Maternal-fetal water exchange in human pregnancy

A literature and human clinical study

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Table of contents

Abstract

Background

The transfer of water between the maternal and fetal unit is of vital importance for the developing fetus. We aimed to study physiological mechanisms of this transfer, and to estimate the net transplacental water transfer in vivo in human term pregnancies.

Design

Literature study and cross sectional human in vivo study.

Method

Systematic searches were performed in PubMed, Web of Science and Google Scholar, using the following search terms to find eligible articles: [placenta] AND [water] AND/OR [maternal], [fetal], [exchange], [transfer], [fluid] and [placenta] AND/OR [water channels] AND/OR [aquaporins].

In healthy term pregnancies blood flow was measured in the uterine artery and the umbilical vein by Doppler ultrasound before blood samples were collected during cesarean section. Hemoglobin and hematocrit concentrations were measured in the maternal radial artery, the uterine vein (n=43) and the umbilical artery and vein (n=31).

Results

The searches in total retrieved 3134 articles which were evaluated by duplicates, title and/or abstract. Human in vivo and in vitro studies and relevant experimental animal studies were included giving a total of 52 articles.

In our human in vivo study, the median [Q1, Q3] estimated water uptake from maternal circulation to the uteroplacental unit was 10.02 [1.67, 13.57] ml/min, and from the placenta to the fetal circulation 10.71 [4.98, 16.99] ml/min.

Conclusion

There are several driving forces and transfer mechanisms implied in placental water transfer, probably working together and the picture remains uncomplete. Our estimations exceed the previous and indicate transfer of considerable amounts of water at term. Existing literature supports bidirectional placental water transfer which may be large under given circumstances.

Key words:

Water, maternal-fetal transfer, placenta, human

Abbreviations

basal membrane (BM)
body mass index (BMI)
colloid osmotic pressure (COP)
cytotrophoblasts (CT)
freezing point depression (FPD)
gestational age (GA)
hemoglobin (Hb)
hematocrit (Hct)
horse radish peroxidase (HRP)
intervillous space (IVS)
lanthanum hydroxide (LH)
microvillous membrane (MVM)
sodium lauryl sulfate (SLS)
syncytiotrophoblast (SCT)
time-averaged maximum velocity (TAMX)
vapor pressure osmometer (VPO)

Introduction

In humans the placenta serves as the interface between the mother and her fetus. As gestation advances intrauterine life is completely dependent on a functional placenta. In postnatal life, several different organs are needed to fulfil the same tasks and functions that the placenta

provides during pregnancy. It holds a great importance and is surrounded by mystique in several cultures. Some groups believe the placenta to have supernatural powers and that it withholds the guardian spirit of the child (1). Other cultures have been known to eat the placenta, burn or bury it so evil spirits or animals will not reach it, to use it as medicine or a contraceptive and to celebrate ceremonies in its honor (1).

The placenta has been considered to be a rather passive component of pregnancy; its function similar of other passive diffusion membranes. For a long period of time, it was quite unrecognized as an independent organ (1). Today we know that the placenta has a complex physiology and governs the maternal adaptions to pregnancy and the intrauterine conditions, and thereby fetal outcome (2). It is the site of maternal-fetal exchange and asserts the regulation of gas exchange, provides all nutrients for the fetus and removes its wastes. In addition, it is an important endocrine organ, a protector from pathogens, teratogens and drugs and an establisher of the first immunological defense. Recently, it has been recognized that the placenta through modulating maternal physiology and the intrauterine conditions for the developing fetus have long-term effects for the future life and health of both mother and offspring (3-7).

Moreover, these placental functions change and adapt during pregnancy to meet the evolving fetal demands and secure fetal growth. Several studies have demonstrated that placental weight and neonatal birthweight are closely correlated and that placental function is of vital importance for fetal growth and intrauterine health (8-10). Because the placenta is a dynamic organ and because fetal needs continuously change during pregnancy, the study of placental physiology is complex and challenging. The interspecies differences in placental anatomy and pregnancy physiology complicate extrapolation of experimental data. The limited access to this organ and the obvious ethical issues of invasive studies further complicates human placental research. In 2015, The National Institute of Health (USA) recognized the placenta as our most poorly understood organ and initiated the Human Placenta Project, where the ultimate goal is greater understanding of the human placental structure, development and function (11). Even as a lot of effort is put in to studying placental pathophysiology important basic functions of the placenta, like the exchange of water, remains ambiguous. Placental water transfer is of great importance for fetal development, likely to influence maternal-fetal nutrient and waste exchange as well as fetal hemodynamics. Altered quantity of amniotic fluid is part of the clinical development in several pregnancy pathologies. This thesis will

focus on placental water exchange between maternal and fetal compartments and consists of a literature study and a clinical human in vivo study.

Anatomy and development of the placenta

The human placenta is a discoid organ, which at term weighs on average 500 g and has a diameter of 15-20 cm. It has a surface area of 10-14 m^2, larger if microvilli are included. The maternal side of the placenta is perfused mainly by the two uterine arteries. The arteries branch into about 100 spiral arteries that open directly into the intervillous space (IVS) where maternal blood, rich in oxygen and nutrients, flows freely. On the fetal side, the two umbilical arteries transport deoxygenated blood and waste from the fetus to the capillaries of the placental circulation. These capillaries extend into the placental villous tree which branches out in the IVS. Thus, placental villi of fetal origin are in direct contact with maternal blood. After gas, nutrient and wastes are exchanged, the oxygenated and nutritious blood is transported back to the fetus through the single umbilical vein. The uterine veins return the remaining deoxygenated and nutrition poor blood back into the maternal circulation (Figure 1).

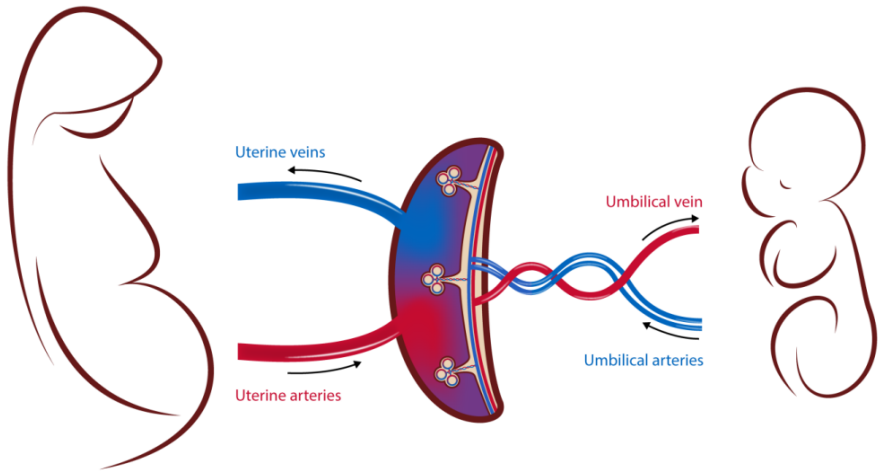

Figure 1. The maternal and fetal placental circulations. Illustration: Øystein H. Horgmo, University of Oslo. Reused with permission from Holme, AM (12).

The majority of maternal-fetal exchange takes place at the terminal branches of the villous tree, the terminal villi (figure 2). It consists of a multinucleated syncytial layer called syncytiotrophoblast (SCT), a dispersed layer of cytotrophoblasts (CT) and the fetal capillary endothelium. Throughout pregnancy the layer of the CT becomes more discontinuous and completely disappears in some areas. At term, only two cell layers separate the fetal and maternal circulation. This is called the vasculosyncytial membrane, where the distance

between maternal and fetal blood is at its smallest (2-3 µm) and where the maternal-fetal exchange will be most efficient.

The SCT is a continuous cell layer in direct contact with maternal blood. It is polarized with a highly folded microvillous membrane (MVM) facing maternal blood and a basal membrane (BM) facing the subjacent CT or the fetal endothelium. Both membranes contain multiple different receptors and transport proteins but demonstrate a distinct polarity. The polarity and the properties of the syncytiotrophoblast are crucial for the maternal-fetal exchange.

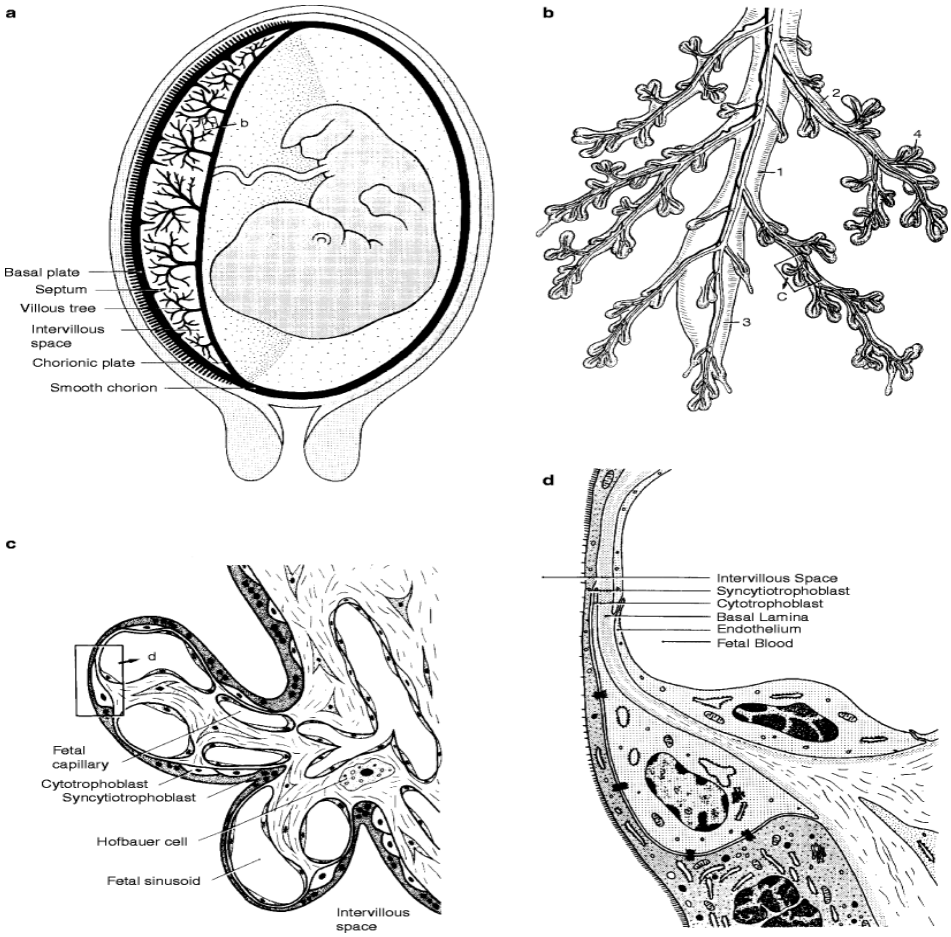

Figure 2. Basic morphology of human placental villi. a) Longitudinal section across the uterus, placenta and fetal membranes in the human. The chorionic sac, consisting of placenta and membranes, is black. b) The mature villous tree protruding into the intervillous space which is bathed in maternal blood. A stem villus (1) continues into a bulbous immature intermediate villus (3). The slender side branches (2) are the mature intermediate villi, its surface densely covered with terminal villi (4). c) Highly simplified section of two terminal villi. d) Schematic section of the vasculosyncytial exchange surface, demonstrating its layers. From left to right: intervillous space (perfused with maternal blood), microvillous membrane (MVM) of the syncytiotrophoblast, the basal membrane (BM) of the syncytiotrophoblast, the basal lamina, the fetal endothelial cell and the fetal capillary (perfused with fetal blood). Reused with permission from Benirschke K et al (13).

Comparative placentation

There are considerable interspecies differences in respect to placental anatomy and physiology. The different types of placentas are classified into three main types, based on the number of layers separating maternal and fetal blood (14). In the epitheliochorial placenta (sheep, goat and pig), the trophoblasts are attached to the uterine epithelium but does not invade it. The placental barrier consists of the maternal endothelial wall, subepithelial connective tissue, uterine epithelium, the trophoblast, a basement membrane and the fetal endothelial wall. In the endotheliochorial placenta (carnivores), the trophoblasts invade and destruct the uterine epithelium. The trophoblast is thus directly opposed to the maternal endothelium. The barrier consists of maternal endothelium, the trophoblast, the basement membrane and the fetal endothelium. In the hemochorial placenta the trophoblast is in direct contact with maternal blood. Hemochorial placentas can be further subdivided according to the number of continuous trophoblast layers separating maternal and fetal circulation. Hemotrichorial placentas (mouse, rat) where there are three layers of trophoblasts between the two circulations, hemodichorial placentas (early human, rabbit) where there are two layers of trophoblasts, and hemomonochorial placentas (mature human, guinea pig) where only one layer of trophoblasts (the syncytiotrophoblast) separates fetal and maternal circulation.

Placental functions

In addition to transport and exchange functions which will be described in more detail, the placenta exerts a number of vital functions that no other single organ can manage alone. The placenta is a major endocrine organ and a successful pregnancy is completely dependent of the hormones produces by the placenta. The placental hormones influence both maternal and fetal conditions, for instance the maternal physiological adaptions to pregnancy are largely initiated and controlled by placental hormones. Events like implantation, placentation, remodeling of spiral arteries, immunomodulation, labor and breastfeeding are also dependent on hormone production (15). The main producer of placental hormones is the syncytiotrophoblast, although other placental cells also contribute. Amongst the most essential pregnancy hormones are human chorion-gonadotropin (hCG), progesterone, estrogens, placental growth hormone and human placental lactogen (hPL). Other types of hormones like leptin and other adipokines, inhibin and activins are also produced. Abnormal levels of placental hormones are implied in several pregnancy-associated complications (15).

The possibility of placental hormones as biomarkers for pregnancy outcome is an object of comprehensive research today.

As earlier noted, in the placenta, cells of fetal origin are in direct contact with maternal blood. Although it contains paternal antigens, foreign to the mother, the composition of the trophoblast surface does not lead to a maternal immune activation. Several mechanisms are believed to be at play to suppress the maternal immune response (16-17). On the other hand, the placenta also protects the fetus from certain infectious pathogens, drugs and xenobiotics and transfers immunoglobulins from maternal to fetal blood. It can thus be considered as the first immune system of the fetus.

Transfer across the placenta

The placental transport of most substances is bidirectional and a result of continuous exchange between mother and fetus. This may result in a net transfer in either maternal or fetal direction. There are two possible routes of transfer across any epithelium; transcellular and paracellular. A unique feature of the syncytiotrophoblast is that it is believed to be a true syncytium without intercellular spaces between the syncytiotrophoblast cells. Thus, the main route of transport for nutrients and ions over the vasculosyncytial membrane is assumed to be transcellular. There is some evidence for narrow water filled channels crossing the syncytiotrophoblast ("transtrophoblastic channels") or temporary breaks in the syncytium which would provide an alternative transfer route between the maternal and fetal compartment, but the occurrence and significance of these channels remains to be settled (16).

The transfer of any substance across a membrane can occur down or against a gradient. The gradient can be osmotic, colloidal, hydrostatic or electrical. Non-mediated transport down a gradient is called diffusion, and the respiratory gases cross the placenta by this pathway. Mediated transport is the transport of a substance through specific transport mechanism, e.g. by membrane proteins. It includes both facilitated and active transport. Facilitated transport does not require energy and happens down a gradient. Glucose is transported across the placenta by facilitated transport through glucose transporters (GLUTs) in the MVM and BM of the SCT. Active transport drives a substance against a gradient and is an energy requiring process. Sodium/potassium is actively transported by the Na/K-ATPase into and out of the cells respectively.

Transfer of substances by endo- and transcytosis is also possible. The MVM or BM of the SCT invaginates and creates vesicles. The vesicle can then be released intracellularly or transported across the SCT, re-fuse with the plasma membrane on the opposite side and release vesicle content outside the SCT. This is the transfer pathway for IgG, iron and some lipoproteins across the placenta. In addition, micro- and nanovesicles are released from the MVM into the maternal circulation. The significance and specific content of these vesicles are currently being explored and they are believed to participate in immunological signaling and intercellular communication.

Concentration differences between the maternal and fetal circulation as well as molecular properties of the transported substance (e.g. size, charge, lipid solubility, molecular weight and grade of protein binding) and the characteristics of the membrane (fluidity and lipid composition, thickness and surface area) are factors that will influence transfer across the membrane. For example, the thickness of the placental membrane decreases with increasing gestational length which will improve transport efficacy. Regarding mediated transport, the expression and action of transporters, channels, enzymes and receptors in the SCT membranes are of importance. The blood flow to the placenta from both mother and fetus influences hydrostatic pressure differences and the availability of substrate and is dependent of maternal and fetal cardiac output. All in all, there is a constant interplay between mother, placenta and fetus to balance the different needs and ensure a successful pregnancy.

Transplacental water transport

When studying maternal-fetal water transfer, different transfer routes must be considered. Transplacental water transfer is believed to be an important route of both fetal water uptake and loss. Other pathways of fetal water uptake are transfer across the fetal membranes, through fetal intake of amnion fluid (drinking) and fetal oxidative metabolism. Fetal water loss happens through the kidneys (urine production) and the lungs. Gain or loss of water from the fetal compartment through the fetal membranes, maternal lymphatic system and muscle tissue are also imaginable routes.

Possible transplacental routes are by transsyncytiotrophoblastic channels, specific aquaporins and diffusion directly through the membrane lipid layer of the SCT. The driving forces could be different gradients between mother and fetus – based on hydrostatic, osmotic, colloidal and

electrical forces. The hydrostatic gradient is the difference between fetal and maternal blood pressure, e.g. blood pressure of IVS and the fetal capillaries emerging from the umbilical vein. An osmotic gradient would be a concentration difference of any solute with an osmotic potential between maternal and fetal blood. This also includes a colloidal osmotic gradient which is the difference in maternal and fetal colloidal osmotic pressure exerted by plasma proteins. An electric gradient would occur if either maternal or fetal side is more electronegative than the other. The difference in electrical potential is produced by transport of ions through different pathways.

Altered water transfer and fluid balance between maternal and fetal compartments is implied in several pregnancy complications. Maternal-fetal water transfer is complex and depend on several factors difficult to study simultaneously. The transfer routes, the driving forces and the volume of transfer remains debated. There are few reviews on the subject and a lack of human data. Thus, I have summarized the existing literature with the focus on maternal-fetal water transfer and mechanisms. Furthermore, we aimed to study maternal-fetal water transfer in the human in vivo, using blood samples from incoming and outgoing vessels both on the maternal and fetal sides of the placenta (4-vessel sampling) (17).

Literature study

Method and material

The literature search was performed from August 2018 to August 2019 in PubMed, Web of Science and Google Scholar. The following search terms were used in a general search strategy [placenta] AND [water] AND/OR [maternal], [fetal], [exchange], [transfer], [fluid]. Searches for [placenta] AND/OR [water channels] AND/OR [aquaporins] were also performed, but the selection of articles were limited to overviews or articles aiming at a general discussion. The searches in total retrieved 3134 articles of which 127 were included based on the title and/or abstract. After correcting for article duplets and a more thorough reading of the articles, a total of 27 articles from the different PubMed searches were included. A more selective search was also performed by selecting, looking up (in PubMed, Web of Science, Google Scholar) and reading references from the articles already included. This led to the inclusion of another 25 articles. Inclusion and exclusion criteria are described in figure 3. The total number of articles included in this review was 52 articles.

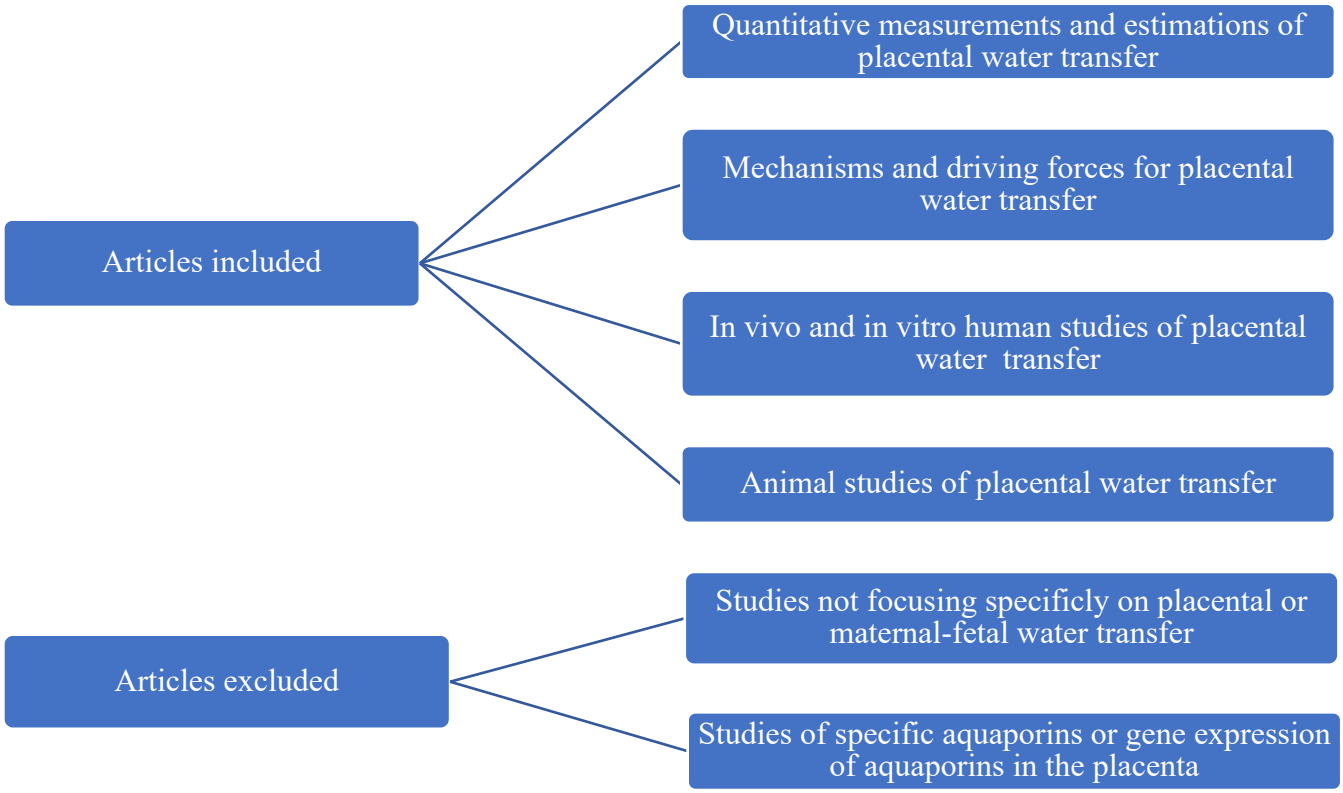

Figure 3. Inclusion and exclusion criteria of the literature search

Results

An overview of the most relevant articles identified by the literature search is presented in supplementary table 1 and 2 differentiated by human and experimental data. The quantitative estimations of placental water transfer are listed in supplementary table 3.

Possible routes of maternal-fetal water transfer

Transtrophoblastic channels

Studies have shown that the human placenta is permeable to small, inert hydrophilic molecules that do not enter cells, e.g. inulin and mannitol (18, 19). The placental permeabilities of these molecules correlated with their diffusion coefficients in water. The diffusion coefficient is the proportionality between molecule flux and concentration gradient. The results were later interpreted by Stulc et al. as proof that the diffusion medium of each sides of the placental membrane is in contact with one another. It was proposed that this could be possible by wide, transtrophoblastic pores crossing the SCT membrane, but such channels have been difficult to demonstrate by electron microscopy (20). In the rabbit and rat, physiologists used permeability studies to mathematically estimate the radius of such pores (21, 22).

In experiments performed with horseradish peroxidase (HRP) as a tracer, the goal was to identify transtrophoblastic channels in the hemochorial placenta of the guinea pig microscopically (23). The hypothesis was that distensible transtrophoblastic channels open when the fetal hydrostatic pressure reached a certain point, thereby assuring that fluid in excess will be recirculated to the mother. Under normal pressure conditions, no transtrophoblastic transfer of HRP was seen. In a parallel experiment where the placenta of the guinea pig was artificially perfused, fetal venous pressure was increased to 20 mmHg. The result was a fluid shift in fetal-maternal direction. HRP-labelled invaginations from the interstitial space into the trophoblast as well as narrow intratrophoblastic tubules were demonstrated (23) (figure 4). HRP was even seen on the IVS surface. The authors meant these findings indicated HRP-containing fluid had crossed the trophoblast layer through these channels. There were also seen labelled pinocytic vesicles in the trophoblast below the IVS surface. When fetal venous pressure was further increased, the channels enlarged considerably. There were plenty cross-sectional views of the assumed channels, but few

longitudinal. Stulc et al. offered the explanation that the channels were contorted and irregular and therefore unlikely to be detected on one single electron micrograph. Instead, a single channel would appear in a number of electron micrographs at the same time (20). When hydrostatic pressure was normalized, the channels reduced in both width and number, and connections to the IVS, pinocytic vesicles and peroxidase on the maternal surface were no longer visible. Recent development in 3D imaging techniques may help elucidate whether the channels actually do exist under physiological conditions, or if they are artefacts of experiments (24-26).

In a human in vitro study by Kertschanska et al., 19 term placental cotyledons were harvested, artificially perfused via fetal circulation and the marker lanthanum hydroxide (LH) was added to the perfusate (27). LH does not penetrate intact plasma membranes but remains extracellular. LH precipitates were found to be concentrated in the basal membrane of the trophoblast and basal invaginations became visible. These invaginations were found deep into the syncytiotrophoblast near the apical (microvillous) membrane, LH-containing vesicles in the syncytiotrophoblast appeared and traces of LH were also visible in the intervillous space. However, no direct connection between invaginations and the intervillous space was detected under normal pressure. The authors discussed the possibility that the transtrophoblastic channels did not open directly into the IVS but were supported by apical vesicular transport. Regarding the function of these channels, the authors suggested them as a route for fetal-maternal transfer from the basal to the microvillous membrane (figure 4).

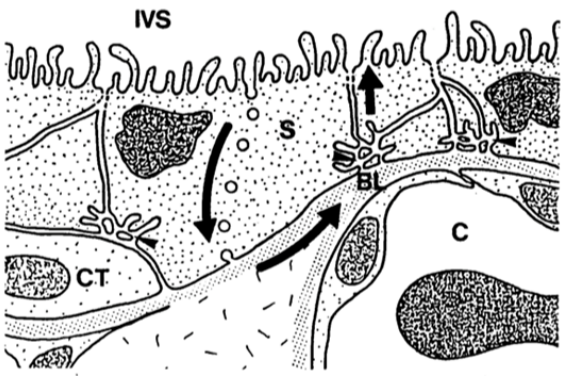

Figure 4. The possible transtrophoblastic channel system. IVS: intervillous space. S: syncytiotrophoblast. CT: cytotrophoblast. BL: basal lamina. C: fetal capillary. Arrowheads: invaginations from basal membrane. Arrows = route of membrane flux. Reused with permission from Kertschanska et al. (27)

Kertschanska et al. later isolated and perfused 56 cotyledons from normal and term human placentas (28). They increased fetal arterial and venous pressure in turn and demonstrated that the transtrophoblastic channels dilated and opened at the apical (microvillous) membrane into

the intervillous space. They further traced LH from the fetal side to the intervillous space. Dilatation of the transtrophoblastic channels were accompanied by stromal edema. The authors suggested that the stromal edema was a result of water transfer out of fetal circulation and into the stroma due to increased hydrostatic or colloid osmotic forces within fetal circulation. When pressure on the venous side was increased, much lower pressure and shorter time periods were needed to produce the same channel dilatation compared to the arterial side. They hypothesized that the wide, low resistance venous vessels influenced the intracapillary pressures more directly than the high resistance arterial vessels. Further they suggested that the transtrophoblastic channels functioned as pressure-dependent valves to help the fetus get rid of excessive water and accordingly they would be important in the regulation of fetal water balance and fetal-maternal water transfer. Similar results were found in the hemotrichorial placenta of rats in a following study; continuous channels were not visible under normal physiological pressure, but appeared when venous pressure was increased (29). However, in studies of the hemomonochorial placenta of the degus it has been questioned if the channels arise because of ischemic injury during preparation or fixation of the placental samples (30).

The transplacental transfer of the inert and radioactive labelled solutes mannitol, EDTA and inulin was measured in maternal-fetal and fetal-maternal direction in rats (22). They found that the transfer of all three substances was much higher from fetus to mother than from mother to fetus. This suggests a net fetal to maternal fluid flow which is not compatible with gestational development as the fetus would be increasingly dehydrated throughout gestation. The authors argued that other compensatory routes in the opposite direction must exist and proposed a hypothesis that there is a transplacental recirculation of fluids which is driven by active transport of solutes (Na^+) from the mother to the fetus. The osmotic gradient then pulls water across the placenta from mother to fetus. Water and NaCl in excess are filtered back to the mother through these wide transtrophoblastic channels in the placenta down a hydrostatic pressure gradient and thus providing a circulation of fluids over the placenta.
Another explanation given for the inconsequent findings regarding transtrophoblastic channels are temporary breaks in the thin human syncytial barrier (31). This could also explain that water does not drain from the fetus towards the mother but is pulled back to the fetus through these gaps in the syncytium, also through osmosis. In the human placenta, fibrin deposits have been identified, suggested to be filling the temporary gaps in the syncytiotrophoblast (32).

Osmotic transfer across the syncytiotrophoblast membrane

In a study by Jansson et al. human syncytiotrophoblast from term placentas were isolated and the two membranes, facing the maternal (MVM) and fetal (BM) side respectively, were separated (33). Placental vesicles of MVM and BM membranes were used to calculate osmotic water permeability at the two sides of the syncytiotrophoblast (34). The authors hypothesized that water moves across the syncytiotrophoblast by direct diffusion through lipid regions in the membrane rather than through transtrophoblastic channels. They found that the BM was significantly more permeable for water than MVM. BM has a different lipid composition and higher membrane fluidity than MVM, and the study demonstrated that the higher the fluidity, the higher rate of water transport. The water permeability of human MVM and BM was similar to other types of membranes that are known not to have water channels, and considerably lower permeability than epithelial membranes with known water channels. Inhibitory agents against water channels were applied to the vesicles, and no reduction in water permeability was seen. The activation energy for water transport in MVM and BM was higher than expected in membranes that contain water channels. Together these findings were taken to support the theory that MVM and BM lack water channels and favor a lipid-mediated pathway across the syncytiotrophoblast.

A following vesicle study showed that water permeability in MVM and BM increased with increasing gestational length, and declined near term (35). This may contribute to the increased water exchange observed in vivo which is described by Hutchinson D.L et al (36). No changes in cholesterol content or membrane fluidity were found to explain the increased water permeability. The authors hypothesized that the higher protein-lipid ratio in both MVM and BM in third trimester and subsequent decrease near term, as earlier described (37), could explain the gestational increase in permeability.

Aquaporins

Aquaporins are a family of small transmembranous proteins that are known to increase the water permeability of a lipid cell membrane and allow rapid transcellular movement of water. Water is transferred through aquaporins by osmosis due to an osmotic gradient which is produced by active transport of solutes. Some aquaporins have been suggested to be part of cell migration and adhesion (38) and of importance for normal placental functions other than water transport (39). To date, there are 13 mammalian aquaporins known. Aquaporin 1, 3, 4,

5, 8 and 9 are found to be present in the placenta and fetal membranes of the human (38). Aquaporin 3 and 9 have been detected in the apical membrane of the human syncytiotrophoblast (40). Emerging evidence also show that aquaporins and their expression may be related to poly- and oligohydramnios (41). Alterations in the expression of aquaporins have also been seen in cases of preeclampsia, chorioamnionitis and maternal undernutrition (42). The role of aquaporins in water transfer in both normal and pathological pregnancies is dubious and the subject of comprehensive research today.

Possible driving forces for transplacental water transfer

As for the route of transfer, the forces driving transplacental water transfer are uncertain. The theoretical driving forces for water transport can be hydrostatic, osmotic and colloidal osmotic pressure gradients between mother and fetus.

Osmotic gradients

Colloidal osmotic pressure (COP) is generated by plasma proteins. Proteins cannot easily cross the placenta, and as such cannot drag the water along with them and participate in bulk flow of water (43). In the human, plasma colloidal osmotic pressure and protein levels are known to be higher in the mother than in the fetus (44, 45). COP and total protein levels in the fetus increase with increasing gestational age (45), but do not exceed the maternal COP or protein levels at any time. Consequentially, placental water transfer cannot be explained by simple osmosis down a colloid concentration gradient. Despite the concentration difference in maternal favor, net water transfer is in maternal-fetal direction during gestation. Therefore, other forces of transfer must exist. Baum et al. suggested higher maternal pressure in the IVS, a greater fetal total osmotic pressure, or an active transport of water from maternal to fetal side against a concentration gradient (45). A later investigation of the maternal and fetal total osmotic pressure in the sheep and goat compared the freezing points of maternal and fetal plasma using a osmometer (46). The total osmotic pressure was equal, and in many cases lower, in the fetal compared to maternal plasma and this could not explain water transfer in fetal direction.

Armentrout et al. measured maternal and fetal osmolality in chronically catheterized sheep (47). They used two different measuring methods, freezing point depression (FPD) and vapor pressure osmometer (VPO). Their results are rendered with permission in table 1. Total

osmolality of the maternal plasma was systematically hypertonic to fetal plasma regardless the method of measurement.

Table 1. Fetal and maternal plasma osmolality in chronically catheterized sheep (control)

Constituent	Maternal Artery Mean ± SD (n)	Uterine Vein Mean ± SD (n)	Umbilical Artery Mean ± SD (n)	Umbilical vein Mean ± SD (n)
Na^+	145.3 ± 1.2 (9)	146.4 ± 1.5 (8)	143.7 ± 1.9 (9)	143.3 ± 1.4 (9)
K^+	4.49 ± 0.10 (9)	4.46 ± 0.11 (8)	3.91 ± 0.14 (9)	3.91 ± 0.14 (9)
Mg^{2+}	0.85 ± 0.08 (9)	0.81 ± 0.06 (6)	0.81 ± 0.05 (7)	0.79 ± 0.05 (7)
Ca^{2+}	2.12 ± 0.07 (7)	2.16 ± 0.08 (8)	2.90 ± 0.05 (9)	2.97 ± 0.04 (9)
Cl^-	107.7 ±1.7 (9)	107.5 ± 1.9 (8)	101.3 ± 2.4 (9)	103.0 ± 2.2 (9)
HCO_3^-	24.5 ± 1.1 (9)	26,9 ± 1.4 (7)	27.4 ± 1.3 (9)	27.5 ± 1.4 (8)
Inorganic phosphate	0.48 ± 0.03 (6)	0.50 ± 0.02 (5)	0.73 ± 0.05 (6)	0.75 ± 0.06 (6)
Blood Urea Nitrogen	6.2 ± 0.7 (8)	6.3 ± 0.8 (7)	7.1 ± 0.8 (8)	7.0 ± 0.7 (8)
Glucose	3.4 ± 0.3 (8)	2.8 ± 0.2 (7)	1.0 ± 0.20 (8)	1.5 ± 0.5 (8)
Total protein g/100 ml	6.60 ± 0.25 (6)	6.70 ± 0.28 (5)	3.43 ± 0.22 (6)	3.40 ± 0.20 (6)
Osmolality by VPO	294.1 ± 1.6 (4)	293.8 ± 2.3 (4)	287.0 ± 1.9 (4)	289.3 ± 2.4 (4)
Osmolality by FPD	301.1 ± 2.9 (8)	302.0 ± 3.3 (7)	300.0 ± 2.3 (8)	299.4 ± 2.8
Osmolalities are in milliosmoles per kilogram**.** Maternal total plasma osmolality is hypertonic to fetal plasma.				

The authors used maternal and fetal arteriovenous blood samples similar to our in vivo human study presented in this assignment. The water flow was calculated both from the total osmolality difference and from the concentration differences of Na^+, K^+ and Cl^-. On average the flow from mother to fetus was found to be 0,061 ml/kg/min per milliosmole per liter osmotic gradient across the placenta when the average fetal weight was 3.41± 0.24 kg. Another observation in the same study was that the total concentration of "active solutes" was higher in the fetal than in the maternal plasma (47, 48). Active solutes are defined as solutes that are transferred to the fetus by active transport, derived from actively transported precursors or transported by carrier mechanisms (48). Such known solutes are lactate, fructose, amino acids, calcium and phosphate. Urea and bicarbonate are produced by the fetus and contributes to increase fetal osmolality. It has been speculated that active solutes are more

powerful osmotic agents than inert solutes (48) and that this could account for the paradox that maternal plasma in total is hypertonic to the fetal plasma despite of a maternal-fetal transfer of water. However, there is no physiological evidence of a fetal concentration rise in actively transported solutes during gestation (48).

The hypothesis that bicarbonate exerts osmotic pressure important for transplacental water transfer has been discussed (49). Increased fetal oxygen consumption would lead to an increase in fetal venous CO2/bicarbonate concentration. Fetal osmolality would then rise and induce increased maternal-to-fetal water transfer. By computer calculations, changes in osmotic pressure exerted by bicarbonate were estimated by summing the number of bicarbonate ions at a given time in the exchange process (49). Along the capillary, fetal osmotic pressure fell about 2,5 mOsm. At the same time, maternal osmotic pressure increased correspondingly (figure 5).

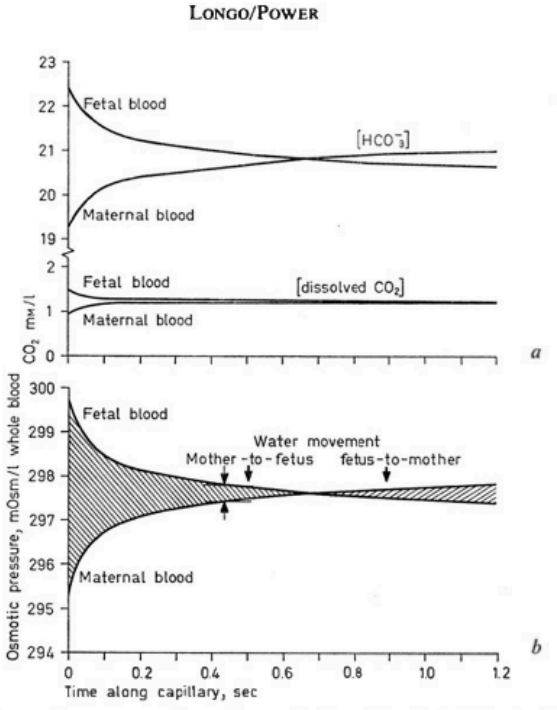

Figure 5. *a)* Concentration changes in osmotically active CO_2 in fetal and maternal blood during one capillary transit. *b)* Change in fetal and maternal osmotic pressure during one single capillary transit. During the first of the capillary transit, water moves in maternal-fetal direction, while it moves in fetal-maternal direction during the last half. Small arrows: the mean osmotic pressure difference that accounts for net movement of water in maternal-fetal direction (7.3 mmHg or 0.37 mOsm). Reused with permission from Longo LD. and Power DD. (49).

These shifts in osmotic pressure could indicate a rapid transfer of water from mother to fetus at the arterial part of capillary, and a rapid transfer back in the opposite direction at the venous part of the capillary (49). This would result in a maternal-fetal net transfer of water sufficient enough to ensure fetal growth. Another mathematical model by Wilbur, Power and Longo came to similar conclusions (50). The model used human parameters when available and otherwise data from other species like the sheep and rabbit. A rapid water transfer in fetal direction of approximately 3.5 ml/min was shown at the start of the placental capillary transit. This flow was gradually reduced, reversed and ultimately an almost equal amount of water returned to the maternal side, resulting in a remaining net flow of 0.014 ml/min. This equals a daily fetal water gain of 20 ml. The initial, rapid water transfer to the fetus was explained by the higher fetal concentrations of bicarbonate and CO_2 (figure 6). In the middle of the capillary transit, the fetal amino acid concentrations became of greater importance than bicarbonate. At the end of the capillary, there was a (slower) transfer towards the mother. The authors hypothesize this shift in direction is due to the glucose in maternal blood. These results support the theory of a bidirectional water transfer.

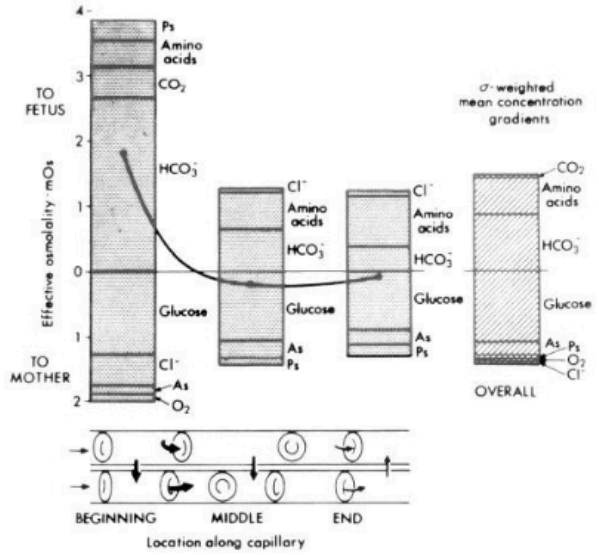

Figure 6. Transplacental osmotic forces at the beginning, middle and end of the capillaries. The solutes are weighted by their reflection coefficients. Gradients created by CO_2 and O_2 rapidly disappear and are negligible by the middle of the capillary. Bar to the right: contribution of various factors to the overall osmotic force during one capillary transit. Reused with permission from Wilbur et. al. (50).

The model also studied how changes in maternal or fetal blood flow would affect water transfer when all other parameters remained the same (figure 7). Fetal water acquisition was close to 20 ml/day when maternal and fetal blood flows were within the normal range. An

increase in maternal and/or fetal blood flow favored water transfer in fetal direction. A reduction of both maternal and fetal flow resulted in water transfer in maternal direction. Based on these results, the authors suggest that imbalance in maternal-fetal blood flow could be a cause of polyhydramnios and hydrops fetalis.

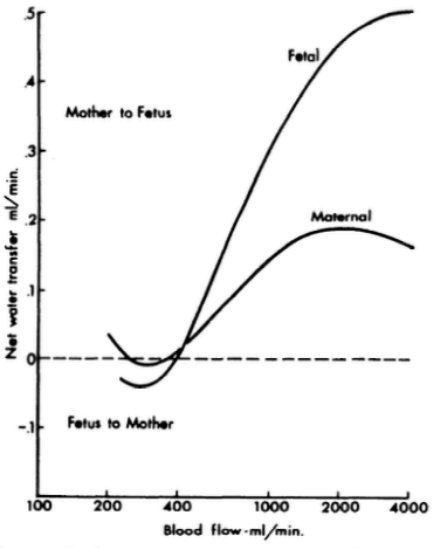

Figure 7. Effects of changing maternal or fetal placental blood flow, all other factors remaining constant. A normal water transfer of 0.014 ml/min in maternal-fetal direction is shown when the flows are within normal values. Reused with permission from Wilbur et al. (50)

A computer simulation based on known experimental data of chronically catheterized, healthy sheep under near normal-physiological conditions explored the bicarbonate hypothesis further (51). This experiment suggested that active solutes and solutes produced by the fetus, such as bicarbonate, are the main driving forces for maternal-fetal placental water transfer. It was also suggested that increased bicarbonate production could be part of a fetal self-regulating mechanism, e.g. when placental blood flow is compromised (51).

In the same computer experiment, they investigated the effect of increasing the placental diffusion permeability of NaCl and decreasing the placental reflection coefficient for NaCl, resulting in higher fetal NaCl concentrations (51). According to the calculations, this led to a increase in total fetal water content from about 4000 ml to more than 12 liters and gross polyhydramnios was produced. This could suggest increased fetal NaCl concentration as an explanation for polyhydramnios. Several experimental studies exploring the osmotic effect of NaCl have been done (52-55). Over 5 days, 7 liters of isotonic saline solution were infused intravenously into sheep fetuses near term (52). In contrast to what might be anticipated, only

a small rise in fetal blood volume and extracellular fluid occurred. There was a significant increase in fetal urine production (6000 ml per day at its greatest) and fetal swallowing of amniotic fluid was doubled. Only a very moderate rise in amniotic and allantoid fluid occurred. At autopsy, only 2 of the 7 liters infused were found in the fetal compartments. The fetus appeared to have a compensatory mechanism which protected it against overhydration when supplied great amounts of fluid. The authors speculated that the extra fluid had recirculated to the maternal compartment, supposedly through the placenta. No changes in osmolality or solute concentration that would favor water transport in a fetal-maternal direction were detected, and the mechanisms of this fluid transfer remains unclear. These results indicate that effective fetal compensation mechanisms to protect the fetal fluid homeostasis do exist.

The same authors later infused fetal sheep with 4L of either isotonic saline solution or lactated Ringer solution over a period of 4 hours (53). There was a moderate increase in fetal arterial and venous blood pressure, but not to the expected extent. Fetal extracellular volume also increased and fetal urine output rose from a baseline of 9,3 ml/min to 16,7 ml/min at the end of the infusion. This is by far the highest urine flow rate ever reported in sheep fetuses (53). Nonetheless, the combined volume of these increases could only account for half of the infused volume. Yet again, this supports the hypothesis that the fetus can transfer excess fluid across the placenta back to the maternal circulation. In this case, as much as 2000 ml must have been transferred. The authors speculate that the placental filtration coefficient for water must have increased 50 – 100 times despite the relatively moderate the increase in blood pressure. It appears as if the small rise in fetal blood pressure may have mediated a substantial rise in placental permeability (53). This experiment suggests that the fetus can regulate its fluid volume over shorter periods of time, and during extreme conditions. Vasopressin has been suggested as a candidate mediator to adjust the placental permeability in response to alterations in fetal blood pressure, but the authors did not confirm a significant change in fetal arginine vasopressin.

In another experiment 5 M NaCl was intravenously infused into late-gestation fetal sheep for 3 days (54). Their hypothesis was that as the fetal osmolality increased due to the infusion, a transfer of water from the mother to the fetus would occur. Because of the great amount of fluids transferred (4.8 liters), an accumulation of water and thereby a development of hydrops fetalis and/or polyhydramnios was expected. The fetal osmolality increased, but in 7 of 8

cases no accumulation of water neither in the fetus nor in the amniotic/allantoid fluid occurred. The latter in spite of a dramatic increase in fetal production of dilute urine. Only one fetus developed a gross polyhydramnios as hypothesized. These results support the hypothesis of fluid transfer back to maternal circulation. The authors suggested increased fetal swallowing of amniotic fluid and intramembranous absorption (described in more detail later) as alternate mechanisms to transplacental transfer (54). They also proposed that there are different NaCl reflection coefficients along the length of the placental capillaries. In the arterial part of the capillaries a high NaCl reflection coefficient would ensure movement of water, but not NaCl from the maternal compartment to the fetal. The vascular volume, and thus the placental capillary pressure would increase. The increased pressure would then promote movement of fluid and NaCl toward the maternal compartment in the venous portion of the placental capillaries which have low NaCl reflection coefficients. A differentiation in transfer along the placental capillary coincides with result from previously mentioned model studies (49-51).

Intravenous infusions of 200 ml 25 % mannitol in the maternal rabbit lead to a mean decrease in fetal water content of 3 % in fetuses of different gestational ages (56). The closer to term, the greater the decrease in water content. This suggests a net transfer of water from fetus to mother. Maternal solute concentration was relatively higher than fetal. Fetal dehydration has also been produced in eight pregnant rhesus monkeys by administrating either a NaCl or a disaccharide solution into the maternal circulation (55). There was a significant reduction in fetal and placental total water content compared to fetuses of similar gestational age in normal pregnancies (57). These studies demonstrate that it is possible to produce transplacental gradients of different solutes across the placenta of the rabbit and rhesus monkeys, and that these solutes are osmotically active over the placenta.

In an experiment using labeled isotopes, transfer rates of water in rats were measured both in maternal-fetal and fetal-maternal direction (58). Maternal rats were injected with tritiated water and transplacental transfer to the fetus was found to be more than 1.5 ml/h. To measure transfer towards the mother a fetus was bathed in an albumin-saline solution containing a radioactive label. The rate of fetal-maternal transfer turned out to be very little compared to maternal-fetal transfer rate. A theory of a unidirectional transfer of water, driven by the higher maternal blood pressure (hydrostatic pressure) was presented. The theory was supported by a lack of significant change in water transfer rates or direction when the albumin-saline solution

surrounding the fetus was replaced by a saline solution only. This was taken to suggest that a colloidal osmotic gradient between the two sides of the amnion is irrelevant. They proposed that water does not return to maternal circulation across the placenta, it is secreted by the fetus into the amnion fluid, whereas an uptake by the endometrial and subsequent uterine vein(s) returns it to the maternal circulation. The transport route will then be maternal arterial blood → placenta → fetal blood → amniotic fluid → amniotic membrane → endometrium → maternal venous blood. Support for an extraplacental recirculation of water from the fetal compartment has been found in experimental studies using chronically catheterized sheep or monkeys (59-61). Several experimental studies have calculated transmembranous absorption or an extraplacental loss of water from the fetal compartment. In chronically catheterized sheep transmembranous absorption was estimated to be between 10 and 30 % of the water transferred over the placenta, but its importance in humans is still unclear (61-63). Absorption of amnion fluid by the fetal membranes and directly into the fetal circulation (intramembranous absorption) have been shown in sheep, and in the rhesus monkey by injecting a radioactive ion into the amnion fluid after ligating the esophagus and registering uptake into fetal circulation (59, 60).

Hydrostatic pressure gradients

It is claimed that in the human placenta, the hydrostatic pressure difference is in fetal-to-maternal direction (31, 64). Water is therefore believed to be transported from mother to fetus against the hydrostatic pressure gradient – a difference in pressure between the intervillous space and the fetal capillary bed. Recent 3D based models demonstrate that the intravillous flow pattern largely affects solute transfer (26) and, as explained above, mathematical models demonstrate that manipulation of maternal and fetal blow flow, largely alters the transfer of water (50). Experimental data of injected labeled nonelectrolytes and tritiated water in maternal guinea pig and sheep demonstrate that placental water transfer is influenced both by blood flow and diffusion (65).

The intervillous space pressure of three pregnant rhesus monkeys near term was measured at rest and during uterine contractions by inserting probes through the uterus into different areas of the placenta including the IVS (66).The IVS pressure at rest varied from about 2 mmHg to 17 mmHg. The pressure in the cotyledon center was higher than the pressure just beneath the chorion. During a contraction, the pressure gradient became steeper as blood was forced from

the center of the cotyledon to the more peripheral parts of IVS. These results demonstrate that the IVS pressure fluctuates within each cotyledon and during uterine contractions. However, it was reported that the mothers were mildly to severely hypotensive during the procedure, which could affect the results, e.g. give a false lower pressure in the IVS. Although there have been measurements of the IVS in the rhesus monkey, to our knowledge there are no similar human data available. Furthermore, we found no measurements of the hydrostatic pressure in the fetal capillary vascular bed. Thus, a comparison of the hydrostatic pressure between fetus and mother remains challenging.

In addition to calculate a net transplacental water transfer of 0.1ml/min in fetal direction, the theory of a fetus driven transfer was presented by Sebire and Talbert in a computer-based model (67). The authors proposed that the water transport was not a passive mechanism of simple diffusion but controlled by the fetus itself. They suggested the fetus monitor the blood volume via atrial and venous stretch receptors and regulate its urine secretion to control fluid volume, and as such cardiac output and blood pressure. The walls of both villous stem arteries and veins have a well-developed layer of smooth muscle which represent a possibility of vasoregulation (figure 8).

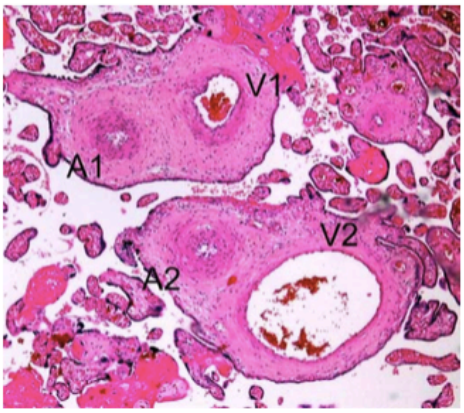

Figure 8. Photomicrograph of the human placenta showing well developed vascular smooth muscle in cotyledonal stem arteries and veins, which makes vasoconstriction possible. A: artery. V: vein. Reused with permission from Sebire N.J. and Talbert D. (67)

Vasoconstriction in villous stem veins would lead to a rise in intravillous capillary pressure and favor water transport to the mother. Relaxation in the same stem veins would favor water transport in the opposite direction. This kind of autoregulation protects the fetus from rapid changes in maternal blood pressure, which could occur during physical activity, changing positions from supine to standing, vasovagal reactions, labor etc. Sebire and Talbert further hypothesized that a fetal release of a vasoconstrictive hormone could induce this kind of vasoregulation in the villous veins. A loss of fluid volume would lead to decreased fetal release of this vasoconstrictive hormone, ensuring relaxation in the stem veins and thus water

transfer to the fetus would be favored (figure 9). Whether this theoretical hormone exists in vivo is yet to be known, but in vitro perfusion of human placental cotyledons have demonstrated that vasoconstrictive agonists in fetoplacental circulation affect venous resistance causing fetomaternal fluid loss (68).

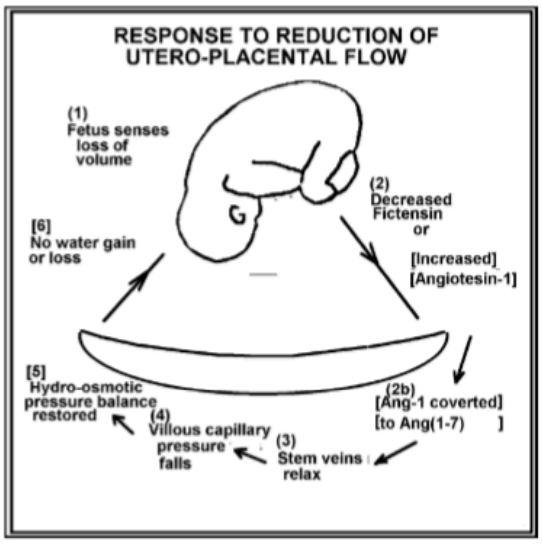

Figure 9. Events following a sudden reduction in uteroplacental flow. Reduced maternal blood pressure will lead to a movement of water in maternal direction driven by intravillous capillary pressure. The volume loss is registered by the fetus which decreases the release of "fictensin". The placental veins relax, and the capillary pressure falls until it again matches that of maternal blood, and fetal water loss to the mother is stopped. Reused with permission from Sebire N.J. and Talbert D. (67)

The authors suggest it would be an upper and lower limit to what pressures the fetus can protect itself from by vasoconstriction/vasorelaxation (67). If the maternal blood pressure increases to such an extent that fetal compensatory mechanisms are overrun, fetal blood pressure rises. This would start a release of anti-natriuretic peptide, increased urine production which again could lead to polyhydramnios. Likewise, if maternal colloidal osmotic or hydrostatic pressure decreased to a lower pressure than the fetus could produce, water would be transferred in maternal direction, leading to oligohydramnios.

Lumbers et al. calculated the rate of net transplacental water transfer to the fetus in chronically catheterized sheep fetuses by measuring the urine and lung liquid flow rates (69). Fetal drinking was prevented by ligating the esophagus. Water produced by oxidative metabolism and the amount of fluid deposited in growing tissue was calculated because this was not possible to measure. They found a mean rate of net transplacental water transfer to the fetus of 0.40 ± 0.09 ml/min/kg, which is a higher estimate than previous studies have reported.

Human in vivo studies of transplacental water transfer

Stenger et al determined the total osmotic pressure difference between maternal and fetal circulation plasma by freezing point depression in 13 women scheduled to delivery by caesarian section (70). 9 of them were healthy and the remaining four suffered from pregnancy complications. Maternal samples were collected from the radial artery and the uterine vein and fetal blood sampled from the umbilical vein after clamping of the cord. In contrast to animal studies (48,25), the total osmotic pressure was consequently (but slightly) higher in the umbilical vein than in the maternal radial artery in all of the 9 cases, i.e. there was a higher fetal osmotic pressure. This would favor water transfer from mother to fetus. It should be mentioned that in the complicated pregnancies, the difference in fetal relative to the maternal osmotic pressure was even greater. The mothers were exposed to acute stress like preeclampsia and eclampsia, severe spinal hypotension and maternal acidosis due to respiratory obstruction under general anesthesia. This finding is interesting – the higher the maternal stress, the bigger the difference in osmotic pressure between mother and fetus.

In the same study, the fetal and maternal plasma concentrations of sodium and potassium were determined. The mean fetal concentrations of both sodium and potassium were higher than corresponding mean maternal concentrations. In the complicated pregnancies, the sodium concentration differences were even greater (e.g. higher fetal sodium concentration), which could explain the increased fetal osmotic pressure earlier described. Interestingly this points to a gradient in osmotic pressure as a possible driving force for maternal-fetal water transfer, and that sodium and potassium may contribute. This is one of the few human in vivo studies of placental transfer and their method is comparable the 4-vessel in vivo study recently performed by Holme et. al (17) in which Hb and Hct were measured to estimate placental transfer of water as presented in this assignment.

In a clinical in vivo study from 1959, Hutchinson et al. studied the water exchange between maternal, amniotic and fetal compartments (36). 9 women of different gestational age from week 12 to term were included, amongst them two polyhydramnios patients at term. The patients were either planned to go through a caesarian section or therapeutic abortions. The amnion fluid was injected with two different hydrogen tracers, deuterium and tritium. The concentration of tracers was then measured in the maternal serum, the amnion fluid and fetal cord blood at different time points. This made calculations of the exchange rates between the

amniotic, maternal and fetal compartments possible. The authors found a continuous rise in the water exchange rate between maternal and fetal compartments until delivery (figure 10). Results demonstrated a rate of about 100 ml/h at week 12 to 3600 ml/h at term in both maternal-fetal and fetal-maternal direction. The maternal-fetal exchange rates in the two polyhydramnios patients were one-to-eight and one-to-twenty compared to the healthy pregnant in both directions.

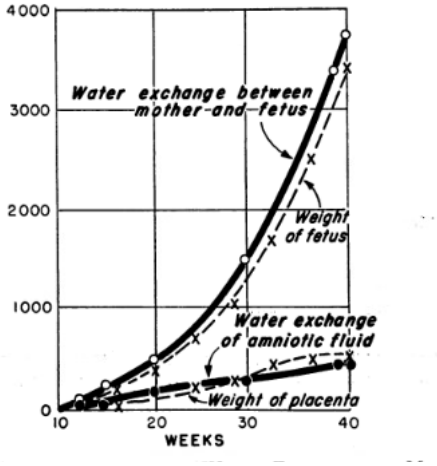

Figure 10. The relation of water exchange in ml/h to gestational length. The values are related to reported average values for fetal and placental weight in grams. Reused with permission from Hutchinson D.L. et al. (36)

While Hutchinson et al. calculated the total water shift over the placenta in both maternal and fetal direction, they did not report the net transfer of water to the fetal compartment. Two normal pregnancies with caesarian section near term (week 38 and 40) were included. If exchange rates listed in the article are used to calculate net transfer rates, the rates in maternal to fetal direction are 145ml/h and -25ml/h respectively. The question of what can explain such a high turnover of water when the net transfer to the fetus is so small remains uncertain.

When studying the transfer of radiolabeled sodium across the human placenta, a 70 time increase in sodium permeability from gestational week 9 to week 36 was uncovered, then there was a rapid decline in permeability from week 36 to term (71).The sodium permeability was thought to increase with placental maturation including a larger and thinner exchange surface. The authors suggest that the sharp terminal decrease in placental permeability is due to deposition of fibrinoid on the villus' surface. Interestingly the authors also observed that in week 12 and 40, the fetus received respectively 160 and 1100 times as much sodium needed for fetal growth. Less than 0,1 % of the sodium which reached the fetus was retained, and the authors hypothesize that the remaining 99,9 % was returned to the maternal circulation via routes unexplained. This corresponds with data available from studies of sheep (26, 52, 53).

CLINICAL STUDY: A human in vivo study of transplacental water transfer

Materials and Methods

Design and study population

This cross-sectional in vivo study was done in three sub cohorts of a larger study including 179 women with healthy term pregnancies (17). For the study in large, women with planned cesarean section were invited to participate. Exclusion criteria were smoking, significant pre-existing comorbidity or medication, pregnancy complications and onset of labor prior to scheduled cesarean section. In a sub cohort of 43 women, venous and arterial concentrations of hemoglobin (Hb) and hematocrit (Hct) was measured on the maternal side of the placenta. Similarly, Hb and Hct in the umbilical artery and vein of 31 fetuses was measured, representing the fetal side of placenta. The last sub cohort used for this study included 128 women in which we performed Doppler ultrasound examinations prior to surgery, but where Hb/Hct was not studied specifically.

Data collection

The method has been roughly described in previous publications including an instructional video (17, 72, 73) and is briefly summarized here. Clinical data were collected at inclusion and the day of delivery (17, 72). The blood flow (Q) in the uterine artery and the umbilical vein was measured by Doppler ultrasound 2-3 hours before cesarean section (17). All sonographic examinations were performed by one examiner (Guttorm Haugen) using the same machine (Acuson Sequoia 512, Mountain View, CA, USA). Internal vessel diameter (D) and time-averaged maximum velocity (TAMX) were measured and blood flow (Q) was calculated as: $Q = h \cdot (D/2)^2 \cdot \pi \cdot TAMX$ where h is the coefficient for the spatial blood velocity.

Blood samples were collected during cesarean section, which was performed in spinal anesthesia. Just prior to the uterine incision, blood was drawn from the maternal radial artery (as a proxy for the uterine artery) and the uterine vein. Immediately after delivery of the infant, but before cord clamping, blood samples were obtained from the umbilical artery. Blood samples from the umbilical vein were drawn directly after cord clamping and intentionally before delivery of the placenta. Maternal whole blood was directly analyzed for hemoglobin and hematocrit concentration in a blood gas analyzer (Radiometer ABL825 Flex).

Hematocrit (Hct) in umbilical blood was calculated as the product of erythrocytes and their mean corpuscular volume (MCV) measured by a cell counter on Sysmex XN-9000/1000. The reported coefficient of variation is 2.4 %. Hemoglobin (Hb) in umbilical blood was analyzed by adding sodium lauryl sulfate (SLS) and through a series of reactions SLS binds to the iron contained in the hemoglobin molecule (SLS-Hb). SLS-Hb is then photometrically measured on Sysmex XN. The reported coefficient of variation is 1.9 %. Both analyses were performed at the Department of Medical Biochemistry, Oslo University Hospital, Rikshospitalet.

Calculations

Assuming similar blood composition in the radial and uterine arteries, the following A-V and V-A concentration differences were calculated:

$$\boldsymbol{Uteroplacental\ AV\ difference}\ [\boldsymbol{Hb}] = [Hb]_{radial\ artery} - ([Hb]_{uterine\ vein})$$

$$\boldsymbol{Uteroplacental\ AV\ difference}\ [\boldsymbol{Hct}] = [Hct]_{radial\ artery} - ([Hct]_{uterine\ vein})$$

$$\boldsymbol{Fetal\ umbilical\ v-a\ difference}\ [\boldsymbol{Hb}] = [Hb]_{umbilical\ vein} - \left([Hb]_{umbilical\ artery}\right)$$

$$\boldsymbol{Fetal\ umbilical\ v-a\ difference}\ [\boldsymbol{Hct}] = [Hct]_{umbilical\ vein} - \left([Hct]_{umbilical\ artery}\right)$$

According to Fick's principle the volume of water (ml/min) taken up by the fetus and by the uteroplacental unit was calculated by multiplying the arteriovenous concentration differences of Hb and Hct with the blood flow in the umbilical vein and uterine artery respectively (74). In 21 women both Doppler blood flow measurement and maternal Hb and Hct concentrations were available, thus the calculations were performed both based on the median blood flows (a) and on the individual measured blood flow (b).

Estimation of uteroplacental water uptake:

a)

$$Volume\ water\ \frac{ml}{min} = \text{Median bilatereal flow a. uterina}\ \frac{\text{ml}}{\text{min}} - \left(\left(\text{median bilateral flow a. uterina}\ \frac{\text{ml}}{\text{min}} \times\ \text{[Hb]a. radialis}\ \frac{\text{g}}{\text{dl}}\right) \div \text{[Hb]v. uterina}\ \frac{\text{g}}{\text{dl}}\right)$$

b)

$$Volume\ water \frac{ml}{min} = \text{Ind. flow a. uterina}\ \frac{\text{ml}}{\text{min}} - \left(\left(\text{Ind. flow a. uterina}\ \frac{\text{ml}}{\text{min}} \times\ \text{[Hb]a. radialis}\ \frac{\text{g}}{\text{dl}}\right) \div \text{[Hb]v. uterina}\ \frac{\text{g}}{\text{dl}}\right)$$

Estimation of fetal water uptake:

$$Volume\ water\ \frac{ml}{min} = \text{Median flow v. umbilicalis}\ \frac{\text{ml}}{\text{min}} - \left(\left(\text{median flow v. umbilicalis}\ \frac{\text{ml}}{\text{min}} \times\ \text{[Hb]v. umbilicalis}\ \frac{\text{g}}{\text{dl}}\right) \div \text{[Hb]a. umbilicalis}\ \frac{\text{g}}{\text{dl}}\right)$$

Statistics

Descriptive data are reported as numbers with percentages, and mean values with standard deviation (SD) if normally distributed, or as median with quartiles [Q1, Q3] if skewed. Comparisons of the paired hemoglobin and hematocrit concentrations in the umbilical vessels and maternal hemoglobin and hematocrit concentrations in the uterine vein and radial artery were performed by paired t-tests. Comparisons of the clinical data between groups were performed by paired t-tests or Wilcoxon signed rank test as appropriate. A two-sided p-value < 0.05 was considered significant. We performed all analyses using Statistical Package for the Social Sciences, Version 25.0 (SPSS Inc., IBM).

Study approval

All participants signed a written informed consent prior to inclusion. The study was approved by the Institutional Review Board at Oslo University Hospital and the Regional Committee for Medical and Health Research Ethics, South East Norway 2419/2011. Application number S-07174a.

Results

Maternal side

Clinical characteristics and demographics are presented in table 1.1.

Table 1.1 Clinical data and demographics

N=43	*N (%)*	*Mean (SD)*	*Median [Q1, Q3]*	*Range*
Age		35.6 (4.2)		28-44
Nulliparous	7 (16)			
BMI			23.1[21.7, 27.4]	18.3-47.6
Systolic BP		109 (11)		90-130
Diastolic BP		68 (8)		50-83
Fasting (hours)			11.0 [9.0, 11.5]	9.0-15.0
Smoking[*]	1			
Higher education	37 (86)			
Partner	41			
5 min Apgar < 7	0			
Gestational age, days		275 (4)		267-294
Birthweight (g)		3488 (378)		2635-4370
Placental weight (g)		622 (109)		335-878
Girls	21			

[*]Stopped in first trimester.

The ultrasound measurements have been published elsewhere (17, 75). Calculated median uterine artery blood flow in the uterine artery was 488.4 [358.5, 604.4] ml/min.

Table 1.2 Hb and Hct in maternal radial artery and uterine vein. (n=43)

	Radial artery	*Uterine vein*	*Mean AV difference (95%CI)*	*p-value*
Hb (g/dL)	11.01 (1.25)	11.22 (1.26)	-0.17 (-0.23, -0.11)	<0.001
Hct	0.336 (0.038)	0.339 (0.039)	-0.005 (-0.007, -0.003)	<0.001

Given a median bilateral blood flow of 488.4 ml/min in the uterine artery, the median [Q1, Q3] estimated volume of water taken up from maternal circulation to the uteroplacental unit based on Hb concentrations (n=43) was 8.28 [0.98, 13.79] ml/min. In 21 women both the Hb concentrations and the blood flow of the uterine artery were measured. Calculation of median [Q1, Q3] volume of water taken up to the uteroplacental unit from maternal circulation based on individual blood flow measurements was 7.70 [0.0, 15.63] ml/min. The same calculations can be performed with Hct as a variable. Based on median blood flow in the uterine artery, the uteroplacental uptake of water was 10.02 [1.67, 13.57] ml/min. Based on individually

measured blood flow of the uterine artery, the uteroplacental uptake of water was 9.67 [1.18, 15.17] ml/min.

Fetal side

Clinical characteristics and demographics are presented in table 2.1. The majority of women in both the maternal and fetal group had a partner and had completed higher education. There were no significant differences between the sub-cohorts except gestational diabetes in two women and a slightly lower maternal body mass index (BMI) in the group with fetal data.

Table 2.1 Clinical data and demographics

N=31	*n*	*Mean (SD)*	*Median [Q1, Q3]*	*Range*
Age		34.6 (4.4)		25-42
Nulliparous	9			
BMI			21.8 [19.0, 24.3]	18.4-33.0
Systolic BP		106 (11)		85-132
Diastolic BP		63 (9)		40-82
Gestational diabetes	2			
Fasting (hours)			10.5[9.2, 12.0]	8.5-13.5
Smoking	0			
5 min Apgar < 7	0			
Gestational age		275 (3)		268-281
Birthweight (g)		3406 (438)		2635-4228
Placental weight (g)		579 (103)		335-740
Girls	14			

The ultrasound measurements have been published elsewhere. Calculated median volume blood flow in the umbilical vein was 196.2 [158.3, 232.2] ml/min.

Table 2.2 Fetal Hb and Hct data (n=31)

	Umbilical artery	*Umbilical vein*	*Mean AV difference (95%CI)*	*p-value*
Hb	15.95 (1.27)	15.21 (1.34)	-0.70 (-1.0, -0.41)	<0.001
Hct	0.48 (0.04)	0.46 (0.04)	-0.026 (-0.034, -0.017)	<0.001

Within the sub cohort where fetal Hb and Hct was sampled specifically, individual measurement of blood flow in the umbilical vein was performed in 5 fetuses only, thus no calculations based on individual blood flow measurements could be done.
Given a median volume blood flow in the umbilical vein of 196.2 ml/min, the median volume of water taken up to fetal circulations from the placenta was 9.03 [1.84, 13.97] ml/min based on differences in Hb concentration and 10.71 [4.98, 16.99] ml/min based on concentration differences in Hct.

Discussion

Our data demonstrates significant arteriovenous and venoarterial concentration differences of hemoglobin and hematocrit on maternal and fetal side respectively. Since no placental uptake or release of hemoglobin is known, we hypothesized that the concentration differences could be explained by a net uptake of water by the placenta and the fetal compartment. Thus, a reduced volume of water the in uterine vein and umbilical artery lead to a higher concentration of Hb in these vessels compared to radial artery and umbilical vein respectively. The Hct data correlate with the Hb data by small margins, and this strengthens the reliability of our study. The discrepancy between Hct and Hb could be explained by water exchange over the membrane of the erythrocytes. Based on these assumptions, we used the concentration differences in Hb and Hct on maternal and fetal side in combination with blood flow measurements to calculate the net transfer of water. Using the Hct concentrations and median volume blood flows, we calculated a net uteroplacental uptake of 10.02 ml/min and a net fetal uptake 10.71 ml/min. Reassuringly, the calculations of the uteroplacental uptake were similar using both individual or median volume blood flow values. Furthermore, the fact that the calculated uteroplacental uptake of water was approximately the same as the calculated fetal uptake strengthens the validity of our results.

Nevertheless, the calculated amount of water transferred is significantly larger than any known estimate or measurement from earlier studies in both animals and human (36, 47, 50, 58, 67, 69).Yet, several studies suggest a placental capacity for water transfer way beyond what is believed to be needed for fetal growth, providing some support to our findings (36, 52-54, 71). The discrepancy in water transfer rates between studies could partly be explained by different measuring methods, timing of measurements, calculations/equations used to create an estimate etc. Our study is cross-sectional and only represents a moment of transplacental water transfer. Most likely, the volume and rate of water transferred will vary throughout the day according to changes in all maternal, placental and fetal driving forces for water transfer. This is a limitation to the estimations of mean or median water transfer rate where variations in the flow rate would not be detected (69).

Our estimates may be influenced by several factors. The mothers had been fasting for a mean of 10.5 hours, thus perhaps in a mildly dehydrated state. Maternal intravenous administration of fluids just prior to surgery might affect net transfer to the fetus. During the caesarian

section, the mothers lay in a supine position and tilted to their left. We cannot exclude that this position could lead to alterations in maternal or fetal arterial and/or venous blood pressure. In particular could impaired maternal venous flow result in increased intravillous perfusion pressure, which again could affect the maternal-fetal transfer of water. Spinal/epidural anesthesia is known to reduce general blood pressure and could also influence the driving forces for maternal-fetal transfer. An intraplacental variation in water transfer rates between different placental cotyledons must also be considered.

Despite of the above-mentioned limitations, our human data were collected in a more near-physiological state than many of the previous in vivo studies have and as such it provides unique information. We cannot exclude that the arteriovenous differences observed, and estimated water transfer might be influenced by movement of water outside of the placenta and that water may return to maternal circulation through other routes than the uterine vein. The high uteroplacental water uptake observed in our study could be explained by transfer of water from the IVS into maternal decidua, and further to maternal venules or lymphatic vessels. This would lead to an overestimation of the water taken up from the maternal side. Along the same line of thought, potential water transfer over fetal membranes to the maternal decidua might be drained the same way (transmembranous absorption) and thus not detected by our sample method. The existing literature of trans- and intramembranous absorption needs further review, as it may represent a significant maternal-fetal transfer route.

The literature search did not reveal many accurate estimations of human placental water transfer at term but demonstrated that placental transfer of water is likely to be dynamic and influenced both by maternal and fetal factors. Transtrophoblastic channels, transfer across the SCT membrane by diffusion or temporary breaks together with aquaporins have been suggested as possible transfer routes. In the human, osmotic gradients either made by proteins or solutes and also hydrostatic pressure gradients could represent driving forces for the transplacental water transfer. The existence of pores or direct channels have been disputed because the syncytiotrophoblast generates and maintains significant concentration gradients for several molecules. Ex vivo perfusion studies, and experimental studies that infused substantial amounts of solutions into the fetal compartment without resulting polyhydramnios, demonstrates a highly dynamic maternal-fetal fluid transfer and may support the involvement of pressure dependent channels. If considerable pressure differences are needed for such channels to open, it is possible that these would not interfere with osmotic gradients operating

in conditions of less hydrostatic pressure differences between maternal and fetal circulations. The complex interplay that keeps maternal-fetal water exchange in physiological balance is still elusive and warrant further research.

Through studying literature references of included articles, literature which might not otherwise have been found was detected. There is a risk that certain authors could refer to each other and have personal, non-objective preferences in choosing their literature. The literature list could be biased and somewhat narrow. Due to reasons of space, this thesis had to limit several areas that unfolded during the research, and each sub-subject in this assignment could have been a study alone. The possible transfer routes through the fetal membranes and the aquaporins are such examples.

The need to study human placental transfer over a longer time span is apparent, but methodological and technological issues makes this a challenge. Due to the great placental interspecies variability, the extrapolation of animal studies to human conditions is questionable. It is plausible that these differences could affect the result of transfer studies, especially since it is suggested that small changes in driving forces and gradients can lead to big alterations in transplacental transfer of water. However, there is reason to believe that studies of different species combined can provide useful information. A lot of the animal studies were quite invasive and required surgery to get access to maternal and fetal vessels in utero. Invasive procedures in the human pregnancy are limited to medically indicated caesarian sections and prenatal diagnostics due to ethical and technical reasons. There have been some earlier human in vivo studies performed during or before caesarian sections (19, 36, 76), but they are few and most of them would be ethically impossible to reproduce today. Furthermore, these human studies were small, with heterogenic study groups and not always representative of normal physiology.

The experimental conditions of human in vitro studies differ significantly from the in vivo intrauterine environment. It is likely that the altered, artificial conditions also influence the function and hemodynamics of the placenta. To extract the results to the in vivo situation would be an underestimate of placental complexity. Studies of in vitro trophoblasts, perfused cotyledons or placentas provide useful information regarding transport mechanisms and driving forces and give us an understanding of the physiology and mechanics. The mathematical and computer derived studies of transplacental transfer are useful because it is

possible to control and change several variables coincidently. This gives a unique opportunity to investigate how the variables influence both one another and transplacental transfer. However, the results of mathematically based studies depend heavily on the biological model for water exchange applied in the experiment. Still, they provide a good basis for further research and might inspire to the development of new in vivo methods.

A large part of the previous experimental studies was done under conditions that did not represent normal physiological conditions. Both animal and human studies have been performed during general anesthesia, although some studies of the chronically catheterized sheep were done in near-normal conditions where the ewe was awake with normal behavior (47, 53, 77). Some of the studies have caused a lot of strain on the research objects, e.g. transferred great amounts of NaCl, ligature of the esophagus, starvation etc. All kind of intervention could possibly influence the transfer of water and as such give unrepresentative and incorrect results. These kinds of experimental studies are interesting because they demonstrate potential compensation mechanisms that come into operation under extreme stress. They also demonstrate what could happen if these mechanisms fail. What they do not give information about, is to which extent these compensation mechanisms exist in a normal state or when and why the mechanisms are activated.

Conclusion

The study of the placenta in its normal physiological state is challenging in vivo due to practical and ethical reasons, but also because there is a limit to how many factors it is possible to explore at once. In sum, the complex interplay between the different driving forces and mechanisms of transplacental water transfer remain difficult to fully explore by the study methods available today.

Our data indicate that considerable amounts of water are transferred. Based on the study of the existing literature, the placental transfer of water is likely bidirectional and larger than what is needed for fetal growth. It is physiological plausible that the fetus regulates transplacental water transfer strictly as it would directly affect fetal cardiac output and blood pressure. It is reasonable to assume there are forceful compensatory mechanisms at play to meet changes in maternal placental perfusion pressure and osmolality amongst other. None of

the studies cited are able to study all aspect of water transfer simultaneously and thus information remains fragmented and diverging.

Given placental transfer of large volumes of water, it is easy to imagine that even small imbalances or alterations in driving forces or transfer mechanisms may have big consequences for water transfer and that this could lead to pathological conditions like oligohydramnios, polyhydramnios and hydrops fetalis. The pathophysiology of these conditions remains somewhat elusive and understanding maternal-fetal water transfer may be of importance.

The 4-vessel method (17) provides a unique opportunity to investigate concentration differences over the placenta in vivo and could help us identify the driving forces of transplacental water transfer. To find the actual volumes of water transferred to the fetus would be important when estimating transfer of many other substances across the placenta. The development of a technique for studying long-time transfer of water and solutes is desirable and will possibly help elucidate the subject of maternal-fetal water transfer.

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Supplementary tables

Supplementary table 1: Overview of human studies of placental water transfer

Article/author	Research objects	Method	Results
HUMAN IN VIVO STUDIES			
Permeability of placenta to inulin **Thornburg KL et al. (19)**	8 maternal-fetal pairs at term (elective cesarean section)	Inulin was infused to the maternal circulation 3 hours before cesarean section. Inulin concentrations were repeatedly measured in samples from maternal peripheral vein, umbilical vein and amnion fluid at the time of delivery.	Placenta permeable to inert molecules which do not enter cells
In vivo permeability of the human placenta to inulin and mannitol **Bain MD et al. (18)**	6 maternal-fetal pairs at term (elective cesarean section)	Maternal intravenous infusion of inulin and mannitol 1-2 h before cesarean section. Samples from maternal arm vein, umbilical vein, amnion fluid and neonatal urine. Calculated net accumulation of inulin and mannitol in the fetus and transplacental concentration gradients.	Mannitol and inulin excreted in neonatal urine
A Comparison of the Freezing Points of Fetal and Maternal Plasmas of Humans **Stenger Vet al.(70)**	13 maternal-fetal pairs at term including 4 with pregnancy complications (elective caesarian section)	Maternal samples from radial artery and uterine vein, fetal sample from umbilical vein after delivery and clamping of the cord. Measurement of freezing pint depression to calculate osmotic pressure om both sides of placenta.	Osmotic pressure in umbilical vein higher than in maternal radial artery. Higher fetal concentration of sodium and potassium than maternal
The permeability of the human placenta to sodium in normal and abnormal pregnancies and the supply of sodium to the human fetus as determined with radioactive sodium **Flexner LB and Cowie DB et al. (71)**	16 maternal-fetal pairs from week 9 to term (caesarian section or surgical abortions)	Injection of radioactive sodium into maternal arm vein, maternal sample from both arm veins, fetal sample 1-2 hours postpartum or from the ash of dead fetuses.	Sodium permeability increased 70 times throughout gestation

HUMAN IN VITRO STUDIES

Is there morphological evidence for the existence of transtrophoblastic channels in human placental villi? Kertschanska S et al. (27)	19 term placentas (delivery method unknown)	Single cotyledons perfused via fetal circulation. Lanthanum hydroxide (tracer) added to perfusate. Villous tissue fixated, dehydrated and ultrathin sections studied by electron microscope.	Invaginations in basal membrane of syncytiotrophoblast
Pressure dependence of so-called transtrophoblastic channels during fetal perfusion of human placental villi Kertschanska S et al. (28)	56 term placentas (delivery method unknown)	Perfused cotyledons under different pressure gradients.	Continuous and distensible transtrophoblastic channels
Paracellular permeability pathways in the human placenta: a quantitative and morphological study of maternal-fetal transfer of horseradish peroxidase Edwards D et al. (32)	21 term placentas (both vaginal and caesarian delivery)	Single cotyledons perfused via fetal and maternal circulations (dual perfusion). Horseradish peroxidase (tracer) added to perfusate. Cotyledon fixated and villous tissue studied microscopically.	Fibrin deposits in the syncytiotrophoblast membrane transferring HRP to intervillous space
Osmotic water permeabilities of human placental micrivillous and basal membrane Jansson T and Illsley NP (34)	Term placentas, exact number unknown (vaginal deliveries)	Osmotic water permeability of MVM[1] and BM[2] vesicles isolated from human placenta was measured by stop-flow/light scattering techniques.	Water more likely transported through lipid layer than water channels
Gestational development of water and non-electrolyte permeability of human syncytiotrophoblast plasma membran Jansson T, Powell, TL and Illsley NP(35)	21 placentas from 16 weeks to term (both vaginal and caesarian delivery)	The rate of change in MVM and BM vesicle volume in response to an osmotic challenge was measured and osmotic water permeabilities and solute permeabilities calculated using stop-flow/light-scattering techniques.	Increasing water permeability of MVM and BM with increasing gestational length

[1] MVM: Microvillous membrane
[2] BM: Basal membrane

Supplementary table 2: Overview of experimental studies of placental water transfer

Article/author	Research objects	Method	Results
Distensible transtrophoblastic channels in the rat Kertschanska S et al. (29)	11 rats near term	Umbilical vein cannulated and perfused, venous pressure was increased	Continuous and distensible transtrophoblastic channels
Asymmetrical transfer of inert hydrophilic solutes across rat placenta Stulc J and Stulcova B (22)	31 rats near term	Radioactive isotope injected both in fetal vitelline vein (fetal-maternal transfer) and maternal vein (maternal-fetal transfer). Maternal sample from carotid artery, fetal arterial sample from fetal axillary artery after removal from uterus.	Netto higher fetal-maternal transfer than maternal-fetal (mannitol, EDTA and inulin)
A comparison of the freezing points of fetal and maternal plasmas of sheep and goat Meschia G et al. (46)	6 goats, 6 sheep from 49 to 145 gestational days	Maternal sample from jugular and uterine vein and uterine artery, fetal sample from umbilical vein and artery. Freezing points measured to calculate osmotic pressure.	Fetal total osmotic pressure equal to or lower than maternal
Osmotic flow through the placental barrier of chronically prepared sheep Armentrout T et al. (47)	4-9 sheep, gestational length unknown	Maternal or fetal plasma was made hypertonic in vivo by infusion of concentrated solutions of mannitol, sucrose, or NaCl. Transplacental water flux was calculated from placental blood flows and arteriovenous differences in water content of the blood. Samples from maternal carotid artery, jugular and uterine vein, fetal femoral artery and umbilical vein, electromagnetic flow sensor in fetal carotid artery.	After three days postoperatively, maternal plasma was consequently hyperosmotic compared to fetal plasma
Fetal blood volume, urine flow, swallowing, and amniotic fluid volume responses to long term intravascular infusions of saline Brace RA (52)	10 sheep around 130 gestational days	Chronically catheterized fetuses. 7 L saline infusion into fetal vein over 5 days.	Very high urine flow rate, increased more than infusion rate. Only 2 of 7 L were found in fetal compartments during the 5 days

Transplacental, amniotic, urinary, and fetal fluid dynamics during very-large-volume fetal intravenous infusions **Brace RA and Moore TR(53)**	10 sheep near term	Chronically catheterized fetuses intravenously infused with 4 L of either isotonic saline or Ringer acetate over 4 hours.	Half of the infused volume must have passed the placenta and into maternal circulation
Fetal fluid responses to long-term 5 M NaCl infusion: where does all the salt go? **Powell TL and Brace RA(54)**	8 sheep near term	Catheter in maternal femoral artery and vein, both fetal femoral arteries and vein. Infusion of 5M NaCl into a fetal vein over 3 days (4,8 L).	Increased fetal osmolality, but no accumulation of fetal, amniotic or allantoid fluid, big increase in fetal urine production
The placental transfer of water from fetus to mother following the intravenous infusion of hypertonic mannitol to the maternal rabbit **Burns PD et al. (56)**	30 rabbits near term	Sample from maternal carotid artery and then umbilical vein during caesarian section, infusion of 200 ml 25% mannitol into maternal right external jugular vein.	Fetal water content decreased by 3 %
Effects of osmotic gradients across the primate placenta upon fetal and placental water contents **Bruns PD et al. (55)**	8 pregnant monkeys, 40-140 gestational days	Maternal infusion of NaCl or disaccharide solution into peripheral leg veins, fetal water content determined after caesarian section and death.	Fetal dehydration was produced as a result of the maternal NaCl infusion
Fluid shift across the placenta: II Fetomaternal transfer of horse-radish peroxidase in guinea pig **Kaufmann P et al. (23)**	18 term guinea pigs	Placenta isolated and perfused. HRP [3]added to perfusion fluid. Placenta cut in pieces and examined by electron microscopy.	Continuous and distensible transtrophoblastic channels

[3] HRP: Horse radish peroxidase

Model study of placental water transfer and causes of fetal water disease in sheep **Faber JJ and Anderson DF (51)**	Model study, sheep from 99-145 gestational days	Computer simulation from existing experimental data	Active solutes and bicarbonate main driving forces for water transfer. Reduction of Na-gradient between mother and fetus
Long-term regulation of fetal cardiac output. A hypothesis on the role of carbon dioxide **Longo LD and Power GG(49)**		Computer simulations	CO_2/bicarbonate essential for osmolality and water transfer

Supplementary table 3: Overview of quantitative placental water transfer.

Article/authors	Research objects	Method	Results
The role of the fetus in the water exchange of the amniotic fluid of normal and hydramniotic patients **Hutchinson DL et al (36)**	9 women from 12 weeks (w) to term, including 2 polyhydramnios (caesarian section or therapeutic abortions)	Amnion fluid injected with hydrogen tracers, samples from maternal serum, AF and fetal umbilical cord blood	Fetal-maternal transfer w12: 111ml/h. Maternal-fetal transfer w12: 100 ml/h. Fetal-maternal transfer w40: 3682 ml/h. Maternal-fetal transfer w40: 3657 ml/h
Osmotic flow through the placental barrier of chronically prepared sheep **Armentrout T et al (47)**	12 maternal-fetal sheep pairs (in vivo), 120-145 gestational days	Tritiated water injected into fetal femoral vein. Placental blood flow measured. Rate of water flow across placenta calculated from arteriovenous difference in the two placental circulations (fetal umbilical vein and femoral artery, maternal carotid artery and uterine vein)	0.061 ml/kg/min in fetal direction Fetal weight 3.41 ± 0.24 kg
Unidirectionality of the water exchange between mother and 19-day fetus in the rat **Romeu A et al (58)**	Maternal-fetal rat pairs near term (in vivo, n unknown)	Maternal injections of tritiated water, maternal-fetal and fetal-maternal water transfer calculated	1ml/h fetal direction Fetal weight unknown
Measurement of net transplacental transfer of fluid to the fetal sheep **Lumbers ER et al (69)**	8 maternal-fetal sheep pairs, 122-133 gestational days	Fetal esophagus ligated and drinking prevented over 3h. Urine and lung liquid flow measured	0,40 ± 0,09 ml/min/kg in fetal direction Fetal weight unknown

Water exchange in the placenta – a mathematical model **Wilbur WJ et al. (50)**	Model study	Mathematical model, parameters from previously experimental human and animal studies	0,0139 ml/min in fetal direction Net fetal water gain 20ml/day
The dynamic placenta: II Hypothetical model of at fetus driven transplacental water balance mechanism producing low apparent permeability in a highly permeable placenta **Sebire NJ and Talbert D (67)**	Model study	Computer simulations	0,1 ml/min in fetal direction