Fetal fluid and protein dynamics

Pasman, S.

4

Origin and function of amniotic fluid albumin: a review of the available evidence

Suzanne A. Pasman, Emile de Heer, Frank F.P.H. Vandenbussche

Abstract

Little is known about the origin of albumin in amniotic fluid. A fetal origin is questionable, because of the low concentration of protein in fetal urine and lung fluid. A maternal origin of proteins in amniotic fluid was already proposed in the 1970s. This review focuses on the possible sources of albumin in amniotic fluid and on the pathways for albumin to enter and leave the amniotic fluid in the second and third trimester. Based on the available evidence, it is unlikely that the fetus makes a large contribution to amniotic fluid albumin. Maternal albumin probably reaches the amniotic fluid through the fetal membranes, though the amniotic membrane itself also produces albumin. The functions of albumin in amniotic fluid are even less understood. Albumin may influence the homeostasis of amniotic fluid volume. Furthermore, intake of albumin, and the fatty acids which albumin carries, could be a substantial part of fetal nourishment. More knowledge on the mechanisms that determine amniotic fluid composition and on the function of albumin in amniotic fluid could provide the basis for new fetal therapy strategies.

Introduction

Although albumin is the most prevalent protein in amniotic fluid [18;25;26], its origin in amniotic fluid remains unclear. Most clinicians in the field of fetal and maternal medicine assume that albumin in the amniotic fluid is of fetal origin, though this assumption has never been established by research. While the amniotic fluid is mainly formed of fetal urine and lung fluid [27], the concentration of protein in fetal urine and lung fluid is many times lower than that in amniotic fluid (Table 1). This suggests that the albumin in amniotic fluid has a different origin, most likely a maternal source. It was already proposed in the 1970s that albumin in amniotic fluid is transported from maternal blood to amniotic fluid without passing through the fetal blood [28]. Using polymorphisms, multiple larger proteins that are found in the amniotic fluid, such as transferrin and alpha1-antitrypsin, have been shown to have a maternal origin [29;30]. Finally, the placenta and even the membranes cannot be ignored, and should be considered as possible sources of albumin in amniotic fluid.

The functions served by albumin in amniotic fluid are even more elusive. Albumin is a protein that hardly shows any differences between species, underlining its importance to sustain life. It surrounds embryo's in abundance, either when they are hatching from an egg or growing inside a uterus. It is the most prevalent serum protein with a crucial role in maintaining intravascular osmotic pressure and functioning as a carrier protein of numerous substances [31;32]. One might expect albumin to have a similar role in amniotic fluid as it has in the blood. This could imply it has an important role in amniotic fluid volume and pressure regulation. Albumin may also provide an important contribution to fetal nourishment. When considering the potential non-fetal origin of albumin, the amniotic fluid can be seen as a high-energy substance, providing prenatal feeding through the gastrointestinal tract. In other words, it could provide a kind of fetal "breast" feeding.

This review considers the current evidence on the origin of albumin in amniotic fluid in the second and third trimester of gestation. It will discuss the possible pathways from a fetal, a maternal and a placental origin, based on present literature. Due to the relative scarcity of human data, results from animal studies will be described where human evidence is absent. We will then speculate on the function of albumin in amniotic fluid, and will finally propose potential directions for future research.

4

Fetal blood as a possible source of amniotic fluid albumin

In the embryonic period, production of albumin starts in the yolk sac [33]. By the second and third trimester, albumin is probably mainly formed in the fetal liver [34-36]. There are a number of possible routes by which fetal serum albumin could be transferred to the amniotic fluid (see Figure 1).

Table 1 Normal values of total protein, albumin and AFP concentration in different compartments during 2nd and 3rd trimesters of human pregnancy.

	Total protein (g/L)‡	Albumin (g/L)‡	Changes during gestation of total protein and albumin:	AFP (g/L)‡	Changes during gestation of AFP:
Maternal serum	**65** [1;2]	**30** [1;3]	lower than in non-pregnant women	**0.00005 - 0.0001** [4-6]	increases until 32 weeks and then decreases
Fetal serum	**25 - 55** [7-10]	**20 - 35** [7-10]	increases between 20 and 35 weeks	**0.05 - 2** [4-6]	rapidly decreases from beginning of 2nd trimester
Fetal lung fluid	A **[0.006 - 0.4]** [11-13]	?	?	?	?
Fetal urine	B **[0.04 - 0.1]** [14;15]	C **[0.002 - 0.06]** [16-18]	?	?	?
Amniotic fluid	**3 - 6** [19-22]	**1.5 - 4** [21-24]	increases until 25 weeks, decreases until 35 weeks; can increase again after 38 weeks	**0.0001 - 0.03** [4-6]	rapidly decreases from beginning of 2nd trimester

‡ Values are given as means. Two values indicate minimum and maximum means at different gestational ages. Two values within [ ] indicate the range found in different publications.
A tracheal fluid of fetal sheep
B fetal bladder punctions in obstructive uropathy
C first voided neonatal urine after birth

Urine

Urine is the main contributor of amniotic fluid, and as such it is the most obvious candidate for the source of amniotic fluid albumin. However, the concentration of total protein and albumin in fetal urine is 25 to 750 times lower than the concentration in amniotic fluid (Table 1). Table 1 shows the range of reported values for (mean) albumin concentration, taken from studies that measured albumin in first voided postnatal urine of premature and term neonates. The range of values reported for (mean) total protein concentration is also shown, obtained from human fetal bladder punctions in obstructive uropathy, in cases with good outcome after birth. Although these urine measurements were not obtained in healthy fetuses, it is more likely that they overestimate the actual values than underestimate them. The low concentration of protein in urine compared to that in amniotic fluid implies that either the amniotic fluid is highly concentrated, with water extracted on a daily basis, or that the albumin in the amniotic fluid has another source.
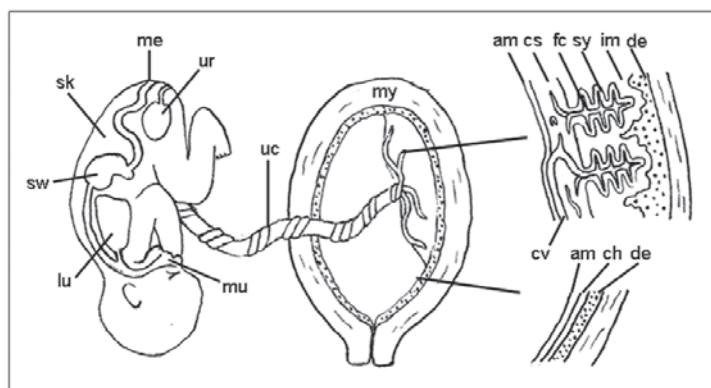
4

Figure 1 Possible pathways to and from human amniotic fluid in 2nd and 3rd trimester. Uterus is depicted, with fetus, placenta and fetal membranes in detail.

Fetus	**Uterus**	**Placenta**	**Fetal membranes**
lu= lung fluid	de= decidua	cs= chorion stroma	am= amniotic
me= meconium	my= myometrium	cv= chorial vessels	membrane
mu= mucous		fc= fetal capillaries	ch= chorionic
membranes		im= intervillous	membrane
sk= ski		maternal blood	
sw= swallowing		sy= syncytio-	
uc= umbilical cord		trophoblast	
ur= urine			

Intra-membraneous pathway: sk,mu,uc,cs/cv↔am
Trans-membraneous pathway: de↔ch↔am

Note that all pathways are bidirectional except fetal urine and meconium.

During the 3rd trimester of pregnancy, the amniotic fluid concentrations of several proteins decrease [18;22;23]. There are several explanations for this. In the case of proteins such as beta2-microglobulin (12 kD), which have a molecular weight lower than that of albumin (67 kD), it has been shown that the concentration in first voided urine after birth is similar to that in corresponding amniotic fluid [18]. The concentration of these proteins in amniotic fluid decreases during pregnancy [18;23]. This suggests that tubular resorption increases strongly from 24 weeks gestation onward [18;23]. The change in the concentration of low molecular weight proteins in amniotic fluid during gestation can thus be explained by renal development. In the case of albumin and proteins with higher molecular weight, it has been shown that albumin concentration in first voided urine after birth is much lower than in the corresponding amniotic fluid [18]. Burghard et al. found that the concentration of postnatal urinary albumin and high molecular weight proteins was only 5% and 15% of that in amniotic fluid [18]. Gitlin et al. estimated that amniotic fluid albumin was less than 5% of urinary origin at term [17]. They calculated that a term fetus would have to produce 8 litres of urine daily to explain the amount of albumin present in amniotic fluid. Urine production and clearance of water (and low molecular weight proteins) are clearly not in this order of magnitude [27;37]. A urinary origin of albumin combined with a strong daily extraction of water thus seems an implausible explanation for the concentration of amniotic fluid albumin. Furthermore, bulk amniotic fluid turnover increases strongly during gestation due to an increase in urine production during pregnancy [37], as well as an increase in the amount of fluid that is swallowed by the fetus [27]. This has led to the conclusion that the decrease of albumin concentration in amniotic fluid in the 3rd trimester is a result of a rise in bulk fluid turnover, which exceeds the inflow of albumin from a separate source [18;28]. This would indeed explain the decrease of amniotic fluid albumin between 25 and 35 weeks of gestation and the low concentration of albumin in urine compared to amniotic fluid. It seems that the observed changes in amniotic fluid albumin concentration during gestation can only be explained by a non-urinary origin of amniotic fluid albumin.

Another indicator that fetal urine is not the main source of amniotic fluid albumin is the ratio between AFP and albumin concentrations. AFP is a fetal protein with a molecular weight similar to that of albumin (AFP, 70 kD; albumin, 67 kD). In normal pregnancies, the fetal urine to fetal blood concentration ratio is roughly similar for AFP and albumin [38]. However, at term, as can be derived from Table 1, the amniotic fluid to fetal blood concentration ratio for AFP is 30 times smaller than that of albumin. This relatively high concentration of albumin, implies a mainly non-urinary source of amniotic fluid albumin. In pregnancies complicated by congenital nephrosis,

fetal proteinuria leads to markedly raised amniotic fluid AFP [39]. Amniotic fluid albumin, however, is only slightly elevated or even normal [40]. Thus, although it is probable that AFP in amniotic fluid originates mainly from fetal urine, it is likely that the albumin in amniotic fluid has another source.

Lung fluid

Lung fluid is the second largest contributor to amniotic fluid. It is estimated that around 15% of the daily production of amniotic fluid is attributable to lung fluid [27]. Although more lung fluid is produced, about half of it is immediately swallowed [41]. However, total protein concentration in fetal lung fluid is many times lower than in the amniotic fluid (Table 1). A number of experiments have been conducted using ovine fetuses to study fetal lung fluid production. When radioactively labelled albumin was injected, hardly any was found to pass from fetal blood to lung fluid [13]. Furthermore, it was shown that chloride is actively secreted by fetal lungs and, along with it, a fluid with a protein concentration much lower than that in fetal lung interstitial fluid [42;43]. Of course, care should be taken when interpreting these above mentioned findings, as they were all derived from studies in ovine fetuses. However, current knowledge of human fetal lung physiology shows similar results to those in ovine studies. For example, using pulmonary Doppler ultrasonography, human fetal lung fluid outflow was shown to increase until 36 weeks of gestation [44], similar to measurements in ovine fetuses [45]. To our knowledge, no data exists on the concentration of albumin in human fetal lung fluid. Unfortunately, neonatal bronchoalveolar lavage measurements can not be compared with the fetal situation, since lung fluid production is strongly decreased after birth mainly due to a strong decrease in active chloride secretion [43]. To conclude, though little is known on human fetal lung fluid composition, based on ovine experimental studies, it is unlikely that lung fluid is a significant contributor to amniotic fluid albumin.

Intestines

Fetal intestines are another possible source of amniotic fluid albumin. It is often assumed that meconium is voided into the amniotic fluid only just before or during birth. However, during fetoscopy, small amounts of meconium have been observed as early as the early second trimester of gestation. In fact, intestinal enzymes are present in amniotic fluid throughout pregnancy [46]. However, fetal continence for meconium seems to increase markedly after 18 weeks of gestation [47]. It has also been shown that after 18 weeks, intestinal enzymes in amniotic fluid decrease significantly [48-50]. Furthermore, it has been shown between 35 and 42 weeks gestation that there is no significant difference in albumin concentration between

amniotic fluids that contain meconium and those that do not [21]. Finally, in an ovine experimental study, iatrogenic jejuno-ileal atresia did not alter albumin concentration in amniotic fluid [51]. In conclusion, although there may be an intestinal contribution to amniotic fluid albumin before 18 weeks, it is very unlikely that there is a significant contribution in the late 2nd trimester or the 3rd trimester.

Skin

The skin is another possible pathway through which albumin could permeate. In 1969, Lind et al. described microvilli on human fetal skin at 14 weeks gestation [52]. Such microvilli are also present on amniotic membrane [53;54] and indicate an important transport function. However, a marked change takes place shortly after 14 weeks. From 25 weeks gestation, human fetal skin consists of multiple layers and is keratinised, making it much less permeable for water and solutes [52;55-57]. In conclusion, it is unlikely that fetal skin is a significant contributor to amniotic fluid albumin in the second half of pregnancy.

Intramembraneous pathway

Finally, the so-called intramembraneous pathway could contribute to the albumin in the amniotic fluid. This is a pathway between the fetal blood and the amniotic fluid, which is thought to explain important fluid shifts in ovine fetuses, and which probably also exists in humans (see Figure 1) [58]. Brace and Gilbert introduced this possible pathway, pointing out that not all uptake of amniotic fluid was due to swallowing, but that part of the reabsorption must take place through the membranes. Therefore, they hypothesised a kind of lymphatic drainage, between the amnion and chorion leave, draining amniotic fluid towards the fetal blood vessels on the fetal surface of the placenta. In ovine pregnancy, an anatomic substrate for fluid drainage is present in the form of micro blood vessels between the amnion and chorion leave. Brace and Gilbert named this the intramembraneous pathway. In humans, though, there are no micro blood vessels between the amniotic and chorionic membranes, but there is a large surface area of fetal blood vessels present in the umbilical cord and at the fetal surface of the placenta adjacent to the amniotic fluid. Using a mathematical model, Mann et al. calculated that human intramembraneous flow of water must be around 390 ml daily [59]. The term intramembraneous pathway has subsequently been proposed for the combination of fetal skin, the mucous membranes (mainly oro-nasal saliva), the umbilical cord surface, and the fetal side of the placenta [27].

The components of the intramembraneous pathway could all contribute to the build-up of albumin concentration in amniotic fluid. The contribution of human fetal skin

has already been discussed. The contribution of mucous membranes in the human situation is unknown. In 3 kg ovine fetuses, fluid secretions from the head amounted to 25 ml daily [27]. The contribution of the umbilical cord is also unknown, but depends on the transport characteristics of Wharton's jelly. Since Wharton's jelly contains mucopolysaccharides, this may facilitate the transport of some amniotic fluid solutes like bilirubin [28]. In fact, albumin has been shown to pass through ovine umbilical cords in vitro [28]. Finally, the main contributor of the intramembraneous pathway is expected to be the fetal surface of the placenta. Both the chorionic tissue and the chorial blood vessels could transport fetal serum albumin to the amniotic fluid. However, animal research has not yet demonstrated albumin transport from the fetus to amniotic fluid through this pathway, but has instead shown an albumin transport in the opposite direction [60;61]. In view of this, it is unlikely that the intramembraneous pathway is a significant contributor to amniotic fluid albumin.

Several investigators have studied the dynamics of transport between amniotic fluid and fetal blood through the intramembraneous pathway. Passive diffusion of solutes seems to occur in both directions over the intramembraneous pathway, limited only by solute size [62]. However, passive diffusion only accounted for a minor part of the flow over the intramembraneous pathway. The majority of intramembraneous flow seems to be due to bulk flow of water and solutes, which is only explainable by unidirectional transport, in the form of active vesicular transport [62-64]. This hypothesis was developed in part due to several studies that used albumin tracers to investigate intramembraneous flow. In 2002, in an ovine study by Faber et al., ^{125}I labelled albumin was injected into the amniotic fluid [60]. All urine and lung fluid was diverted and fetuses were made unable to swallow. They found that ^{125}I-albumin was cleared from amniotic fluid at a rate of 25-30 ml/h bulk flow. In addition, after ^{125}I labelled albumin was injected in fetal serum, hardly any ^{125}I-albumin appeared in the amniotic fluid after 30 hours. Previously, Mann et al. had performed a similar study and obtained comparable results [61]. Besides radioactively-labelled albumin, they also injected radioactively-labelled creatinin into the amniotic fluid. They found that creatinin clearance from amniotic fluid was greater than albumin clearance, 5 hours after injection. Notably, they found a higher radioactivity in fetal blood compared to maternal blood. However, caution should be made in extrapolating data from ovine studies. Osmolality is higher in ovine than in human amniotic fluid, and differences in osmolality and composition suggest that human and ovine amniotic fluid have different regulating mechanisms [65]. Nonetheless, vacuolation was recognised in human amnion with electron microscopy [54;66], which might support the hypothesis of active vesicular transport. It has also been proposed that aquaporins play an

4

important role in intramembraneous flow [67]. These are small transcytotic channels that transport water and in some cases small molecules such as 0.1 kD creatinin. However, aquaporins are unable to transfer larger 67 kD albumin. Human studies on the intramembraneous pathway are scarce. In 1972, Gitlin et al. injected radioactively-labelled protein into human amniotic fluid in 2 cases after the occurrence of intrauterine fetal death [17]. Birth took place 2 days after administration. As would be expected, no ^{125}I-albumin was retrieved in the stomach. Nonetheless, 10% of the injected ^{125}I-albumin was still cleared from the amniotic fluid. This was believed to have taken place through the fetal membranes, since the highest radioactivity was registered in the membranes. After birth, some radioactivity could also be measured in the cord blood and some in the maternal blood. In conclusion, it does not appear that albumin is transported from fetal blood to amniotic fluid through the intramembraneous pathway in significant amounts. It is, however, plausible that the intramembraneous pathway is in part responsible for the transport of amniotic fluid albumin to the fetal blood.

Summary

Based on the available evidence from the literature, it is unlikely that the fetus contributes significantly to the amniotic fluid albumin. Data from human and animal experiments seem to rule out fetal urine, lung liquid, skin or intestines as a large source of albumin. Finally, studies on the intramembraneous pathway have shown uptake of albumin rather than excretion into the amniotic fluid.

Maternal blood as a possible source of amniotic fluid albumin

Most clinicians think the transfer of molecules between mother and fetus takes place exclusively from maternal blood to fetal blood over the trophoblast. However, there are other possible pathways that albumin could take to leave the maternal blood and build up a concentration in the amniotic fluid.

Via fetal blood

First of all, the possibility should be considered that albumin is transferred via the trophoblast from maternal to fetal blood, after which it enters the amniotic fluid. In 1997, Malek et al. performed a study in a dually-infused human placental lobe [68]. ^{14}C-labelled albumin and IgG were injected into the maternal side of the placental lobe. Only small traces of albumin were retrieved at the fetal side of the placenta.

After 1 hour, this low concentration of albumin remained constant for the study period of 4 hours. In contrast, after an internalization phase of 2 hours, the concentration of IgG kept increasing markedly in the fetal blood. Although albumin and IgG are both bound by the so-called neonatal Fc receptor (FcRn), this phenomenon could be explained by the observation that a specific Fc receptor (FcgammaRIIb) is present in fetal capillary endothelium, which can transfer IgG into the fetal blood, but not albumin [69]. In the previous chapter, we already saw the unlikelihood that fetal serum accounts for significant amounts of the albumin in the amniotic fluid. In conclusion, it is unlikely that maternally derived albumin would be transported via fetal blood and then contribute significantly to amniotic fluid albumin.

There are, however, other possible pathways that albumin could take to move from the maternal compartment to the amniotic fluid, without first entering the fetal blood.

4

Transmembraneous pathway

The transfer of albumin from maternal blood to amniotic fluid and vice versa can theoretically take place through the amnion and chorion leave. The exchange of fluid and solutes through the amniotic and chorionic membrane towards the decidua parietalis and the maternal uterus is frequently referred to as the transmembraneous pathway (see Figure 1). In ovine experiments, the transmembraneous flow of water, in second and third trimester, seems to be directed from amniotic fluid towards the decidua. It is estimated that at term, about 1% of all outward flow from the amniotic fluid takes place via the transmembraneous pathway [70]. This is in contrast to early pregnancy, when the chorion is the major pathway for inward flow of water and solutes into the coelomic fluid [71]. In 20 and 42 day old human embryos, albumin has been histochemically shown to be present in the yolk sac, chorion stroma and amnion [72]. In 1982, Wang et al. identified breaks in the basement membrane in fresh full term human amnion with electron microscopy. Based on this finding, and the fact that intercellular occluding junctions are absent in the amnion, they proposed the possibility of a transmembraneous transfer of proteins into the amniotic fluid [53]. In 1983, Wang and Bartels supported this hypothesis with the finding that the intercellular channels in human chorion also contain very few occluding junctions, making a paracellular route for proteins plausible [73;74]. In conclusion, the transmembraneous pathway is a possible route for the build-up of amniotic fluid albumin concentration.

Via placental tissue

Finally, though it is seldom considered, the placental tissue could serve as a direct pathway. It is theoretically possible that a direct exchange takes place from the

maternal blood in the placenta, through the chorion tissue, through the amniotic membrane on the fetal side of the placenta, to the amniotic fluid (see Figure 1). Passive diffusion of molecules through the placenta can only take place up to 0.6 kD [75]. Active transport to the amniotic fluid, however, may be possible, through this part of the intramembraneous pathway. For example, an ovine experimental model suggested that there is an active transport of glucose and lactate through the intramembraneous pathway into the amniotic fluid [63]. It is therefore conceivable that there is also an active transport of albumin through the fetal side of the placenta. Different receptors, for example FcRn, could be involved in binding albumin [76]. Binding receptors can theoretically either concentrate albumin on the cell surface, induce formation of endocytose vesicles, or they can save albumin from degradation in the acid environment of the endosomes, thus recycling albumin back to the apical cell surface or facilitating transcytotic transport. Though several receptors that can bind albumin have been shown to be present in the human placenta [77-79], it is not known to what extent these receptors contribute to the transport of albumin through the placenta. In 2006, Lambot at al. showed that in the term human placenta, uptake of maternal albumin takes place in the trophoblast, via clathrin-mediated vesicular transport [78]. In their study, however, a quarter of the albumin was rapidly recycled towards the maternal side of the trophoblast. Most of the internalized maternal albumin was degraded, and it did not seem to reach the villous stroma or cross the endothelium of fetal capillaries. However, this may not rule out a pathway for albumin directly through the chorion stroma in the placenta to the amniotic fluid. Lambot et al. suggested that 150 grams of albumin enters the trophoblast layer daily [78]. Even if most of this is recycled to the maternal blood or degraded, a significant amount could still remain, which could be transported through the placenta to the amniotic fluid. It is worth noting that a study of adult mice showed that FcRn saved as much albumin from degradation in the endosomes as the liver produced daily [80]. It is imaginable that such a recycling mechanism also takes place on the fetal side of the placenta. Thus, after uptake of amniotic fluid albumin via vesicular transport in the intramembraneous pathway, albumin may be released back into the amniotic fluid, thus increasing its concentration there. In conclusion, we can only speculate on the contribution of maternal albumin transferred via placenta tissue to the amniotic fluid. Recycling of amniotic fluid albumin on the fetal side of the placenta should also be considered.

Studies indicating a maternal origin
The first indication for the maternal origin of amniotic fluid albumin came from several studies using radioactively-labelled albumin. In an experiment by Gitlin et al. in 1964,

[131]I labelled albumin was injected into human maternal blood at term [81]. After 3 to 9 hours, radioactivity and albumin concentration were measured in maternal blood, fetal blood and amniotic fluid. A 3 to 5 times higher specific activity per mg albumin was found in amniotic fluid than in fetal blood. In contrast, radioactively-labelled IgG, injected into maternal serum, showed a 2.5 times lower specific activity in amniotic fluid than in fetal blood after 4 hours. A month after the injection of the ^{131}I labelled albumin (25 and 32 days), there was no protein-bound radioactivity left in the amniotic fluid, although there was still radioactivity present in fetal and maternal blood. In a comparable study, performed by Dancis et al. in 1961, radioactively-labelled albumin was injected into human maternal blood at three months gestation [82]. After 24 hours, radioactivity in amniotic fluid was lower than that in fetal blood. However, since radioactivity was only measured per ml and not per mg albumin in this study, specific activity per mg albumin may have been higher in amniotic fluid, as was found by Gitlin et al. Theoretically, measurement of radioactivity per mg is a preferable measure, since it reflects the proportion of maternally derived albumin compared to fetally produced albumin, ignoring the difference of the fluid volume in which the tracer is dissolved. In 1986, Tomoda et al. published a study where ^{125}I-labelled albumin was injected into the amniotic fluid of ovine fetuses [83]. It was shown that as the fetus digested the albumin, most radioactive iodine was spliced from the albumin and cleared via the placenta to the mother, and was subsequently retrieved in maternal urine. Fetal urine was measured for 9 days, using a catheter, before recycling it to the amniotic fluid. Hardly any ^{125}I re-entered the amniotic fluid through fetal urine. Based on Gitlin et al.'s findings, in 1975, Sutcliffe argued that if the albumin in the amniotic fluid had been of fetal origin, its specific activity would not have exceeded that in the fetal serum. Thus, he concluded, most of the albumin in amniotic fluid at term must be of maternal origin [28]. Dancis et al. came to the opposite conclusion, but it is not possible to say whether this is because the transfer mechanism is different at different gestational ages (Dancis' study was conducted at three months gestation, whereas Gitlin's study was conducted at term), or whether it is due to the difference in measurement method (per ml instead of per mg). Finally, Tomoda et al. pointed out that the results of studies with radioactively-labelled albumin should be interpreted with caution since ^{125}I-albumin can be spliced when digested by the fetus. Dancis et al. also addressed this problem, pointing out that the possibility that radioactive iodine is spliced in the placenta can not be ruled out. This may explain why hardly any protein-bound radioactivity was measured in the amniotic fluid after 25 or more days. In conclusion, radioactive labelling studies at term endorse the idea that maternal albumin is transported directly from maternal blood to the amniotic fluid without passing through fetal blood.

4

The second indication for the maternal origin of amniotic fluid albumin is the fact that proteins with a molecular weight of over 260 kD are not detected in amniotic fluid [25;28]. This suggests that proteins enter the amniotic fluid by ultrafiltration and that they are only able to permeate the fetal membranes when they are below a certain molecular weight. One striking example is haptoglobin 1-1, with a molecular weight of 85 kD, which is found in amniotic fluid, whereas the other forms 2-1 and 2-2, with a molecular weight of over 260 kD, are not. Thanks to the difference in the isoforms of haptoglobin, it has been possible to prove the maternal origin of haptoglobin 1-1 in amniotic fluid in some cases [84]. Since albumin has a molecular weight of 67 kD, it should be able to permeate the membranes. In 1960, Abbas et al. found that at term, the electrophoretic protein pattern of amniotic fluid was very similar to that obtained by dialysing maternal serum through the fetal membranes [85]. In conclusion, based on its molecular weight, it is likely that albumin enters the amniotic fluid by passive diffusion through the membranes.

The third indication for the maternal origin of amniotic fluid albumin comes from measurements of protein concentration ratios. It has been hypothesised that if serum proteins enter the amniotic fluid by ultrafiltration, then proteins of similar molecular weights will diffuse into the amniotic fluid at approximately the same rate. Thus, the relative concentration of proteins would be similar in two compartments. Derrington et al. investigated the ratio of transferrin to albumin and found that, at term, the ratio of transferrin to albumin in amniotic fluid was more similar to that in maternal blood than to that in fetal blood [87]. Transferrin has already been shown to be of maternal origin by comparing polymorphisms of the protein in amniotic fluid and in fetal and maternal blood [29]. Both Sutcliffe et al. and Johnson et al. studied the change in protein concentration during the course of pregnancy, comparing the concentrations of proteins in amniotic fluid to the concentrations in fetal and maternal serum [38;86]. The albumin concentration ratios during pregnancy were similar to the ratios of proteins like orosomucoid and Gc-globulin that are of proven maternal origin, and were very different from the ratios of AFP, which is of proven fetal origin.

Several of the studies discussed above were already mentioned in an extensive review published in 1975 entitled "The nature and origin of the soluble protein in human amniotic fluid," in which R.G. Sutcliffe concluded that albumin in amniotic fluid must be, to a large extent, of maternal origin [28].

Summary
Based on the available evidence, it is likely that amniotic fluid albumin is, to a significant degree, of maternal origin. From in vivo and in vitro experiments, it seems

probable that albumin is transported via the transmembraneous pathway. It is not known to what extent the fetal side of the placenta contributes to maternally derived albumin transport or to recycling of amniotic fluid albumin.

Placenta or fetal membranes as a possible source of amniotic fluid albumin

Another possible explanation for the relatively high concentration of albumin in amniotic fluid compared to fetal urine and lung fluid, which are the main sources of amniotic fluid, is that the placenta or fetal membranes produce the albumin that enters the amniotic fluid.

Placenta
Several studies have investigated the synthesis of albumin in the placenta. In baboon fetuses, no albumin mRNA was detected in the placenta or the amniotic membrane [36]. In the term human placenta, however, McKinnon et al. identified albumin mRNA in the syncytiotrophoblast by RT-PCR and albumin protein by immunohistochemistry [87]. Similarly, AFP synthesis was detected in the trophoblast, though only in the first trimester and not in the term human placenta [88]. In conclusion, albumin does seem to be synthesized in the placenta, but it is not known to what extent this reaches the amniotic fluid.

Fetal membranes
To our knowledge, there are no reports on the synthesis of albumin in the chorionic membrane. However, the amniotic membrane itself is a plausible contributor to amniotic fluid albumin. The amniotic membrane consists of a single layer of epithelial cells on a thicker basement membrane and a collagen spongy layer containing mesenchymal cells. Albumin gene expression has been observed in human amniotic epithelial cells [89]. Moreover, Takashima et al. demonstrated that these cells can synthesize and excrete albumin at term. This synthesis was 30 fold greater in intact amnion compared to a cultivated monolayer of amniotic epithelial cells [90]. Furthermore, albumin mRNA expression was observed in mesenchymal cells from human amniotic membrane. However, production of albumin and AFP only increased significantly when these cells were induced to differentiate to hepatocyte type cells in vitro [91]. In conclusion, it is likely that the amniotic membrane contributes to the albumin in the amniotic fluid, although it is not known to what extent.

4

Summary

Based on the available literature, it is not known if the placenta or the chorionic membrane contribute to amniotic fluid albumin. It is, however, likely that amniotic fluid albumin is to a certain extent of amniotic membrane origin.

The function of amniotic fluid albumin

Human serum albumin is a relatively small protein that accounts for around 75% of protein molecules and about half of total protein mass in the plasma of healthy adults [32]. In fetal blood, albumin forms an even larger fraction of total protein (Table1). Because of its relatively large contribution to the plasma protein pool, albumin is responsible for approximately 75% of plasma colloid oncotic pressure in adult serum [32]. Besides this, its main function is to act as a carrier protein: e.g., for fatty acids, steroid and thyroid hormones, calcium, nitric oxide, bilirubin, and numerous types of toxins and drugs [31;92]. Serum albumin has other functions, among them an antioxidant function and an enzymatic function, and it also modulates inflammatory response [32].

Volume regulation

We can only speculate about the function of albumin in amniotic fluid. As in blood, albumin in amniotic fluid is the major component of total protein [18;25;26]. Albumin in amniotic fluid may also have a function in maintaining osmotic pressure, and it could therefore have a regulatory function in the volume and pressure of the fluid. Albumin concentration in maternal blood, has been shown to influence human placental lactogen secretion by the placenta [93;94]. Lactogen receptors in the amniotic membrane, in turn, may be involved in amniotic fluid volume regulation [95]. Albumin may also influence vesicular transport in the intramembraneous pathway, since it has been shown that albumin concentration regulates caveolin-1 production in the human liver [96]. Caveolin-1 is essential in forming caveolae and has been found in fetal endothelium in the human placenta [97].

Carrier protein

Albumin may play an important part in recycling and detoxifying solutes in amniotic fluid. As a carrier protein, it binds numerous solutes that enter the amniotic fluid and it can hold toxins, rendering them harmless [31]. Uptake and degradation can then take place in the fetal gastrointestinal tract or in the fetal side of the placenta.

Nutrition

Considering its non-fetal origin, the albumin in amniotic fluid may have another important function. We know that the fetus at term swallows and digests about 80% of amniotic fluid every 24 hours [27]. In a study by Gitlin et al. in 1972, radioactively-labelled proteins (such as ^{125}I-albumin) were injected into the amniotic fluid, hours or days before birth [17]. In live fetuses, two thirds or more of the amniotic fluid volume was cleared of protein per day and most of this could be retrieved in the stomach after birth. Swallowing prior to birth can thus add significantly to fetal protein intake. Albumin could serve as an extra source of amino-acids and energy, especially considering its carrier function of fatty acids in aqueous solution. Fetuses that lack this extra source because of an oesophageal or intestinal obstruction are more often growth restricted. In a fetal rabbit experiment, oesophagus ligation restricted growth by 10 to 15% [98]. A retrospective study in fetuses with gastro-intestinal malformations showed that 38% were small for gestational age [99]. This growth retardation was found to be most significant in the last weeks of gestation [100]. Since more than 90% of fat deposition in the fetus occurs in the last ten weeks of pregnancy [101], a large amount of fatty acid transfer is required during this period.

Regulation of amino acid uptake from mother to fetus is not yet fully understood [102]. If the albumin in amniotic fluid is indeed of maternal origin, it is clear this would contribute to daily amino- and fatty acid uptake. Also, it is possible that fatty acids or other ligands are brought into the amniotic fluid through the fetal membranes, along with albumin. Maternal diet has already been shown to influence fatty acid composition in the amniotic fluid and fetal intestinal membrane in rats [103]. Further, even if the albumin in amniotic fluid has an amniotic membrane or placental origin, its amino-acids must still come from the mother. Placental insufficiency might then be not just a matter of diminished transport but also of diminished production of albumin. In conclusion, it is important to realize that the fetal membranes could actively participate in the exchange processes between the maternal and fetal compartment.

Summary

Based on the known functions of albumin in blood, it is probable that albumin in amniotic fluid influences the homeostasis of amniotic fluid volume. It is also possible that it functions as a carrier protein, for example for fatty acids. Finally, it is very likely that albumin in amniotic fluid forms a substantial part of fetal nourishment.

4

Future research

To date, research has not brought forth solid conclusions regarding the origin of albumin in amniotic fluid. In recent years, the proteins in the amniotic fluid have again been studied extensively, this time in the field of proteomics [25;26;104]. However, the possible maternal origin of these proteins has hardly been addressed. Research on the transport mechanism that regulates albumin concentration in amniotic fluid may have wider implications, ultimately leading to new treatment modalities involving the amniotic fluid.

Albumin polymorphisms

In the case of a maternal or paternal abnormality or polymorphism of albumin, the type of albumin in amniotic fluid could be compared to that in maternal serum to establish evidence for the fetal or maternal origin. There are several polymorphisms known in human albumin that could easily be measured with tandem mass spectrometry [105]. Unfortunately, polymorphisms are rare, with frequencies reported to lie between 1 in 1700 to 1 in 3000 in humans [106;107]. However, in case an amniocentesis would be performed in a mother with a known albumin polymorphism, it would become possible to measure the proportion of maternal and fetal contribution to amniotic fluid albumin at a given gestational age.

Albumin tracing

Labelling studies could be designed without the use of radioactivity or toxins, to study the tracer dynamics of maternally injected albumin into the fetal blood and amniotic fluid. For example, stable isotope tracers could be measured with gas chromatography/ mass spectrometry (GC/MS) or GC-combustion-isotope ratio mass spectrometry (GC-C-IRMS). This method has already been used to study surfactant metabolism in premature neonates [108]. Intrauterine transfusion would be a unique situation where simultaneous samples could be obtained from maternal blood, fetal blood and amniotic fluid at different gestational ages.

Albumin synthesis

The production of albumin in the placenta, and chorionic and amniotic membranes could also be investigated. Real time-PCR of mRNA and immunohistochemistry of albumin and its precursors, can be compared at different gestational ages [87;88]. In addition, mesenchymal stem cells or other free floating cells that are present in the amniotic fluid could be considered as a source of albumin.

Albumin transport

The transport mechanisms through the chorion and amnion can be studied, to gain insight into the pathways that albumin takes and the functioning of the intra- and transmembraneous pathway in humans. When doing this, a bidirectional transfer of albumin must be considered. Passive diffusion and active excretion, as well as active vesicular transport should be considered. Albondin, caveolin, megalin, cubilin or clathrin-mediated vesicle transport and macropinocytosis can be studied in human in vitro placenta and membrane models [78]. Finally, the possibility should also be considered that albumin is recycled after vesicular transport, due for example to binding to FcRn [80].

Poly- and oligohydramnios

Another possibility to gain insight into the intramembraneous pathway, is to investigate the concentration of amniotic fluid albumin in poly- and oligohydramnios, of different types of causes. Since these pathological situations represent a dysfunction of the homeostatic properties of the intramembraneous pathway, comparison between different causes and non-pathological cases may tell us about how this pathway functions.

Transamniotic nutrition

Transamniotic feeding of amino- and fatty acids may be a complement to transplacental feeding and can therefore be considered in investigations on intrauterine growth restriction and fetal therapy for this problem. Since the importance of prenatal nutrition on adult metabolism has become apparent in recent years, transamniotic feeding should also be considered in the research on the developmental origins of health and disease.

Conclusion

From the currently available evidence, it appears that the fetus does not make a large contribution to albumin in the amniotic fluid in the second and third trimesters. It seems that a significant amount of the albumin in the amniotic fluid is of maternal origin, and this maternal albumin probably reaches the amniotic fluid through the fetal membranes. In addition to passive diffusion, it is possible that there is a form of active transport or even recycling of albumin over the intra- or transmembraneous pathway. The amniotic membrane itself also produces albumin. At present, we do not know with certainty what the relative contribution is of each of the possible sources for

amniotic fluid albumin. These relative contributions presumably change during the course of the pregnancy.

The albumin in amniotic fluid has a number of possible functions. These include volume regulation and transport of either toxins or nutrients. Intake of non-fetal albumin, and the fatty acids which albumin carries, could be a substantial part of fetal nourishment.

Different suggestions have been proposed in this review to investigate the origin, the transport mechanisms, and the function of albumin in amniotic fluid. This could lead to the development of new fetal therapy strategies, including relatively simple measures for otherwise hard-to-treat conditions such as poly- and oligohydramnios and intrauterine growth restriction.

Acknowledgements

References

1   Robertson EG, Cheyne GA. Plasma biochemistry in relation to oedema of pregnancy. J Obstet Gynaecol Br Commonw 1972; 79(9):769-776.
2   Pilsczek FH, Renn W, Hardin H, Schmulling RM. Clinical laboratory values during diabetic pregnancies. J Ayub Med Coll Abbottabad 2008; 20(1):3-6.
3   Larsson A, Palm M, Hansson LO, Basu S, Axelsson O. Reference values for alpha1-acid glycoprotein, alpha1-antitrypsin, albumin, haptoglobin, C-reactive protein, IgA, IgG and IgM during pregnancy. Acta Obstet Gynecol Scand 2008; 87(10):1084-1088.
4   Habib ZA. Maternal serum alpha-feto-protein: its value in antenatal diagnosis of genetic disease and in obstetrical-gynaecological care. Acta Obstet Gynecol Scand Suppl 1977; 61:1-92.:1-92.
5   Forestier F, Daffos F, Rainaut M, Bruneau M, Trivin F. Blood chemistry of normal human fetuses at midtrimester of pregnancy. Pediatr Res 1987; 21(6):579-583.
6   Mizejewski GJ. Levels of alpha-fetoprotein during pregnancy and early infancy in normal and disease states. Obstet Gynecol Surv 2003; 58(12):804-826.
7   Moniz CF, Nicolaides KH, Bamforth FJ, Rodeck CH. Normal reference ranges for biochemical substances relating to renal, hepatic, and bone function in fetal and maternal plasma throughout pregnancy. J Clin Pathol 1985; 38(4):468-472.
8   Takagi K, Tanaka H, Nishijima S, Masaoka N, Miyake Y, Sakata H et al. Fetal blood values by percutaneous umbilical blood sampling. Fetal Ther 1989; 4(2-3):152-160.
9   Weiner CP, Sipes SL, Wenstrom K. The effect of fetal age upon normal fetal laboratory values and

venous pressure. Obstet Gynecol 1992; 79(5 (Pt 1):713-718.

10   Gozzo ML, Noia G, Barbaresi G, Colacicco L, Serraino MA, De SM et al. Reference intervals for 18 clinical chemistry analytes in fetal plasma samples between 18 and 40 weeks of pregnancy. Clin Chem 1998; 44(3):683-685.

11   Boston RW, Humphreys PW, Normand IC, Reynolds EO, Strang LB. Formation of liquid in the lungs of the foetal lamb. Biol Neonat 1968; 12(5):306-315.

12   Mescher EJ, Platzker AC, Ballard PL, Kitterman JA, Clements JA, Tooley WH. Ontogeny of tracheal fluid, pulmonary surfactant, and plasma corticoids in the fetal lamb. J Appl Physiol 1975; 39(6):1017-1021.

13   Olver RE, Schneeberger EE, Walters DV. Epithelial solute permeability, ion transport and tight junction morphology in the developing lung of the fetal lamb. J Physiol 1981; 315:395-412.:395-412.

14   Muller F, Dommergues M, Bussieres L, Lortat-Jacob S, Loirat C, Oury JF et al. Development of human renal function: reference intervals for 10 biochemical markers in fetal urine. Clin Chem 1996; 42(11):1855-1860.

15   Johnson MP, Corsi P, Bradfield W, Hume RF, Smith C, Flake AW et al. Sequential urinalysis improves evaluation of fetal renal function in obstructive uropathy. Am J Obstet Gynecol 1995; 173(1):59-65.

16   Awad H, el-Safty I, el-Barbary M, Imam S. Evaluation of renal glomerular and tubular functional and structural integrity in neonates. Am J Med Sci 2002; 324(5):261-266.

17   Gitlin D, Kumate J, Morales C, Noriega L, Arevalo N. The turnover of amniotic fluid protein in the human conceptus. Am J Obstet Gynecol 1972; 113(5):632-645.

18   Burghard R, Pallacks R, Gordjani N, Leititis JU, Hackeloer BJ, Brandis M. Microproteins in amniotic fluid as an index of changes in fetal renal function during development. Pediatr Nephrol 1987; 1(4):574-580.

19   Queenan JT, Gadow EC, Bachner P, Kubarych SF. Amniotic fluid proteins in normal and Rh-sensitized pregnancies. Am J Obstet Gynecol 1970; 108(3):406-414.

20   Wolf PL, Bloch D, Tsudaka T. Biochemical profile of amniotic fluid to assess fetal maturity. Clin Chem 1970; 16(10):843-844.

21   Fennefrohn B. [Examinations of the amniotic fluid in the last weeks of pregnancy. 3. Determination of calcium, anorganic phosphorus, calcium-phosphorus quotient, albumin, and total proteins]. Z Geburtshilfe Perinatol 1974; 178(3):183-190.

22   Legras B, Esvant JY, Mention JE, Cloarec L. [Alterations in the proteins found in the amniotic fluid in the course of normal pregnancy. A study carried out by immunoprecipitation tests on the amniotic fluid (author's transl)]. J Gynecol Obstet Biol Reprod (Paris) 1978; 7(4):793-800.

23   Oliveira FR, Barros EG, Magalhaes JA. Biochemical profile of amniotic fluid for the assessment of fetal and renal development. Braz J Med Biol Res 2002; 35(2):215-222.

24   Pasman SA, Sikkel E, Le CS, Oepkes D, Roelandse FW, Vandenbussche FP. Bilirubin/albumin ratios in fetal blood and in amniotic fluid in rhesus immunization. Obstet Gynecol 2008; 111(5):1083-1088.

25   Hassan MI, Kumar V, Singh TP, Yadav S. Proteomic analysis of human amniotic fluid from Rh(-) pregnancy. Prenat Diagn 2008; 28(2):102-108.

26   Cho CK, Shan SJ, Winsor EJ, Diamandis EP. Proteomics analysis of human amniotic fluid. Mol Cell Proteomics 2007; 6(8):1406-1415.

27   Brace RA. Physiology of amniotic fluid volume regulation. Clin Obstet Gynecol 1997; 40(2):280-289.

28   Sutcliffe RG. The nature and origin of the soluble protein in human amniotic fluid. Biol Rev Camb Philos Soc 1975; 50(1):1-33.

29   Seppala M, Ruoslahti E, Tallberg T. Genetical evidence for maternal origin of amniotic fluid proteins. Ann Med Exp Biol Fenn 1966; 44(1):6-7.

30   Massi G, Cotumaccio R, Orsini G, Bellati U, Noia G, Auconi P. Evidence for the maternal origin of

4

alpha1-antitrypsin in amniotic fluid. J Pediatr 1981; 99(6):961-962.

31   Fasano M, Curry S, Terreno E, Galliano M, Fanali G, Narciso P et al. The extraordinary ligand binding properties of human serum albumin. IUBMB Life 2005; 57(12):787-796.

32   Quinlan GJ, Martin GS, Evans TW. Albumin: biochemical properties and therapeutic potential. Hepatology 2005; 41(6):1211-1219.

33   Jauniaux E, Gulbis B, Jurkovic D, Campbell S, Collins WP, Ooms HA. Relationship between protein concentrations in embryological fluids and maternal serum and yolk sac size during human early pregnancy. Hum Reprod 1994; 9(1):161-166.

34   Jones CT, Rolph TP. Metabolism during fetal life: a functional assessment of metabolic development. Physiol Rev 1985; 65(2):357-430.

35   Zhang H, Miao Z, He Z, Yang Y, Wang Y, Feng M. The existence of epithelial-to-mesenchymal cells with the ability to support hematopoiesis in human fetal liver. Cell Biol Int 2005; 29(3):213-219.

36   Naval J, Calvo M, Laborda J, Dubouch P, Frain M, Sala-Trepat JM et al. Expression of mRNAs for alpha-fetoprotein (AFP) and albumin and incorporation of AFP and docosahexaenoic acid in baboon fetuses. J Biochem 1992; 111(5):649-654.

37   Touboul C, Boulvain M, Picone O, Levaillant JM, Frydman R, Senat MV. Normal fetal urine production rate estimated with 3-dimensional ultrasonography using the rotational technique (virtual organ computer-aided analysis). Am J Obstet Gynecol 2008; 199(1):57-5.

38   Johnson AM, Umansky I, Alper CA, Everett C, Greenspan G. Amniotic fluid proteins: maternal and fetal contributions. J Pediatr 1974; 84(4):588-593.

39   Rapola J. Why is congenital nephrotic syndrome associated with a rise in the concentration of alpha-fetoprotein in the amniotic fluid? Pediatr Nephrol 1990; 4(2):206.

40   Hogge WA, Hogge JS, Schnatterly PT, Sun CJ, Blitzer MG. Congenital nephrosis: detection of index cases through maternal serum alpha-fetoprotein screening. Am J Obstet Gynecol 1992; 167(5):1330-1333.

41   Brace RA, Wlodek ME, Cock ML, Harding R. Swallowing of lung liquid and amniotic fluid by the ovine fetus under normoxic and hypoxic conditions. Am J Obstet Gynecol 1994; 171(3):764-770.

42   Bland RD. Pathogenesis of pulmonary edema after premature birth. Adv Pediatr 1987; 34:175-221.:175-221.

43   Olver RE, Walters DV, Wilson M. Developmental regulation of lung liquid transport. Annu Rev Physiol 2004; 66:77-101.:77-101.

44   Kalache KD, Chaoui R, Marks B, Wauer R, Bollmann R. Does fetal tracheal fluid flow during fetal breathing movements change before the onset of labour? BJOG 2002; 109(5):514-519.

45   Harding R, Hooper SB. Regulation of lung expansion and lung growth before birth. J Appl Physiol 1996; 81(1):209-224.

46   Hakguder G, Ates O, Olguner M, Riza SA, Akgur FM. Is induction of fetal diuresis with intraamniotic furosemide effective for the removal of intestinal waste products from amniotic fluid? Eur J Pediatr Surg 2002; 12(5):293-298.

47   Abramovich DR, Gray ES. Physiologic fetal defecation in midpregnancy. Obstet Gynecol 1982; 60(3):294-296.

48   Potier M, Melancon SB, Dallaire L. Developmental patterns of intestinal disaccharidases in human amniotic fluid. Am J Obstet Gynecol 1978; 131(1):73-76.

49   Mulivor RA, Mennuti MT, Harris H. Origin of the alkaline phosphatases in amniotic fluid. Am J Obstet Gynecol 1979; 135(1):77-81.

50   Pocknee RC, Abramovich DR. Origin and levels of trypsin in amniotic fluid throughout pregnancy. Br J Obstet Gynaecol 1982; 89(2):142-144.

51   Touloukian RJ. Composition of amniotic fluid with experimental jejuno-ileal atresia. J Pediatr Surg 1977; 12(3):397-402.

52   Parkin FM, Lind T, Cheyne GA. Biochemical and cytological changes in liquor amnii with advancing

gestation. J Obstet Gynaecol Br Commonw 1969; 76(8):673-683.

53 Wang T, Schneider J. Location of anions in human amnion. Evidence for a non-placental route for the transfer of proteins into amniotic fluid. Arch Gynecol 1982; 231(4):269-277.

54 Schmidt W. The amniotic fluid compartment: the fetal habitat. Adv Anat Embryol Cell Biol 1992; 127:1-100.:1-100.

55 Evans NJ, Rutter N. Development of the epidermis in the newborn. Biol Neonate 1986; 49(2):74-80.

56 Hashimoto K, Gross BG, DiBella RJ, Lever WF. The ultrastructure of the skin of human embryos. IV. The epidermis. J Invest Dermatol 1966; 47(4):317-335.

57 Parmley TH, Seeds AE. Fetal skin permeability to isotopic water (THO) in early pregnancy. Am J Obstet Gynecol 1970; 108(1):128-131.

58 Gilbert WM, Brace RA. The missing link in amniotic fluid volume regulation: intramembranous absorption. Obstet Gynecol 1989; 74(5):748-754.

59 Mann SE, Nijland MJ, Ross MG. Mathematic modeling of human amniotic fluid dynamics. Am J Obstet Gynecol 1996; 175(4 Pt 1):937-944.

60 Faber JJ, Anderson DF. Absorption of amniotic fluid by amniochorion in sheep. Am J Physiol Heart Circ Physiol 2002; 282(3):H850-H854.

61 Mann SE, Lee JJ, Ross MG. Ovine intramembranous pathway permeability: use of solute clearance to determine membrane porosity. J Matern Fetal Med 2001; 10(5):335-340.

62 Adams EA, Choi HM, Cheung CY, Brace RA. Comparison of amniotic and intramembranous unidirectional permeabilities in late-gestation sheep. Am J Obstet Gynecol 2005; 193(1):247-255.

63 Brace RA, Vermin ML, Huijssoon E. Regulation of amniotic fluid volume: intramembranous solute and volume fluxes in late gestation fetal sheep. Am J Obstet Gynecol 2004; 191(3):837-846.

64 Gesteland KM, Anderson DF, Davis LE, Robertson P, Faber JJ, Brace RA. Intramembranous solute and water fluxes during high intramembranous absorption rates in fetal sheep with and without lung liquid diversion. Am J Obstet Gynecol 2009.

65 Albuquerque CA, Nijland MJ, Ross MG. Human and ovine amniotic fluid composition differences: implications for fluid dynamics. J Matern Fetal Med 1999; 8(3):123-129.

66 Armstrong WD, Wilt JC, Pritchard ET. Vacuolation in the human amnion cell studies by time-- lapse photography and electron microscopy. Am J Obstet Gynecol 1968; 102(7):932-948.

67 Beall MH, van den Wijngaard JP, van Gemert MJ, Ross MG. Regulation of amniotic fluid volume. Placenta 2007; 28(8-9):824-832.

68 Malek A, Sager R, Lang AB, Schneider H. Protein transport across the in vitro perfused human placenta. Am J Reprod Immunol 1997; 38(4):263-271.

69 Takizawa T, Anderson CL, Robinson JM. A novel Fc gamma R-defined, IgG-containing organelle in placental endothelium. J Immunol 2005; 175(4):2331-2339.

70 Brace RA. Progress toward understanding the regulation of amniotic fluid volume: water and solute fluxes in and through the fetal membranes. Placenta 1995; 16(1):1-18.

71 Jauniaux E, Gulbis B. Fluid compartments of the embryonic environment. Hum Reprod Update 2000; 6(3):268-278.

72 Jacobsen GK, Jacobsen M, Henriksen OB. An immunohistochemical study of a series of plasma proteins in the early human conceptus. Oncodev Biol Med 1981; 2(6):399-410.

73 Wang T, Schneider J. Fine structure of human chorionic membrane. Ultrastructural and histochemical examinations. Arch Gynecol 1983; 233(3):187-198.

74 Bartels H, Wang T. Intercellular junctions in the human fetal membranes. A freeze-fracture study. Anat Embryol (Berl) 1983; 166(1):103-120.

75 Unadkat JD, Dahlin A, Vijay S. Placental drug transporters. Curr Drug Metab 2004; 5(1):125-131.

76 Chaudhury C, Brooks CL, Carter DC, Robinson JM, Anderson CL. Albumin binding to FcRn: distinct from the FcRn-IgG interaction. Biochemistry 2006; 45(15):4983-4990.

77 Leach JL, Sedmak DD, Osborne JM, Rahill B, Lairmore MD, Anderson CL. Isolation from human

4

placenta of the IgG transporter, FcRn, and localization to the syncytiotrophoblast: implications for maternal-fetal antibody transport. J Immunol 1996; 157(8):3317-3322.

78   Lambot N, Lybaert P, Boom A, ogne-Desnoeck J, Vanbellinghen AM, Graff G et al. Evidence for a clathrin-mediated recycling of albumin in human term placenta. Biol Reprod 2006; 75(1):90-97.

79   Lundgren S, Carling T, Hjalm G, Juhlin C, Rastad J, Pihlgren U et al. Tissue distribution of human gp330/megalin, a putative Ca(2+)-sensing protein. J Histochem Cytochem 1997; 45(3):383-392.

80   Kim J, Bronson CL, Hayton WL, Radmacher MD, Roopenian DC, Robinson JM et al. Albumin turnover: FcRn-mediated recycling saves as much albumin from degradation as the liver produces. Am J Physiol Gastrointest Liver Physiol 2006; 290(2):G352-G360.

81   Gitlin D, Kumate J, Urrusti J, Morales C. The selectivity of the human placenta in the transfer of plasma proteins from mother to fetus. J Clin Invest 1964; 43:1938-51.:1938-1951.

82   Dancis J, Lind J, Oratz M, Smolens J, Vara P. Placental transfer of proteins in human gestation. Am J Obstet Gynecol 1961; 82:167-71.:167-171.

83   Tomoda S, Brace RA, Longo LD. Fate of labeled albumin and erythrocytes following injection into amniotic cavity of sheep. Am J Physiol 1986; 251(4 Pt 2):R781-R786.

84   Usategui-Gomez M, Morgan DF, Toolan HW. A comparative study of amniotic fluid, maternal sera and cord sera by disc electrophoresis. Proc Soc Exp Biol Med 1966; 123(2):547-551.

85   Abbas TM, Tovey JE. Proteins of the liquor amnii. Br Med J 1960; 1(5171):476-479.

86   Sutcliffe RG, Brock DJ. Immunological studies on the nature and origin of the major proteins in amniotic fluid. J Obstet Gynaecol Br Commonw 1973; 80(8):721-727.

87   McKinnon B, Li H, Richard K, Mortimer R. Synthesis of thyroid hormone binding proteins transthyretin and albumin by human trophoblast. J Clin Endocrinol Metab 2005; 90(12):6714-6720.

88   Duc-Goiran P, Mignot TM, Robert B, Machavoine F, Mondon F, Hagnere AM et al. Expression and localization of alpha-fetoprotein mRNA and protein in human early villous trophoblasts. Placenta 2006; 27(8):812-821.

89   Sakuragawa N, Enosawa S, Ishii T, Thangavel R, Tashiro T, Okuyama T et al. Human amniotic epithelial cells are promising transgene carriers for allogeneic cell transplantation into liver. J Hum Genet 2000; 45(3):171-176.

90   Takashima S, Ise H, Zhao P, Akaike T, Nikaido T. Human amniotic epithelial cells possess hepatocyte-like characteristics and functions. Cell Struct Funct 2004; 29(3):73-84.

91   Tamagawa T, Oi S, Ishiwata I, Ishikawa H, Nakamura Y. Differentiation of mesenchymal cells derived from human amniotic membranes into hepatocyte-like cells in vitro. Hum Cell 2007; 20(3):77-84.

92   Baker ME. Albumin's role in steroid hormone action and the origins of vertebrates: is albumin an essential protein? FEBS Lett 1998; 439(1-2):9-12.

93   Cirelli N, Lebrun P, Gueuning C, ogne-Desnoeck J, Vanbellinghen AM, Graff G et al. Physiological concentrations of albumin stimulate chorionic gonadotrophin and placental lactogen release from human term placental explants. Hum Reprod 2001; 16(3):441-448.

94   Lambot N, Lebrun P, Cirelli N, Vanbellinghen AM, ogne-Desnoeck J, Graff G et al. Colloidal effect of albumin on the placental lactogen and chorionic gonadotrophin releases from human term placental explants. Biochem Biophys Res Commun 2004; 315(2):342-348.

95   Wintour EM, Shandley L. Effects of fetal fluid balance on amniotic fluid volume. Semin Perinatol 1993; 17(3):158-172.

96   Botos E, Klumperman J, Oorschot V, Igyarto B, Magyar A, Olah M et al. Caveolin-1 is transported to multi-vesicular bodies after albumin-induced endocytosis of caveolae in HepG2 cells. J Cell Mol Med 2008; 12(5A):1632-1639.

97   Lyden TW, Anderson CL, Robinson JM. The endothelium but not the syncytiotrophoblast of human placenta expresses caveolae. Placenta 2002; 23(8-9):640-652.

98   Cellini C, Xu J, Buchmiller TL. Effect of esophageal ligation on small intestinal development in normal and growth-retarded fetal rabbits. J Pediatr Gastroenterol Nutr 2006; 43(3):291-298.

99   Tarnok A, Mehes K. Gastrointestinal malformations, associated congenital abnormalities, and in-trauterine growth. J Pediatr Gastroenterol Nutr 2002; 34(4):406-409.

100 Blakelock R, Upadhyay V, Kimble R, Pease P, Kolbe A, Harding J. Is a normally functioning gastrointestinal tract necessary for normal growth in late gestation? Pediatr Surg Int 1998; 13(1):17-20.

101 Haggarty P. Placental regulation of fatty acid delivery and its effect on fetal growth--a review. Placenta 2002; 23 Suppl A:S28-38.:S28-S38.

102 Cleal JK, Lewis RM. The mechanisms and regulation of placental amino acid transport to the human foetus. J Neuroendocrinol 2008; 20(4):419-426.

103 Friesen R, Innis SM. Maternal dietary fat alters amniotic fluid and fetal intestinal membrane essential n-6 and n-3 fatty acids in the rat. Am J Physiol Gastrointest Liver Physiol 2006; 290(3):G505-G510.

104 Tsangaris G, Weitzdorfer R, Pollak D, Lubec G, Fountoulakis M. The amniotic fluid cell proteome. Electrophoresis 2005; 26(6):1168-1173.

105 Amoresano A, Andolfo A, Siciliano RA, Cozzolino R, Minchiotti L, Galliano M et al. Analysis of human serum albumin variants by mass spectrometric procedures. Biochim Biophys Acta 1998; 1384(1):79-92.

106 Carlson J, Sakamoto Y, Laurell CB, Madison J, Watkins S, Putnam FW. Alloalbuminemia in Sweden: structural study and phenotypic distribution of nine albumin variants. Proc Natl Acad Sci U S A 1992; 89(17):8225-8229.

107 Madison J, Galliano M, Watkins S, Minchiotti L, Porta F, Rossi A et al. Genetic variants of human serum albumin in Italy: point mutants and a carboxyl-terminal variant. Proc Natl Acad Sci U S A 1994; 91(14):6476-6480.

108 Bohlin K, Patterson BW, Spence KL, Merchak A, Zozobrado JC, Zimmermann LJ et al. Metabolic kinetics of pulmonary surfactant in newborn infants using endogenous stable isotope techniques. J Lipid Res 2005; 46(6):1257-1265.

4