Fetal fluid and protein dynamics
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This thesis describes our research on fetal pathophysiology in hemolytic anemia and hydrops fetalis. Measurements performed in fetal blood as well as in amniotic fluid, before or during intrauterine red blood cell transfusion, where used for our studies.

In chapter one, the motives leading to the studies presented in this thesis are described. As a tertiary fetal therapy centre, the LUMC provides a unique possibility to study fetal pathophysiology. Many prenatal potentially treatable diseases are accompanied by amniotic fluid volume abnormalities and the final stages are almost inevitably associated with hydrops fetalis. The final common pathway of these pathophysiological phenomenons is a shift in fluids and proteins between the different fetal compartments (intra- and extravascular). One specific question that arose from clinical practice was to understand the background of the diagnostic use of the bilirubin concentration in amniotic fluid to predict fetal anemia. Another question was, to understand the fetal reaction to severe anemia and intrauterine blood transfusions, and thus to improve the safety of this procedure. Background information is presented about fetal hemolytic anemia, the diagnosis of fetal anemia, intrauterine transfusion and hydrops fetalis.

In chapter two, in order to understand the Queenan or Liley chart, and thus the relation between fetal anemia and the rise in bilirubin concentration in amniotic fluid, we studied the relationship between the bilirubin concentration in amniotic fluid and in fetal blood. In 68 nonhydropic fetuses that received a first intrauterine blood transfusion, we compared amniotic fluid, taken shortly before transfusion, with the initial blood sample taken at the commencing of transfusion. All fetuses were anemic and had an increase in bilirubin concentration in their blood, although there was not a strong correlation between this concentration and the severity of the anemia. Most important finding was that the amniotic fluid/ fetal blood ratio of bilirubin in these affected cases was in accordance with the amniotic fluid/ fetal blood ratio of normal physiological bilirubin values. This amniotic fluid/ fetal blood ratio was dependent on gestational age. This can be explained by the fact that this ratio is determined by the binding of unconjugated bilirubin to albumin and thus by the amniotic fluid/ fetal blood ratio of albumin, during gestation. We hypothesized that of all the possible pathways, it is most plausible that bilirubin exchanges between fetal blood and amniotic fluid over the intramembraneous pathway. Exception to the normal amniotic fluid/ fetal blood ratio of the bilirubin concentration is described in 6 severely hydropic fetuses.
In **chapter three**, we tested our hypothesis that the bilirubin concentration in amniotic fluid is determined by both the bilirubin concentration in fetal blood and the albumin concentration in fetal blood and in amniotic fluid. We measured bilirubin and albumin concentration in fetal blood and in amniotic fluid in 30 fetuses that received a first intrauterine blood transfusion. A strong correlation was found between the bilirubin to albumin ratio (BAR) in fetal blood and that in amniotic fluid, confirming our hypothesis. The BAR in fetal blood was consequently higher than the BAR in amniotic fluid though, possibly due to a difference in binding capacity of albumin in amniotic fluid compared to blood, for example due to a difference in pH. The slope in Queenan’s and Liley’s chart that has been used for years to predict the severity of fetal anemia, can now be understood: it can be explained by the corresponding slope of the albumin concentration in amniotic fluid during gestation. Exception to the consequent relation between the BAR in fetal blood and the BAR in amniotic fluid is described in 3 severely hydropic fetuses.

In **chapter four**, we focused on the question what the origin of albumin is in the amniotic fluid. We reviewed the literature to assess the available evidence on the fetal, the maternal, and the placental or membrane origin of amniotic fluid albumin. Also, we speculated on the function of albumin in amniotic fluid. From the available evidence, we concluded that a fetal contribution is minimal in second and third trimester, mainly because of the low concentration of protein in fetal urine and lung fluid. A maternal contribution seems plausible, since other large proteins in amniotic fluid were already shown to be of maternal origin. Transfer can take place directly through the fetal membranes. Finally, the amniotic membrane itself also contributes to amniotic fluid albumin, although it is not known in what quantity. The concentration of amniotic fluid albumin may influence the volume of amniotic fluid through maintenance of osmotic pressure or through regulation of receptors involved in amniotic fluid amount and composition. Albumin could have an important role as a carrier protein, for example for fatty acids. Considering its non-fetal origin it could also be an important addition to prenatal transplacental nutrition. Different suggestions are proposed to investigate the origin, the transport mechanisms, and the function of albumin in amniotic fluid.

In **chapter five**, we studied the role of a low albumin concentration in fetal blood on the development of hydrops fetalis. Data was collected from 224 fetuses that received a first intrauterine blood transfusion due to Rh-D alloimmunization. We included 161 nonhydropic, 44 mildly hydropic and 19 severely hydropic fetuses. Relative hemoglobin deficit and relative albumin deficit, both corrected for gestational age, were determined.
at commencing of the procedure. A decrease in albumin concentration occurred only at a hemoglobin deficit below 8 standard deviations below the normal mean. Although the percentage of fetuses with a low albumin concentration was highest in severely hydropic fetuses (63%), it was much less in mildly hydropic fetuses (14%) and also occasionally present in nonhydropic fetuses (6%). Overall 73% of the hydropic fetuses had a normal albumin concentration. It therefore was concluded that a low albumin concentration in fetal blood is most likely a secondary effect and not the initial cause of hydrops in fetal anemia. In our study, both a low concentration of albumin and presence of severe anemia were independently predictive for the presence of hydrops.

In chapter six, the relationship between severity of anemia or presence of hydrops and the fetoplacental blood volume was assessed. We calculated fetal total blood volume in 86 fetuses that received a first intrauterine blood transfusion. The blood volume was calculated on the basis of a dilutional principle, namely of the fetal hemoglobin that was present at the beginning of the transfusion with the adult (donor) hemoglobin. The average fetal total blood volume was 123 ml/kg in nonhydropic fetuses and 144 ml/kg in hydropic fetuses. There was no relation between severity of anemia and blood volume, corrected for fetal weight. This implies that fetuses maintain their blood volume even in severe anemia and compensate for the loss of red cells with an equal increase in plasma volume. In hydropic fetuses, there even seems to take place an overcompensation of plasma volume. This is in accordance with the hypothesis that congestive heart failure plays a role in the pathophysiology of hydrops in anemic fetuses.

In chapter seven, we aimed to study the fluid shift that takes place out of the fetal circulation during an intrauterine blood transfusion. The effect of volume and speed of transfusion, and the severity of anemia and presence of hydrops were analyzed. In 95 fetuses, we calculated fluid shift at first intrauterine transfusions, by determining initial and final blood volumes. We found that on average 36% of the transfused volume already leaves the circulation during transfusion. Volume of fluid shift was related to the volume of donor blood that was administered. The fluid shift was however inversely related to the speed of transfusion, implying that this shift takes time, making it probable that this process continues in the hours after the transfusion. Severity of anemia and presence of hydrops surprisingly had no evident effect on the amount of fluid shift, possibly because they can have different contradictory effects on the cardiovascular system. Further, we found that at low gestational age, fetuses had been unintentionally burdened with relative high volume and speed of
transfusion. Transfusion policy should therefore be adjusted to gestational age. It should also be considered that the hematocrit still increases after transfusion, potentially leading to unintentional high hematocrit values and hyperviscosity of the fetal blood during the days following transfusion.

In chapter eight, the new insights acquired in this thesis are summarized. Among others, the differences between the fluid and protein dynamics in fetuses and in neonates are discussed. Our findings led to several implications for current practice. Finally, proposals for future research are described.