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State of the Art

Amniotic Fluid: Not Just Fetal Urine Anymore

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Amniotic fluid (AF) is a complex substance essential to fetal well-being. This article reviews recent discoveries and the current understanding of the origin and circulation of AF and its nutritive, protective, and diagnostic functions. Future directions for AF research are also discussed.
Journal of Perinatology **25,** 341–348. doi:10.1038/sj.jp.7211290

INTRODUCTION

Amniotic fluid (AF) is a marvelously complex and dynamic milieu that changes as pregnancy progresses. AF contains nutrients and growth factors that facilitate fetal growth, provides mechanical cushioning and antimicrobial effectors that protect the fetus, and allows assessment of fetal maturity and disease. This article will review the development, content, and clinical significance of AF and its essential role in helping the fetus become a newborn.

DEVELOPMENT OF AF AND THE AF CIRCULATION

A fluid-filled extracelomic cavity which will eventually become the amnionic space is identified near the time of implantation, even before the embryo is recognizable. During embryogenesis, AF volume increases faster than embryonic size. The water in AF originally comes from maternal plasma and passes through the fetal membranes based on hydrostatic and osmotic forces. As the placenta and fetal vessels develop, water and solute from maternal plasma pass across the placenta to the fetus and then to the AF. In the early fetal period, AF volume and fetal size are related in a linear fashion. AF volume increases from about 25 ml at 10 weeks to about 400 ml at 20 weeks. During this period, AF composition is similar to fetal plasma. There is rapid bi-directional diffusion between the fetus and the AF across the not-yet-keratinized fetal skin and the surfaces of the amnion, placenta, and umbilical cord, each being freely permeable to water and solutes. During this phase of pregnancy, the AF serves both as a physiologic buffer and an extension of the fetal extracellular compartment. By 8 weeks of gestation, the urethra is patent and the fetal kidneys make urine. Shortly thereafter fetal swallowing begins; however, neither fetal urination nor swallowing contributes significantly to the content or volume of AF until the second half of pregnancy. Keratinization of fetal skin begins at 19 to 20 weeks of gestation and is usually complete at 25 weeks after conception. When keratinization is complete, the relationship between fetal size and AF volume is no longer linear. By 28 weeks of gestation, AF volume reaches a volume of $\sim$800 ml where it plateaus near term gestation and thereafter declines to $\sim$400 ml at 42 weeks.[1]

After the fetal skin is fully keratinized, AF volume is determined by factors that comprise the AF circulation. Five pathways of exchange have been identified between the amniotic space and the surrounding tissues (see Figure 1). Production of AF is predominately accomplished by excretion of fetal urine ($\sim$300 ml/kg fetal weight/day or 600 to 1200 ml/day near term) and the secretion of oral, nasal, tracheal, and pulmonary fluids ($\sim$60 to 100 ml/kg fetal weight/day).[2] Fetal breathing movements contribute to the efflux of lung fluid into the AF, but about half of the effluent is swallowed rather than entering the AF. While volume changes with each fetal breath are small, $<$5 ml per breath, and fetal breathing occurs only for 20 to 30 min of each hour in late gestation, the overall contribution of fetal breathing to AF volume is significant. Removal of AF is predominately accomplished by fetal swallowing ($\sim$200 to 250 ml/kg fetal weight/day). Additionally, a significant intramembranous pathway transfers fluid and solutes from the amniotic cavity to the fetal circulation across the amniotic membranes.[3] The human amnion is a single layer of epithelial cells separating the amniotic cavity from the vascularized chorion. Early in gestation these amniocytes are flattened, but as pregnancy progresses they become cuboidal and have increasing numbers of microvilli on their apical surface. Tortuous intercellular channels exist between the tight junctions of amniocytes. The amount of fluid that passes through the intramembranous pathway is highly variable and has been estimated at 200 to 500 ml/day.[4] The transmembranous pathway, the movement of AF across the fetal membranes and into the maternal circulation within the lining of the uterus, affects AF volume only minimally. While this process has not been directly measured, it is estimated to be $\sim$10 ml/day at term.[2] Sherer[5] is an excellent review of AF dynamics.

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Journal of Perinatology 2005; 25:341–348

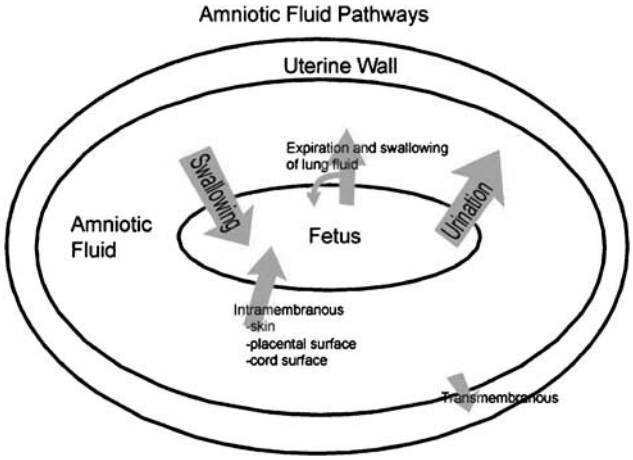

Figure 1. Amniotic fluid pathways.

The relative stability of AF volume in spite of large fluid shifts suggests that control mechanisms exist. It is noteworthy that only half of fetuses with esophageal atresia, and two-thirds of fetuses with duodenal or proximal jejunal atresia develop polyhydramnios; this suggests that other mechanisms besides swallowing are involved in AF volume regulation. Compensation via the intramembranous pathway is the best explanation for the significant number of fetuses with upper intestinal atresia who do not develop polyhydramnios. Compensation is evident in sheep where ligation of the esophagus leads to increased absorption of AF into the fetal circulation with no change in the total AF volume. Intramembranous absorption occurs against a hydrostatic gradient and had been assumed to be driven by passive diffusion due to an osmotic gradient. More recent studies show that passive diffusion accounts for only part of the intramembranous fluid absorption and that many solutes diffuse in the opposite direction (from fetus to AF). It is likely that much larger shifts of fluid and solutes occur by bulk transfer of AF with all of its dissolved solutes into the fetal circulation perhaps via a trans-cellular vesicular transport mechanism.[6] Vascular endothelial growth factor (VEGF) in the ovine fetal membranes appears to be a mediator of this process. VEGF promotes blood vessel development within the amnion and influences the permeability of the microvessels, which perfuse the fetal and placental surfaces.[7] The demonstration of aquaporin proteins in fetal membranes suggests the possibility of water channels as another potential regulator.[8]

Hormonal changes may also play a role in AF volume regulation. There are not significant numbers of receptors for estrogen or progesterone in fetal membranes after early pregnancy. Receptors for decidual prolactin, however, are widely expressed by both fetal and maternal tissues with increasing numbers as pregnancy progresses. There is evidence that decidual prolactin has an effect on amniotic permeability, although this is probably not the only hormonal or growth factor-related mechanism.[9]

Uterine perfusion also impacts AF volume. Maternal dehydration leads to increased fetal plasma osmolality and increased fetal production of arginine vasopressin. This causes an increase in the osmolality of both fetal urine and to a lesser extent AF. When arginine vasopressin is injected directly into ovine AF, fetal urine and AF osmolality increase and fetal urine output decreases significantly, and yet AF volume does not change suggesting reverse intramembranous flow from the isotonic fetal circulation to the hypertonic AF.[10]

The composition of AF changes with gestational age. In the second half of pregnancy, there is a decrease in sodium and chloride concentrations, an increase in urea and creatinine concentrations, and an overall decrease in AF osmolality. Many studies suggest that AF composition is more highly regulated than AF volume. Wintour and Shandley[4] has an excellent summary of these studies.

NUTRITIVE FUNCTIONS OF AF

AF contains carbohydrates, proteins and peptides, lipids, lactate, pyruvate, electrolytes, enzymes, and hormones. Prior to keratin production in fetal skin, amino acids diffuse from the placenta through the placental membranes into AF and from the fetal circulation through the fetal skin into AF. Later in pregnancy diffusion through the placental membranes persists and is augmented by fetal urinary excretion of amino acids.[11] Like breast milk, AF is rich in taurine which is found in greater quantity in AF than in maternal serum, while most other amino acids have lower concentrations in AF than in maternal and fetal blood. Glutamine is an essential precursor for nucleic acid biosynthesis in all cells and is particularly important in rapidly dividing cells such as intestinal mucosa cells. In fetal sheep, the uptake of glutamine from the AF by the fetal intestine is an active process.[12] Arginine also plays an essential role in fetal and placental development. Arginine is hydrolyzed to ornithine, which is then converted into the polyamines, putrescine, spermine, and spermidine, which are key regulators of placental angiogenesis, trophoblast growth, and embryogenesis. In sheep, the concentrations of arginine, ornithine, and polyamines increase rapidly in both allantoic and amniotic fluids early in gestation and remain elevated in AF throughout pregnancy. As gestational age increases, the swallowed polyamines in AF support proliferation and differentiation of intestinal epithelial cells.[13]

The role of swallowed carbohydrates and lipids in AF is less well defined. Growth-restricted rabbit fetuses were given infusions of dextrose or dextrose with amino acids directly into AF, and there was no improvement in growth, while an infusion of bovine AF did improve organ and somatic growth.[14] In a fetal rabbit model with esophageal ligation, the infusion of graded amounts of glucose or glucose with amino acids into AF enhanced organ weights and fetal

growth.[15] No studies have yet demonstrated reversal of fetal growth restriction (FGR) by intra-amniotic infusion of nutrient solutions.

Ligation of the esophagus in fetal rabbits to prevent swallowing followed by infusion of various solutions into the gut distal to the ligature has been performed to demonstrate the nutritive value of fetal swallowing. Those animals infused with lactated Ringer's solution had poor gut development whereas those infused with bovine AF showed more normal gut maturation.[16] Improved fetal organ growth with esophageal infusions of AF has also been shown in fetal sheep.[17] Trophic effects of AF have further been demonstrated on cultured human fetal small intestinal cells.[18] These studies suggest that growth factors found in AF, comparable to those in human milk, play a role in fetal growth and development. These trophic mediators are discussed below.

High levels of *epidermal growth factor* (EGF) are found in human milk and AF but not in standard infant formula. The concentration of EGF in amniotic fluid is four-fold higher than that found in fetal urine suggesting that the site of production is the amnionic membranes. EGF in human AF increases significantly during the second trimester, but is reduced in FGR. The function of EGF in the human fetus is largely unknown. In monkeys, in utero treatment with EGF improves lung maturity.[19] In fetal rabbits, enteral infusions of EGF reverse the effects of esophageal ligation.[16] EGF injected into the AF of pregnant rabbits increases small intestinal length and lactase and maltase activity compared to controls.[20] EGF receptors are present in the human stomach from the 18th week of gestation onward.

Transforming growth factor alpha (TGF-α) has a structure similar to EGF and binds to the same receptor. TGF-α is present in AF and human milk and, like EGF, is found in higher concentrations in human milk from women delivering prior to 27 weeks of gestation compared to those delivering after 27 weeks.[21] TGF-α is also produced in the fetal intestine. Recombinant TGF-α elicits a synergistic trophic response on cultured intestinal cells when combined with recombinant EGF, insulin-like growth factor-1, fibroblastic growth factor, and hepatocyte growth factor, but the trophic response is not as strong as either AF or breast milk.[18] The amnion cells of the umbilical cord express EGF, TGF-α, and the functional EGF/TGF-α receptor suggesting the possibility of a regulating role of the amnion in fetal growth and development. EGF and TGF-α have also been shown to stimulate the production of surfactant components.

Transforming growth factor beta-1 (TGF-β1) is found in rat AF and human breast milk, but is found in human AF only during the late stages of gestation. TGF-β1 is believed to induce terminal differentiation of intestinal epithelial cells and to accelerate the rate of healing of intestinal wounds by stimulating cell migration. TGF-β1 may also stimulate IgA production. Thus, TGF-β1 may prepare the fetal intestine for the extrauterine environment that is experienced after parturition at term.

Insulin-like growth factor I (IGF-I) is found in human milk and AF. When infused into the esophagus of fetal sheep, IGF-I improves somatic growth, spleen weight, and bowel wall thickness compared to control.[22] A single injection of labeled IGF-I into ovine AF demonstrated sustained delivery of IGF-I from the AF to the fetal gut and then into the systemic circulation over a 7-day period.[23] IGF-I and IGF-II receptors, as well as insulin receptors, are found throughout the human neonatal gut. IGF-I in AF may also increase the uptake of swallowed glutamine by the ovine gut.[12]

Erythropoietin (EPO) is found in human AF, colostrum, and mature milk. In the neonatal rat, enteral EPO is absorbed, stimulates erythropoiesis, and is a trophic factor for intestinal growth. The role of swallowed EPO in the human fetus and neonate is not clear. It is puzzling that concentrations of EPO are significant in AF and actually increase in human milk with the length of breast feeding, yet EPO is not absorbed from the gastrointestinal tract even though it is protected from digestion in the stomach.[24] This suggests the possibility of a local intestinal effect.

Granulocyte colony-stimulating factor (G-CSF) is found in human AF. When given enterally to suckling mice G-CSF enhances intestinal growth, suggesting that swallowed G-CSF in AF, colostrums, and breast milk may act as a topical growth factor in the fetal and neonatal intestine.

PROTECTIVE ROLE OF AF

AF plays an important protective role by providing a supportive cushion allowing fetal movement and growth. The oligohydramnios sequence and its related fetal deformations demonstrate the importance of this protective cushion.

AF also has a significant defensive role as a part of the innate immune system. The innate immune system is the first line of defense against pathogens and includes anatomic and physiologic barriers, enzymes and antimicrobial peptides, as well as phagocytosis and release of proinflammatory mediators by neutrophils and macrophages. Many of the substances that comprise the innate immune system have been identified in AF and vernix and have been shown to have significant antimicrobial properties; these include the α-defensins [HNP1-3], lactoferrin, lysozyme, bactericidal/permeability-increasing protein, calprotectin, secretory leukocyte protease inhibitor, psoriasin [S100A7], and a cathelicidin [LL-37].[25–27] These potent antimicrobials show broad-spectrum activity against bacteria, fungi, protozoa, and viruses. Perhaps the most important of these are the α-defensins [HNP1-3], which are found in significant concentrations in AF of women without evidence of infection and likely originate from the fetal skin and lung. AF concentrations of HNP1-3 increase with preterm labor, preterm premature rupture of membranes (PPROM), and chorioamnionitis probably due to release from neutrophils.

Lactoferrin (LF) is a glycoprotein with two binding sites for ferric ion. LF is found in human milk and appears in human AF at 20 weeks gestation increasing in concentration with gestation. Elevated levels of LF have been noted with preterm labor and with amnionitis. In pregnancies complicated by intra-amniotic infection (IAF), LF is likely secreted by neutrophils in the AF and by amniotic cells. LF has both bacteriostatic activity, due to sequestration of iron which is then unavailable for microbial growth, and bacteriocidal activity, due to binding to bacterial outer membranes triggering release of lipopolysaccharide. Enzymatic digestion of LF at acid pH releases a potent cationic, microbicidal peptide called lactoferricin. Lactoferricin shows antimicrobial effects against viruses, protozoa, and fungi.[28] Lactoferrin levels decrease with the onset of term labor.

The activity of the ''cellular'' innate immune system within AF as a protective mechanism for the fetus is less well defined. The numbers of mononuclear phagocytes (i.e., monocytes, macrophages, histiocytes) in AF are limited in normal pregnancies, while their numbers are increased in fetuses with neural tube defects. Whether these macrophages are present to prevent infection because of a disruption of the fetal skin or as scavenger cells to clean up neural debris is uncertain. Neutrophils are not normally identified in the AF of healthy fetuses, but are useful as a marker of AF infection. These cells are fetal in origin and appear to originate in the fetal vessels of the chorionic plate. It is interesting that meconium stained AF shows chemotactic activity for neutrophils in utero, although the meconium itself is not the likely chemotactic factor.[29] Two hematopoietic growth factors, G-CSF and macrophage colony-stimulating factor (M-CSF), are found in AF of healthy term and preterm fetuses. G-CSF is elevated in the serum of women with subclinical chorioamnionitis, in the cord blood of neonates with infection, fetal distress, premature rupture of membranes, and meconium staining of AF, and in the AF, neonatal urine and neonatal bronchoalveolar fluid of newborns after IAI. Whether G-CSF and M-CSF actually play a preventive host defense role in the AF or are just excreted by-products of the immune response during infection is not known.

There may also be nonimmune components of AF that protect the fetus from injury. For example, amniotic fluid may protect the fetal gut from the effects of platelet activating factor (PAF). PAF is a potent vasoconstrictor and has been strongly implicated in the pathophysiology of necrotizing enterocolitis in preterm infants.[30] PAF levels in human AF are low throughout gestation, but at term, PAF content undergoes an eight-fold increase with the onset of labor. PAF is elevated in AF of preterm fetuses whose mothers have failed tocolysis as well as AF of complicated pregnancies. The major PAF degrading enzymes are platelet activating factor acylhydrolase and platelet activating factor acetyl transferase; both show activity in AF, although their exact role is still unclear.[31] In addition, significant amounts of polyamines are found in AF; these have a cationic charge and may play both a nutritive and an antimicrobial role.

AF AS A DIAGNOSTIC MEDIUM

Amniocentesis has been a valuable tool in assessing fetal well-being since the 1970s. The most common evaluation of AF in the US is assessment of fetal chromosomes. Amniocentesis is commonly offered to women who will be at least 35 years of age at the time of full-term delivery or who have other risk factors for a chromosomal abnormality. As the diagnosis of aneuploidy moves into the first trimester with ultrasound assessment of nuchal translucency and more useful maternal serum markers, the use of amniocentesis will decrease with a corresponding increase in chorionic villus sampling. Amniocentesis is also offered when a previous child has a chromosomal abnormality, a parent carries a balanced chromosomal rearrangement or an autosomal recessive disorder, a mother carries an X-linked disorder, or a major structural abnormality or group of anomalies is identified on ultrasound. Assessment of AF is also helpful in the prenatal diagnosis of neural tube defects and an impressive array of inborn errors of metabolism and hematologic and genetic diseases (excellent reviews can be found in Wilson[32] and Kramer and Cohen[33]).

Evaluation of AF bilirubin level based on optical density has been an important tool to predict the severity of fetal hemolysis in red-cell alloimmunized pregnancies. Currently, the combination of amniocentesis to assess optical density, Doppler flow studies of the intra-hepatic umbilical vein and the middle cerebral artery and fetal blood sampling by cordocentesis are recommended to closely monitor the isoimmunized anemic fetus.[34] Allele-specific polymerase chain reaction of AF fetal cells can also be used to identify fetuses at risk for hemolytic disease of the newborn due to minor blood group incompatibilities.[35]

AF assessment has been studied in patients with preterm labor and/or PPROM to investigate possible IAI. AF indicators suggestive of infection include elevated levels of matrix metalloproteinase (e.g., MMP-9), interleukins (e.g., IL-6 and IL-1β), tumor necrosis factor (TNF-α), G-CSF, elevated white blood cell count, low glucose, and the presence of bacteria identified by Gram stain or culture. When preterm labor occurs with intact membranes, the rate of documented IAI is consistently lower than when preterm labor occurs with PPROM. While routine amniocentesis in preterm labor/PPROM has not been shown to be effective in decreasing perinatal mortality, there is still disagreement as to its optimum role in identification of IAI. Amniocentesis has also been helpful in prenatal diagnosis of cytomegalovirus, toxoplasma and parvovirus B-19 infection; this has become particularly relevant with the increasing use of the polymerase chain reaction allowing earlier diagnosis.

Assessment of fetal lung maturity by determination of the lecithin/sphingomyelin ratio and/or the presence of phosphatidyl glycerol in AF has become a well-accepted procedure. The assessment of lamellar body counts in AF,[36] the surfactant to albumin ratio in AF,[37] and electrical conductivity of AF[38] have

more recently been proposed as potentially superior methods for evaluation of fetal lung maturity.

A search for substances in AF that indicate fetal well-being has been ongoing since the 1980s. Changes in levels of inhibin-related proteins in both maternal serum and AF throughout pregnancy have been proposed as indicators of good fetal health. While the studies are contradictory, elevated levels of inhibin-A and activin-A may be useful markers related to fetal well-being during pre-eclampsia, trisomy 21, preterm delivery, and intrauterine growth restriction.[39] More research in this area is needed. A recent review of evaluation of AF S100B protein concentration as an early marker for brain injuries and/or brain maturation also merits further study.[40]

WHEN AF BECOMES PROBLEMATIC

Human AF may also contain substances that are potentially harmful. Perhaps the most concerning AF contaminant is meconium. There is good evidence that defecation in utero is a universal phenomenon occurring occasionally in the second trimester and frequently in the third trimester.[41] This is the likely explanation for the presence of bile pigments and enteric enzymes in AF. Meconium-stained AF occurs in about 13% of live deliveries. Most of these babies do well without associated acidosis or clinical illness. The combination of perinatal asphyxia, passage of meconium, and fetal gasping may lead to meconium aspiration syndrome (MAS), a potentially life threatening pulmonary disease caused by the combination of mechanical obstruction, inflammatory response, disruption of surfactant function, and often pulmonary hypertension (a recent review of MAS is found in Gelfand et al.[42]). MAS is uncommon in preterm infants but when present is associated with an increased risk of intraventricular hemorrhage. Meconium may also play a role in stimulating bacterial growth in the AF, perhaps by serving as an exogenous iron source. A recent study found a correlation between the presence and severity of meconium-stained AF and the rates of both chorioamnionitis and endomyometritis.[43]

AF demonstrates an irritant effect to exposed neural tissue, particularly after 34 weeks gestation. The precise identity of the irritant(s) is unclear, but there are several candidates. Tissue factor (TF), a procoagulant and initiator of disseminated intravascular coagulation, is found in high concentrations in AF at term, while TF pathway inhibitor, a natural inhibitor of TF, is found in relatively low concentrations. It is likely that TF plays a significant role in the devastating effects of AF embolism.[44] Late in pregnancy, elevated levels of activin-A and inhibin-A stimulate production of prostaglandin E2. As noted above, AF of pregnancies with premature rupture of membranes contains elevated levels of inflammatory cytokines (e.g. IL-1, IL-6, TNF-α, and interferon gamma). Whether this represents a fetal immune response or a preparatory step for the initiation of labor is not yet clear. The presence of PAF in AF with the onset of labor has been previously noted. Late in gestation AF contains vernix. While vernix contains antimicrobial substances and may be a contributor in protecting the fetus from IAI, it also has potent inflammatory properties and has been implicated as a cause of maternal antenatal peritonitis.[45] TGF-β, present late in gestation, may also play a role as a potential irritant.[46]

AF plays a major role in the gastrointestinal inflammatory changes associated with gastroschisis. An aseptic peritonitis leads to a fibrous peel, which has also been referred to as perivisceritis. The result is edema and thickening of the serosa, subserosa, and submucosa. This process has been attributed to an increase in the concentration of urea and nitrogenenous products and a decrease in the sodium and osmolality of AF that occurs at $\sim$30 weeks of gestation. Gastrointestinal waste products (i.e., bilirubin, bile acids, and meconium) have been shown to be elevated in the AF of human gastroschisis patients.[47] and in animal models are partly responsible for the perivisceritis seen in gastroschisis.[48] Amnioinfusion[49] and serial amnioexchanges[47] have both been performed in an attempt to minimize gastrointestinal inflammatory changes with preliminary results that are encouraging.

OTHER INTERESTING ASPECTS OF AF

Human AF contains factors that appear to minimize scarring.[50] It is interesting that a fetal incision made early in gestation will heal without a scar whereas one made in late gestation heals with scar formation. Two theories predominate: the first is that hyaluronic acid, which is found in high levels in AF, inhibits collagen synthesis. This hyaluronic acid-rich environment is due to a relative lack of hyaluronidase in AF and to the presence of hyaluronic acid-stimulating factor in AF. In one study looking at the effect of AF on proteases important to wound healing, human AF was shown to enhance collagenase activity, but to inhibit activities of hyaluronidase, elastase, and cathepsin.[51] The second theory is that TGF-β, which is absent from AF early in gestation but present late in gestation, plays a major role in scar formation.[46] Disagreement remains as to whether healing occurs without scar formation during early pregnancy because of a favorable fetal environment (i.e., fetal serum and AF) or because of the properties of fetal skin.

AF has been investigated as a potential way to deliver therapeutic agents to the fetus. Instillation of antibiotics, thyroxine, nutrients (i.e., dextrose, amino acids, and lipids), glucocorticoids, growth factors, surfactants, and beta-adrenergic-receptor agonists directly into the AF for delivery to the fetal circulation by either fetal swallowing or via the intramembranous route has been tried with mixed results. A 1999 National Institutes

of Health (NIH) conference on AF biology superbly summarizes this field.[52]

Human AF also contains factors that alter metabolism of opiates. Placental opioid enhancing factor has been found in placentae and AF of rats, and in placentae of humans and dolphins.[53] In cows and rats, maternal ingestion of AF enhances opioid-mediated analgesia. This effect has not been studied in humans.

Human AF has been evaluated as a source for stem cells with initial encouraging results.[54] The potential to develop a noncontroversial source of stem cells may stimulate research in this area.

UNANSWERED QUESTIONS AND FUTURE DIRECTIONS FOR AF-RELATED RESEARCH

Many research questions about AF remain unanswered. The 1999 NIH conference sponsored by the National Institute of Child Health and Development reviewed the current understanding of AF biology and important future directions for research. The conference summary called for more research in the areas of polyhydramnios, oligohydramnios, AF pressure determinations, embryonic and early fetal kidney development and function, control of lung liquid secretion, development of fetal swallowing and gastrointestinal motility, the dynamics of intramembranous absorption at the cellular and molecular level, AF pharmacokinetics and the potential therapeutic use of the amniotic space, and computer and mathematical models of AF dynamics.[52]

The functions and significance of individual growth factors in human AF remain incompletely described. It is interesting to note that some infants with esophageal atresia have malabsorption of intestinal nutrients. Other infants have a well functioning gut at birth without having swallowed significant amounts of AF. This disparity suggests that there is a redundancy of mediators that promote fetal gut growth with some effectors being swallowed in AF, while others arrive via the hematogenous route. Investigators have speculated that components of AF may protect the preterm infant against NEC or enhance intestinal recovery when NEC is in its healing stages. Components of AF that may promote these effects include glutamine,[55] arginine,[56] EGF,[57] EPO,[58] PAF-AH,[59] and LF.[60] Could harvested or synthetic AF be used as an enteral infusion in the preterm neonate at risk for or recovering from NEC? Would scarring of the gut be decreased? A recent ''simulated AF'' containing G-CSF and EPO was fed enterally to human neonates and was ''well tolerated'' at a dose of 20 ml/kg/day.[61] A follow-up study by the same investigative group showed infants tolerated simulated AF as an initial feeding when they were recovering from NEC.[62]

The skin is a major barrier to bacterial infection except in very preterm infants. Whether harvested or synthetic AF could be used to bathe and protect the not-yet-keratinized skin of the extremely preterm neonate is an appealing question. There is also much to be learned about the immunoprotective properties of AF and whether these can be enhanced to prevent IAI. There is really little information regarding how the innate host defenses of AF interact with the adaptive immune system of the mother and fetus.

Can significant amounts of AF be harvested at elective caesarean section in non-laboring women without harm to the fetus? Would this harvested AF be safe and free of infectious agents or could AF be processed (e.g., pasteurization) to render it free of infectious agents without inactivation of the desired host defense molecules? Storage and processing of AF has been investigated.[63] It is unclear whether trophic factors in AF would survive processes such as pasteurization, freezing, and storage. Given the apparent ease with which the fetus can absorb large volumes of AF in utero, would babies who are unable to tolerate regular enteral feeding (e.g. short gut, lymphatic disruption sequence, gastroschisis) be able to tolerate enteral AF infusion and thus nourish and stimulate the mucosa and minimize villous atrophy? The value of early trophic feedings in preterm infants has been well established. It is also clear that human milk is superior to premature infant formulas for these feedings. Unfortunately, breast milk is not always available. Preterm infants for whom breast milk is not available might benefit from a formula containing growth factors like those in AF and/or human milk.

Finally, as the role of VEGF in control of human AF volume becomes clear, it may be feasible to assess the role of VEGF inhibitors (e.g. bevacizumab) in the treatment of oligohydramnios and the role of VEGF receptor agonists in the treatment of polyhydramnios.

SUMMARY

AF is a wonderfully complex and unique body fluid that nourishes and protects the fetus. Just as breast milk is the optimum beverage for the newborn, AF is the ideal, germ-free bath, cushion and liquor for the fetus. Based on the significant contributions of AF to fetal and neonatal health, additional research is needed to better understand its functions and correct its disorders.

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