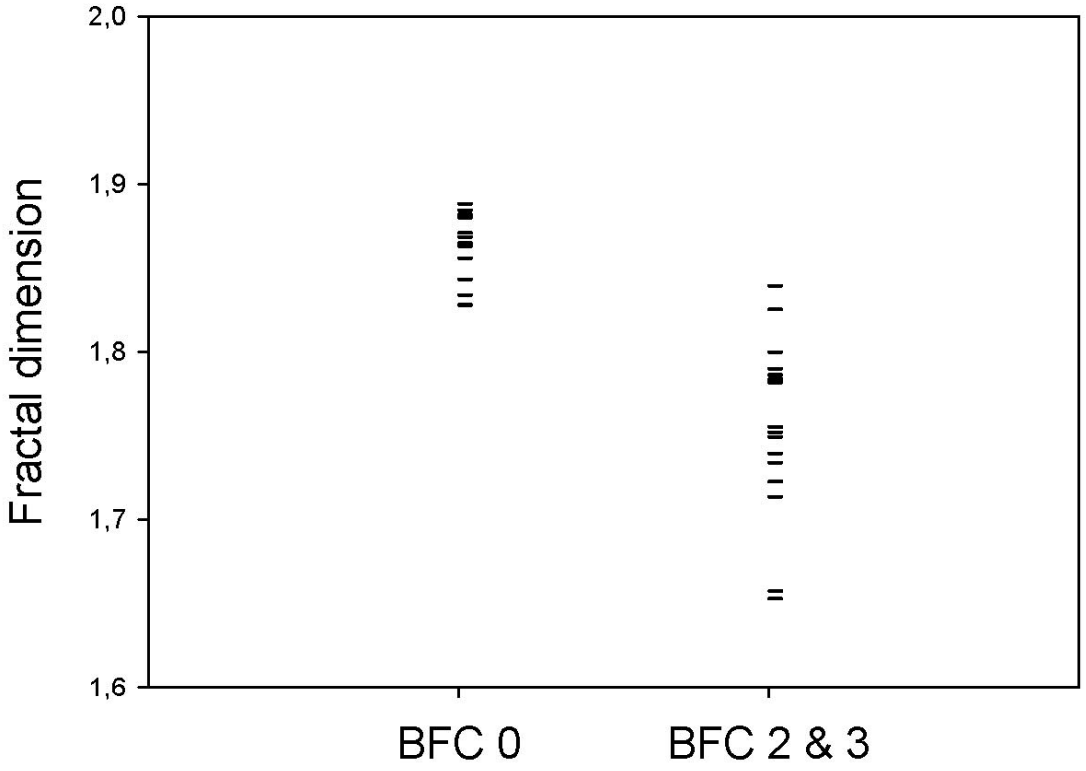


Figure 18. Graphic plot of fractal dimensions for placentas in the BFC 0 and the BFC 2-3 group respectively.

Hyrtl's anastomosis (IV, V)

Paper IV is an investigation of the varying anatomic appearance of the only connection between the two umbilical arteries, Hyrtl's anastomosis, in a controlled normal group consisting of full term pregnancies resulting in AGA infants. Paper V deals with the anastomosis in SGA pregnancies, divided in an asphyctic and a non-asphyctic group.

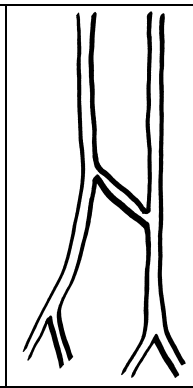
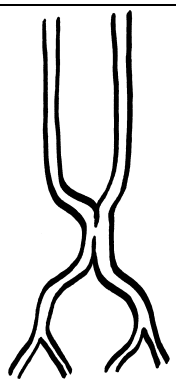
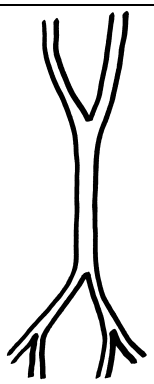
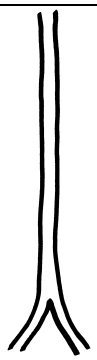
SGA was associated with smaller placenta size and in a higher degree with placental developmental abnormalities than AGA, but there was no statistically significant difference between the asphyctic and the non-asphyctic groups. In the vast majority of both AGA and SGA placentas there was an anastomosis present between the umbilical arteries. For mean values and prevalences respectively, see table IV.

Table IV. Placental parameters for AGA fetuses versus SGA non-asphyctic and asphyctic fetuses.

	AGA (n=67)	SGA non-asph (n=32)	SGA asph (n=32)
Placental weight g, median	428	298	268
Placental area cm^2, median	310	231	208
Extrachorial plac.	21%	31%	47%
Marginal or vel. insertion	4.5%	41%	25%
Anastomosis / no anastomosis	61 / 6	30 / 2	30 / 2
Anastomosis / no anast. %	91 / 9	94 / 6	94 / 6

The anastomosis (or lack of anastomosis) could be categorized into five types. The breakdown by type is presented in table V. No difference was found between the groups of infants.

Table V. Type of anastomosis in placentas from AGA and SGA infants. SGA infants divided in non-asphyctic ($SGA_{non\text{-}asph}$) and asphyctic (SGA_{asph}).

					
	Vessel	**Fenestration**	**Fusion**	**Absent**	**SUA**
AGA (n=67)	56 (84%)	4 (6%)	1 (3%)	4 (6%)	2 (3%)
$SGA_{non\text{-}asph}$ (n=32)	29 (91%)	0 (0%)	1 (3%)	0 (0%)	2 (6%)
SGA_{asph} (n=32)	27 (84%)	2 (6%)	1 (3%)	1 (3%)	1 (3%)

Figures 19 and 20 display the two most frequent different types of anastomosis in angiograms. Usually, however, the anastomosis is not as clearly identifiable in a single image.

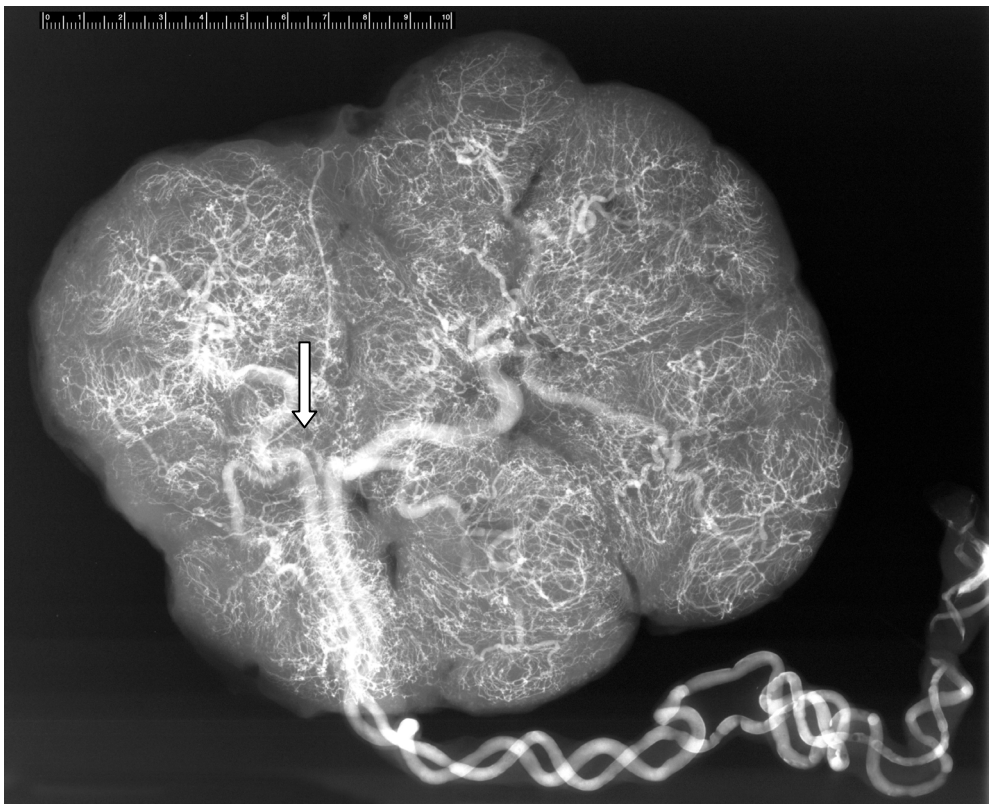


Figure 19. Anastomosis by a separate vessel.

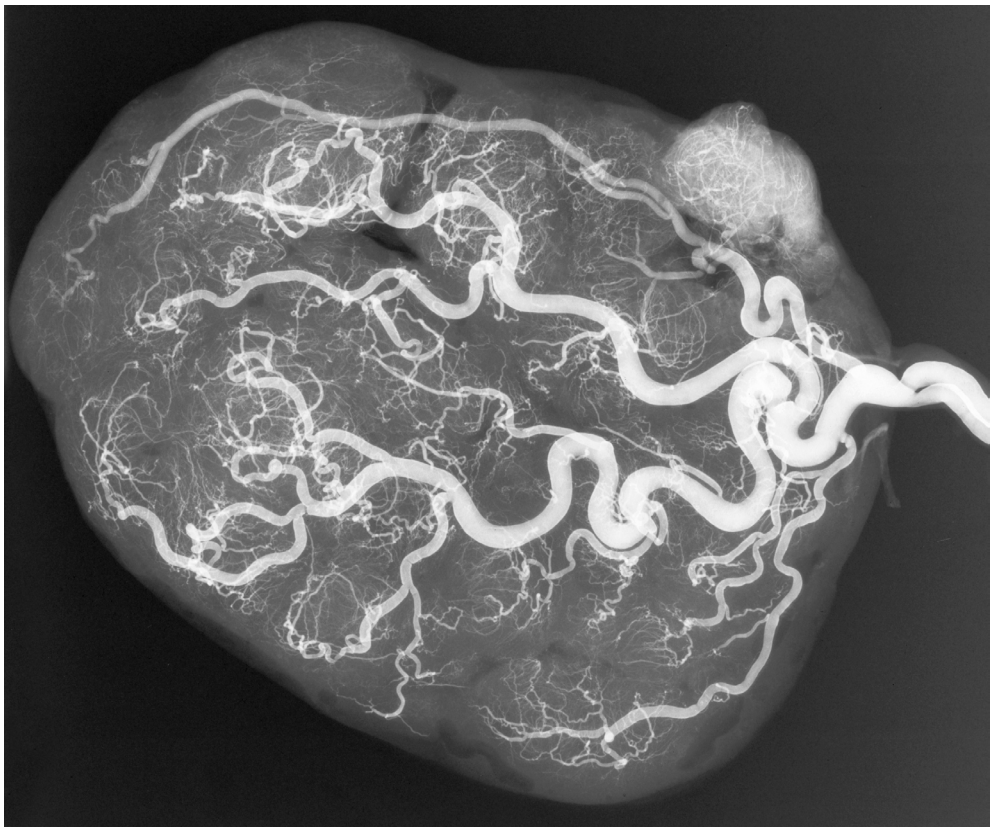


Figure 20. Anastomosis by fusion of the umbilical arteries for a short distance before the cord insertion at the right.

Only one relation was found between the appearance of Hyrtl´s anastomosis and the anatomy of the placenta in other respects, see table VI. This is a relation between the relative width of the anastomosis, compared with the width of the umbilical arteries it connects and the degree of symmetry between their supply areas.

Table VI. The width of Hyrtl's anastomosis related to the mean symmetry values in per cent of total placental area supplied by branches of each umbilical artery. Wide Hyrtl refers to an equal or larger diameter than that of the umbilical arteries whereas thin Hyrtl means a smaller diameter. Symmetry values differed significantly ($p<0.001$) between wide, thin and absent anastomosis in all groups.

	Wide Hyrtl	**Thin Hyrtl**	**No Hyrtl**
SGA_{asph}	27/73 % (n=9)	38/62 % (n=19)	45/55 % (n=1)
$SGA_{non\text{-}asph}$	31/69 % (n=7)	40/60 % (n=22)	--- (n=0)
AGA	26/74 % (n=17)	41/59 % (n=41)	45/55 % (n=4)

Discussion of results

Magistral allantochorial vessel pattern, velamentous or marginal cord insertion and placenta extrachorialis are placental gross developmental anomalies, which have all been related to IUGR (Busch, 1972, Fox and Sen, 1972, Sandstedt, 1979, Nordenvall et al., 1988 and Nordenvall and Sandstedt, 1990), but these relations have also been disputed by others (Shordania, 1929, Benson and Fujikura, 1969). In paper I, placental gross developmental factors, including allantochorial vessel pattern and cotyledons visualized by angiography, has for the first time to our knowledge been related to the umbilical FVW. Earlier studies have focused on histopathologic findings related to IUGR in combination with abnormal FVW (Fok et al., 1990, Laurini et al., 1994, Kreczy, Fusi and Wigglesworth, 1995 and Karsdorp et al., 1996).

It is a novel and interesting finding that nearly all placentas from fetuses with diastolic zero flow had a magistral pattern. Shordania (1929a) used the hydrodynamic formula of Poisseuille to work out that the magistral vessel type would have considerable advantages over the dispersed type. He observed an increasing infant size from dispersed vessel pattern to mixed pattern and further to magistral pattern. As stated by Crawford (1962) this has never been confirmed. Our results were contradictory to those of Shordania. One must rather consider the causal mechanisms instead of the resulting hydrodynamics. Dispersed vessel pattern seems to be the result of regular vessel branching over a decidua providing optimal spiral artery supply.

According to the theory of trophotropism (Krone, 1961 and Benirschke, 1965), when there are uterine areas less suited for placental growth, it expands eccentrically into the region best suited. Such eccentric expansion has been registered ultrasonographically (Pretorius et al., 1996 and Di Salvo et al., 1998). My current view is that a magistral vessel pattern is an expression of such unfavourable conditions, though genetic factors may also play a role.

Magistral vessel pattern is also related to velamentous cord insertion, which per se is an adverse factor through the risk for compression of free vessels in the membranes, or even for vasa previa (Kouyoumdjian, 1980), i.e. vessels in the membranes before the fetus, which may rupture before or during labour.

Different opinions have been proposed about the significance of placenta extrachorialis. Benson and Fujikura (1969) after prospective study of nearly 40,000 pregnancies concluded that there was minimal correlation

between extrachorial placenta and clinical abnormalities, but Fox and Sen (1972) found significant association between circumvallates and fetal growth retardation among other complications, and in our material (paper I), 5 out of 6 extrachorial placentas showed diastolic zero flow.

There are explanations for some exceptions from the majority of the results for the group with normal FVW. The individuals with a normal systolic/diastolic flow (S/D) ratio also showed normal anatomy if the four cases with a slightly increased S/D ratio were not reckoned with. These four cases had a low number of cotyledons. In two infants with normal gross placental anatomy and IUGR, the latter could be explained by a chronic infection, villitis.

Our results strongly indicate a previously unknown relation between pathologic FVW and gross anatomy including gross arterial vessel pattern, as evaluated by conventional methods. In view of these facts, we went on to find an objective method to quantify aberrations in vessel pattern.

Our fractal analysis was the first application of this method to the placental arterial tree for determining the fractal dimension. Fractal geometry gave a model, which was in accordance with the arterial vessel pattern of the placenta in a certain interval. Paar, Pavin and Rosandić (2001) state that fractals are self-similar at all scales in mathematical idealization, but the biological fractals are truncated, i.e. their self-similarity extends at most over a few orders of magnitude. One of the most extensive fractal systems found in nature comprises the sedimentary rocks, with the range of length scales extended to over three decades (Radlinski et al., 1999).

Among 96 reports on fractality of a wide range of natural systems, the range of appropriate scaling properties for declared fractal objects centred around 1.3 orders of magnitude (Avnir et al., 1997). We found the placental arterial tree to scale as closely as exact fractal over a one decade wide interval. Box lengths between 20 and 200 pixels (i.e. $1.3 \leq \log r \leq 2.3$) were used. Above this interval, the data points fell above the regression line, due to the fact that the arterial tree has essentially no holes as the size of boxes approaches the size of the entire structure.

Besides providing a tool for placental investigation, fractal geometry also suggests that there is an underlying regular mechanism behind vasculogenesis**.**

The relation between lower fractal dimension and impaired placental function, here reflected in fetal growth restriction and pathologic FVW, is in accordance with other findings in pathologic conditions. Table VII shows some examples.

Table VII. Decreased fractal dimension is related to pathology in different organs.

		Control group	Pathologic group
Honda et al., 1992	Parotid ducts	1.67	1.36
Cross et al., 1993	Renal arteries	1.61	1.55
Boxt et al., 1994	Pulmonary arteries	1.62	a) 1.50
			b) 1.44
Ullberg et al., III	Placental arteries	1.86	1.76

Honda et al. (1992) found lower fractal dimension of the parotid ducts in patients with Sjögren's syndrome. Cross et al. (1993) demonstrated lower fractal dimension of renal angiograms in congenital renal dysplasia, renal artery stenoses and recurrent thromboembolic lesions respectively. Boxt et al. (1994) obtained lower values for pulmonary arteries in pulmonary hypertension, induced by hypoxia (group a) as well as by hyperoxia (group b), see table VII.
The results are also in accordance with the visual impression of thinner pattern in pathologic conditions, among the studies referred to above most pronounced in the parotid ducts in patients with Sjögren's syndrome. These had a pruned appearance with visible reduced complexity compared to normal controls.

Increased fractal dimension has on the contrary been reported in histology of epithelium after exposition to carcinogen (Landini et al., 2000) and in high resolution computed tomography of lungs in interstitial abnormalities (Kido et al., 1995), both states increasing tissue complexity.

The fractal dimension is an overarching measure, which reacts for any abnormality, which changes the complexity of the structure (Boxt et al., 1994). This gives an opportunity for detecting abnormalities, which have not previously been defined or even discovered.

It could be argued, that fractal geometry should have been less applicable to the placentas with pathologic FVW than to the normals. Goldberger et al. (2002), on basis of heart beat dynamics, discuss that fractal geometry

breaks down with disease and aging. In placentas, which have not developed freely according to basic mechanisms for vasculogenesis, the structural self-similarity is less pronounced, but they did not to any significant extent scale less closely according to a power-law than the normals.

The second main aim of this thesis was to study the variable anatomy of Hyrtl's anastomosis in controlled series as the method of choice for surveillance of risk pregnancies is Doppler assessment of umbilical artery FVW, preferably performed in the vicinity of the cord insertion.

Young 1972 stated that it so far had been impossible to identify any factor linking placental shape or type with the presence or absence of the anastomosis.

However, in the present study a relationship between on the one hand the occurrence and width and on the other hand the symmetry between the territories supplied from each umbilical artery (table VI). Studies related to the relative placental areas supplied by each umbilical artery are sparse. According to Bacsich and Smout (1938) the umbilical arteries are always of equal calibre in the cord whatever the relation of the areas supported by them. Priman (1959) reported the anastomosis to be at an acute angle to the long axis of the umbilical arteries, and most often appearing to be directed "from the stronger to the weaker" artery. This interpretation is not unequivocal.

Szpakowski (1974) observed that in oblique Hyrtl's anastomosis there was a distinct difference in the size of the areas of the placenta supplied by each umbilical artery, but did not present any numbers. Hitschold et al. (1990, 1992) reported asymmetric territories in two cases in which lack of the anastomosis was a prerequisite for discordant umbilical artery flow.

Prenatal ultrasonographic detection including Doppler assessment has recently been performed demonstrating a pulsatile blood flow in the anastomosis (Raio et al., 1999). A slight difference in resistance between the two umbilical arteries was detected, which was higher at the placental side than at the fetal side of the anastomosis. This demonstrated for the first time in vivo the important function as a pressure-equalizer. The flow in the anastomosis was in all cases unidirectional towards the umbilical artery with a lower resistance index.

The occurrence and anatomic variants of Hyrtl's anastomosis has been investigated in strictly defined study groups (paper V). Many studies

were performed several decades, even more than one century, ago, on unselected material. Since SGA placentas often display an aberrant arterial pattern, one would expect divergent anatomy of Hyrtl's anastomosis. However, we now found an anastomosis of the same types and frequencies is present in placentas from full term AGA infants with normal umbilical artery FVW, as in placentas from SGA infants, both asphyctic and non-asphyctic. However, there might be a difference in multiple gestations. Some authors have studied a mix of single and multiple gestations, like Szpakowski (1974), but our present study deals solely with single gestations. In a separate study of 52 pairs of twins, an increased frequency of velamentous cord insertion (12/104 = 12 %) was observed, which seems to be related to a higher frequency of the fusion type of anastomosis (Ullberg, unpublished data).

A survey of different studies is shown in table VIII. One must keep the empty panels in mind when judging the data. The omission of data in one column is not certainly a zero value, since it might alternatively stand for exclusion of such cases from the study group. All studies agree that the vast majority has a vessel type anastomosis. There are differences in the reported frequency of anastomosis by fusion, which varies from 3% to 32%. In our material it was present in 4.6%. Possibly different definition have been applied.

In the studies showing high frequency of fusion, one could speculate about whether a wide anastomosis in some cases might have been interpreted as a fusion after giving off a weaker branch, but this cannot entirely explain the overrepresentation.

Table VIII. Types of anastomosis, comparison between studies.

Author	Year	Nr of cases	Vessel	Fusion	Fenestr.	Absence	SUA
Hyrtl	1870	200	90.5 %	3%	2.5%	2%	
Shordania	1929	194	76.3 %	23.7%	0%	(3/197)	
Bacsich & Smout	1938	50	86%	14%	0	0	
Priman	1959	70	90%	10%		2 (2,9%)	1(1.4%)
Kedzior	1965		68%	32%			
Bhargava & Raja	1970	167	70.7%	24.0%		5.4%	
Szpakowski	1974	170	84.5%	14.3%			
Schellmann et al.	1976	100				0	
Benirschke (rev.)	1998					4%	
Oszukowski	1998	203	88.26%	9.57%		2.17%	
Raio et al.	2001	41	87.8%	12.2%			
Ullberg et al.	2003	131	85%	4.6%	2.3%	3.8%	3.8%

The relation between symmetry of the umbilical artery supply areas and the relative width of the anastomosis was equivalent for AGA and SGA placentas. This points to the need for equalizing flow resistance between the two umbilical arteries is more important for the appearance of Hyrtl's anastomosis than other factors. On the other hand, the presence of an anastomosis may be of greater importance for the well-being of a growth-retarded fetus, lacking placental reserve capacity, than in an uncomplicated pregnancy.

As to the number of anastomoses, we found two anastomoses in one of 131 cases investigated. Hebenstreit (1737), the first to describe the presence of an anastomotic channel, thought they were multiple, but this misunderstanding has later been confuted (Young, 1972). Certainly there are double anastomoses in some animals. Young, Bacsich and Boyd (1962) reported a frequency of 24.7% for cattle and 1.56% for sheep. Numerous anastomoses are reported to occur in human placentas between the third and fourth gestation week (Stieve and Strube, 1933, Patten, 1946) and a 3.5 mm embryo has shown 3 anastomoses (Young, 1972). More than one anastomosis at term is rare. Szpakowski (1974) reported 2 cases with double anastomoses among 170 placentas, Priman (1959) found 1/70 and Oszukowski (1998) 1/203. Kedzior (1965) reported one case in a material of unknown number. Hyrtl (1870) failed to find any case with double anastomoses among his 200 investigated placentas, and so did Arts (1961) among 65 and Bacsich and Smout (1938) among 50 specimens. In summary (the study of Kedzior excluded) double anastomoses were present in 5 cases out of 889 investigated placentas, which is equal to 0.6%. The fact that the few placentas lacking Hyrtl's anastomosis were highly symmetric might suggest a regression of even the last anastomosis when there is no physiological need for it.

A transverse histological section of a vessel type anastomosis showed a considerably thinner muscle layer, with only a circular layer, compared to the connected umbilical arteries (paper V). This suggests that an anastomosis of this type is able to react in the same way to stimuli, but probably not as powerfully, as the umbilical arteries.

Discussion of methods

Freezing

Deepfreezing of the placentas immediately after parturition counteracts blood clot formation, making the contrast filling of the arterial tree possible without preceding rinsing of the vessels, which would be traumatic and increase the risk of contrast leakage. It also has the advantage of permitting storage of the placentas for later study, as shown by Krohn, Ivemark and Salo (1970). They reported poor filling with barium sulphate suspension in 14/26 placentas investigated >1 hour after birth but in none of 10 placentas investigated or freezed within 1 hour. Other authors, e.g. Gudmundsson et al. (2000) first perfused the organ with a fluid containing heparin to prevent blood coagulation, but this procedure also had to be performed immediately after delivery.

Freezing and thawing followed by blood drainage through the unclamped umbilical cord also has the advantage of giving more comparable placental weight data. A potentially negative effect would be the vessel diameters being influenced, but this was overcome by measuring relative vessel widths. The width of Hyrtl's anastomosis after freezing and thawing was remarkably similar to in vivo conditions as reported by Raio et al. (2001), with a mean between 2 and 3 mm.

Angiography

The discoid shape of the placenta, with the allantochorial arteries radiating along the surface and the cotyledons lying side by side without overlapping to any greater extent, is suited for angiography, each image being a two-dimensional projection of the vessels. Post partum placentas can be imaged with high quality and without magnification due to the lack of other organs overprojecting and of being able to place the film in close contact to the object. One great advantage over the cast-corrosion techniques extensively used in former investigations of placental vessels (e.g. Spanner 1935, Bacsich and Smout 1938, Stieve 1941, Wilkins 1954, Crawford 1962, Bhargava and Raja 1970) is that all the placental tissue is preserved allowing samples to be taken for microscopic (including histomorphometric), chemical, immunologic and other analyses.

As stated in the methods section, the arterial vessel trees were filled with barium water suspension down to arteries in the tertiary stem villi, sized 0.03 – 0.05 mm, with passage to neither the capillaries nor the veins. Thereby the arterial phase was visualized in a static way, avoiding the need for exact timing of imaging and allowing subsequent slicing and perpendicular projections.

The injection of contrast medium through one of the umbilical arteries was performed under 100 mm Hg pressure. This pressure has been used by other authors using barium suspension (Sandstedt, 1974, Björk, 1982, Hitschold et al., 1992). This is above the physiologic pressure. However, Gudmundsson et al. (2000) perfused placentas at 74 mm Hg and then injected water-soluble contrast medium at "low pressure", but they were forced to complete with selective catheterization of arteries since all were not filled in the first séance. Injection of plastic solutions to make corrosion casts is reported to demand significantly higher pressures, up to 200 mm Hg (Arts, 1961), with higher risk of leakage and influence on vessel measurements.

An alternative radiological method to classic angiography used for this study might have been computed tomography, CT. The use of CT renders three-dimensional representations of the subjects, giving the possibility to assess volumes, i.e. volumes supplied by individual vessels or of placental lesions. The comparatively higher irradiation dose is no problem in *in vitro* studies. However, CT compared to conventional radiography implies reduced spatial resolution and increased sensibility to attenuation differences. The former makes CT less suited for fractal analysis. Increased attenuation sensibility in post partum placenta studies offer no advantage per se, and necessitates the use of a less attenuating contrast medium to avoid artifacts.

A great disadvantage with the radiologic methods is that they involve ionizing irradiation. It is not justifiable to expose a fetus for in placental studies *in vivo,* and therefore radiological studies necessarily must be performed *post partum*.

Magnetic resonance imaging (MRI), including dedicated vessel studies designated magnetic resonance angiography (MRA), is an interesting, fast developing alternative to x-ray examination. It has the advantage of making placental studies, including flow measurements , possible in vivo. Assessment of placental growth has been shown to be feasible using echo-planar imaging by Duncan et al. (2001). Moore et al. (2000) by the same technique have been able to measure blood movement in the placenta. Still, MR presents much less spatial resolution for detailed structural vessel mapping. Ultrasonography also makes in vivo studies including flow measurements possible, but with limited spatial resolution. There is great interest of in vivo placenta studies including the possibility of longitudinal studies, but in addition to the problem of limited spatial resolution, there are problems tied to the in vivo setting. Concerning MRI, fetal movements give rise to motion artifacts,

restricting the use to fast sequences. With ultrasonography accessibility may be diminished as a consequence of placental site and fetal position (Raio et al. 2001). While alternative methods are of great interest for studies of placental morphology and physiology, there are ethical and practical limitations attached to the in vivo situation. Fractal analysis requires the high spatial resolution given by post partum x-ray angiography.

Besides, post partum angiography has been shown to be of great value for evaluation of findings from ultrasound studies, (Gudmundsson et al., 2000), and the same is true for MR.

Fractal analysis

Image processing

Binary representation of the arterial tree was attained by first digitizing by scanning and thereafter identifying the vessels by edge-detection. The inbuilt image-processing in our brains make us percieve the angiograms as white vessels on a grey background. In reality, the angiogram consists of the representation of vessels of various diameters, consequently of different nuances on the grey-scale, against a background of the placental tissue, which, more frequently in the pathologic placentas, varies in thickness and thereby in attenuation. The result is an image, in which some vessels may be of lesser intensity than some of the tissue background in other parts of the same angiogram, thus making simple threshold detection for identifying the vessels impossible. Edge-detection instead traces shifts between different levels on the grey-scale.

An alternative method for producing a binary representation of a biological structure visualized by radiography was used by Honda et al. (1991, 1992), who traced sialograms of the parotid duct system by hand. Boxt et al. (1994) were able to use simple thresholding for making angiograms binary when investigating pulmonary arteries. In that case the background level must have been rather homogenous since they dealt with post mortem contact angiograms of rat lungs, in which the airways were filled with water.

The two-dimensional model

We have determinated the fractal dimension by box-counting in a two-dimensional model. This is well-suited to the architecture of the placental arterial tree since after the in vivo curved surface of the placenta has been flattened out on the x-ray film, the allantochorial arteries between the membranes branch in a two-dimensional plane. The primary cotelydonary arteries given off by the allantochorial arteries dive

vertically into the chorion and thereafter branch into secondary and tertiary cotyledonary arteries. These run in a three-dimensional way, so the angiographic image is just a two-dimensional projection of them. However, since the cotyledons lie side by side without overlapping to any greater extent, the two-dimensional model can be considered to be representative.

Hyrtl's anastomosis

The angiographies were performed as contact radiograms, and therefore without magnification. Notwithstanding that, there may be a discrepancy between the measured vessel diameters compared to the in vivo conditions owing to the post mortem state and the freezing and thawing procedure.These factors were common for all placentas making all vessels in all placentas consequently comparable to each other. The measured dimensions were, however, remarkably similar to in vivo conditions, as reported by Raio et al.(2001), with a mean diameter of the anastomosis between 2 and 3 mm in both studies.

The measurement of the length of the anastomosis when consisting of a separate vessel in a two-dimensional projection gives falsly too low values. The length reported therefore should be regarded just as a gross hinting about the magnitude. The length has also been characterized in types, i.e. "straight way between the umbilical arteries", "oblique" or "coiled".

Summary and conclusions

1. Diastolic zero flow in the umbilical artery is highly associated with gross placental developmental abnormalities.
2. The fractal dimension of the placenta's arterial tree offers an objective and reproducible measure of its complexity. The fractal model is well suited to the placenta's arterial vessel pattern in placentas from uncomplicated pregnancies with normal umbilical blood flow velocity waveforms
3. The fractal dimension is lower in the arterial tree of placentas with pathological umbilical blood flow velocity waveforms compared to normal controls. The former more often showed magistral allantochorial arterial vessel pattern and marginal cord insertion. The placentas with low fractal dimension and pathological FVW also showed other abnormalities; low weight, small area, few cotyledons and non-uniform cotyledonary size.
4. Fractal geometry suggests that there is an underlying regular mechanism beneath vasculogenesis.
5. Hyrtl's anastomosis is usually a single connection between the two umbilical arteries at the cord insertion, all branches distal to that being end arteries. In less than ten percent of cases there is no anastomosis, half of which because of a single umbilical artery. Rarely two anastomoses were present (0.8%).
6. Hyrtl's anastomosis shows great anatomical variation. It most frequently is represented by a separate vessel between the two umbilical arteries. Some placentas show a fenestration through the walls of the umbilical arteries, in these cases adjacent, and no vessel structure. The third variant is a short fusion of the umbilical arteries. There is also a variation in length and width of the anastomosis.
7. The type or relative width of the anastomosis did not differ between the placentas from AGA infants and from SGA infants.
8. The occurrence and width of the anastomosis in all settings related to the symmetry in size between the supply areas of each umbilical artery, both in the AGA group and in the SGA group, even in spite of gross differences in type of placentation and cord insertion. The results suggest that the relative size of the umbilical artery supply areas, and thereby the demand for shunting of blood, influences the anatomy of Hyrtl's anastomosis more than other factors do.

Future perspectives

The present investigations have raised many new questions.

1. Intra-uterine growth retardation is common in multiple gestations, and in many cases the birth weight is significantly unequal. This inequality is only in part explained by different sex and transfusion syndrome respectively. We have observed that the placenta (or the part of a fused placenta) belonging to the smaller individual of an unequal pair of twins often shows developmental abnormalities including divergent arterial vessel pattern (unpublished data). Here is a field for more detailed study.
2. Twin gestations are also interesting regarding their umbilical cord insertions. Further studies of the relation of the cord insertions to the placental mass would be useful for evaluation of the theory of trophotropism.
3. Ultrasonography of the placenta is a still developing field, partly due to the introduction of 3 D imaging and further improvement of Doppler assessments. Following up of these examinations with post partum angiography would be of great value.
4. Magnetic resonance imaging (MRI) has recently been taken into clinical use for fetal diagnostics when further investigation beyond ultrasonography is required. MRI also gives an excellent option for investigation of the placenta in vivo. Studies with comparison of MRI findings to post partum angiography with pathological examination as a reference standard have yet to be done.
5. Because the placenta may have a heterogeneous appearance, more often in complicated pregnancies, the location of specimens taken for microscopic morphology or chemical analysis should be related to the macroscopic appearance both for routine diagnosis and for further studies.

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