Administration of autologous fetal membranes: Effects on the coagulation in pregnant mini-pigs

Georg A. Petroianu, MD; Linda M. Toomes, MD; Wolfgang M. Maleck; Cora Friedberg, MD; Wolfgang F. Bergler, MD; Roderich Rüfer, MD

Objective: **A hallmark of the so-called amniotic fluid embolism is the induction of coagulation defects. Entry of meconium-free autologous amniotic fluid into the circulation, however, is innocuous. Little is known about the true causative agent or agents. The purpose of this study was to assess the effects of homogenized autologous fetal membranes (FM) on the coagulation system in the mini-pig model.**

Design: **Laboratory study.**

Setting: **University institute animal laboratory.**

Subjects: **Six near-term pregnant, Göttingen-bred mini-pigs.**

Interventions: **After induction of general anesthesia, FM were collected from all animals by cesarean section. Animals received 2 g FM (shredded and suspended in lactated Ringer's solution) via an ear vein.**

Measurements: **Blood samples were taken from a central vein before administration (baseline), immediately after administration, every 10 mins until 90 mins after administration, and every 20 mins until 150 mins after administration. The following parameters were measured: platelets, partial thromboplastin time, prothrombin time index, fibrinogen, factors II, V, VII, VIII, IX, X, XI, antithrombin III, and protein C. The values relative to baseline in the FM group were compared with a historical control group by rank order test. A $p < .05$ was considered significant.**

Main Results: **In the FM group (compared with the control group), platelets were lower; partial thromboplastin time was prolonged; fibrinogen was lower; prothrombin time index was lower (ie, prothrombin time was prolonged); protein C and antithrombin III were lower; and activity levels of factors V and VII were lower. The levels of factors II, VIII, IX, X, and XI showed a trend toward lower activity in the FM group, but the differences were not statistically significant.**

Conclusions: **FM can activate coagulation in mini-pigs. The laboratory parameter changes seen are typical for disseminated intravascular coagulation. However, the full clinical picture of amniotic fluid embolism and disseminated intravascular coagulation could not be elicited despite the high dose of FM used. (Pediatr Crit Care Med 2000; 1:65–71)**

KEY WORDS: amniotic fluid embolism; coagulation; fetal membranes; mini-pigs; platelets; hemodynamics; factor activities

Amniotic fluid embolism (AFE) is a rare but devastating complication of pregnancy and birth. It is characterized by respiratory distress, circulatory collapse, hemorrhage, and severe coagulation defects (1). Its disastrous effects are reflected in the high maternal mortality, ranging from 61% to 86% (2, 3). Despite its rarity, AFE is one of the most common causes of direct maternal deaths (4, 5). In the search for any predisposing factors of AFE, Clark et al. (2) analyzed the National Registry, but no such factors could be identified. As the rarity and unpredictability of AFE preclude clinical studies, many investigators have tried to reproduce this syndrome in animal-based studies (6–20). Unfortunately, most of the studies are flawed by the use of homologous or even xenologous amniotic fluid in nonpregnant animals.

As the pathophysiological mechanisms are not yet completely understood, and detailed coagulation analyses are rare, a new model of AFE in mini-pigs was established in a previous study at our institute (21). To investigate the effects, native autologous amniotic fluid (including meconium) and clear supernatant after centrifugation (excluding meconium) were administered intravenously into pregnant mini-pigs. Both the native amniotic fluid and (to a much lesser extent) the clear supernatant caused an activation of coagulation in mini-pigs. As amniotic fluid might not be the only component entering the maternal circulation during childbirth, it remained to establish if another substance has the power to trigger AFE. As it is conceivable that fragments of fetal membranes (FM) can also enter the maternal circulation, we decided to investigate FM as a possible agent causing AFE.

The purpose of the study was to assess the effects of intravenous administration of FM on the coagulation system in anesthetized mini-pigs by measuring platelet count, partial thromboplastin time (PTT), prothrombin time index (PTI), fibrinogen, activities of factors II, V, VII, VIII, IX, X, XI and the natural anticoagulants antithrombin (AT) III and protein C.

MATERIALS AND METHODS

The study was done with the consent of the state animal care committee (Tierschutzkom-

From the Institute of Pharmacology (Drs. Petroianu, Toomes, and Rüfer), Department of Gynecology (Dr. Friedberg), and Department of Oto-rhino-laryngology (Dr. Bergler) of the University of Heidelberg at Mannheim, and Department of Anesthesiology (Mr. Maleck) of the Klinikum, Ludwigshafen, Germany.

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Address requests for reprints to: Georg Petroianu, MD, Institute of Pharmacology and Toxicology, University of Heidelberg at Mannheim, Maybachstrasse 14–16, D-68169 Mannheim, Germany. E-mail: petroia@rumms.uni-mannheim.de

mission Baden-Württemberg) according to German federal animal protection law (Tierschutzgesetz).

Six pregnant Göttingen mini-pigs (Ellegaard Göttingen Minipigs, Dalmose, Denmark), gestation day 105 out of 110, were used for the study.

All animals were anesthetized according to the "ZEro-STRess-ANaesthesia-Induction" (ZESTRANI) protocol (22).

Animals fasted for 12 hrs were premedicated with fentanyl (100 μg kg^{-1}) and clonidine (10 μg kg^{-1}) given orally on sugar cubes. Ketamine (10 mg kg^{-1}) and flunitrazepam (150 μg kg^{-1}) were then applied intramuscularly. Animals were weighed, connected to an electrocardiograph and a pulse oximeter, and preoxygenated by mask, and the left ear vein of each was cannulated (1 mm inner diameter). Additional narcotics and paralytics, fentanyl (5 μg kg^{-1}), lidocaine (1 mg kg^{-1}), and alcuronium (200 μg kg^{-1}), were given intravenously. The animals were orotracheally intubated with a 7-mm (inner diameter) tube and mechanically ventilated (Siemens Servo 900 C, semiopen system, Siemens-Elema, Erlangen, Germany). A F_{IO_2} of 50% with 0.4% to 1.0% halothane and ~50% N_2O was used. Respiratory rate was fixed at 15 breaths/min. Tidal volume was initially 10 mL kg^{-1} and later varied to achieve an arterial P_{CO_2} of between 30 and 40 mm Hg (4.0 and 5.3 kPa) (ie, normocapnia in pregnancy). If oxygen saturation fell <90%, the F_{IO_2} was increased.

Catheters were introduced into the left carotid artery and left external and internal jugular veins after surgical exposure. Arterial blood pressure was measured continuously via the carotid catheter, and central venous pressure was measured intermittently via the internal jugular catheter. A continuous administration of fentanyl (20 μg hr^{-1} kg^{-1}) was started. A rectal temperature probe was inserted as well. The monitoring (electrocardiograph, oximetry, arterial and venous pressure, and temperature) was done with a Sirecust 1260 monitor equipped with a Sirem module (Siemens, Erlangen, Germany). Oximetry was performed at the lower lip and/or the tail (23).

At the following 16 predefined times, blood was collected anaerobically from the carotid artery and jugular vein, measurements with the blood were performed, and the continuously monitored parameters were noted.

t1, after introduction of the catheters

t2, after administration of 500 mL Ringer solution before C-section

t3, before adminstration of FM

t4, 0 mins after administration of FM

t5–13, at 10 min intervals until 90 mins after administration of FM

t14–16, at 20 min intervals until 150 mins after adminstration of FM

Table 1. Hematocrit, arterial oxygen saturation and partial pressure of CO_2

Time (mins)	Hematocrit (%)	Arterial Oxygen Saturation (%)	Arterial P_{CO_2} (mm Hg)
Baseline	27 ± 2	99.8 ± 0.1	33.8 ± 5.1
0	28 ± 4	99.3 ± 1.2	36.6 ± 6.8
10	27 ± 2	99.8 ± 0.1	35.4 ± 6.0
20	26 ± 2	99.8 ± 0.1	34.7 ± 2.3
30	28 ± 3	99.8 ± 0.1	34.7 ± 4.0
40	27 ± 4	99.8 ± 0.1	36.9 ± 5.2
50	27 ± 4	99.8 ± 0.1	35.4 ± 5.3
60	28 ± 3	99.8 ± 0.1	35.7 ± 4.1
70	28 ± 3	99.7 ± 0.1	37.5 ± 3.1
80	28 ± 4	99.7 ± 0.1	36.6 ± 4.1
90	27 ± 3	99.8 ± 0.1	37.7 ± 1.7
110	28 ± 2	99.7 ± 0.2	37.7 ± 2.5
130	26 ± 2	99.7 ± 0.1	37.4 ± 3.8
150	26 ± 3	99.7 ± 0.1	38.2 ± 5.6

Time, time after administration of fetal membranes.

Table 2. Electrolytes

Time (mins)	Sodium (mmol/L)	Potassium (mmol/L)	Calcium (ion.) (mmol/L)
Baseline	148 ± 4	3.8 ± .2	1.38 ± .08
0	148 ± 5	4.0 ± .5	1.44 ± .06
10	148 ± 6	3.7 ± .2	1.44 ± .04
20	148 ± 4	3.6 ± .3	1.42 ± .06
30	149 ± 5	3.7 ± .3	1.41 ± .06
40	148 ± 5	3.7 ± .3	1.41 ± .06
50	148 ± 4	3.7 ± .4	1.40 ± .07
60	149 ± 5	3.8 ± .4	1.41 ± .05
70	149 ± 5	3.7 ± .3	1.38 ± .07
80	149 ± 5	3.7 ± .2	1.40 ± .05
90	150 ± 4	3.7 ± .2	1.41 ± .04
110	149 ± 4	3.7 ± .2	1.42 ± .04
130	151 ± 6	3.7 ± .2	1.42 ± .06
150	150 ± 5	3.8 ± .3	1.44 ± .04

Time, time after administration of fetal membranes.

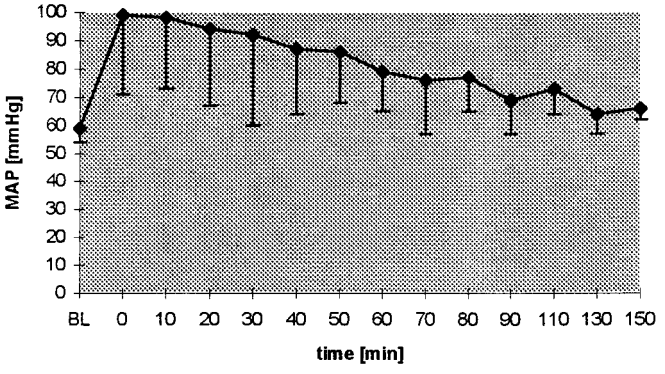

Figure 1. No statistical difference in blood pressure was found between the fetal membranes group and the historical control group. This result was attributable to the therapeutic protocol described in the text.

Arterial and venous blood gases and oxygen saturation were determined with an ABL 500 blood gas analyser/OSM 3 co-oximeter combination (Radiometer, Copenhagen, Denmark) (Table 1). Venous sodium and potassium were measured with a KNA 2 analyzer (Radiometer, Copenhagen, Denmark), and ionized calcium with an ICA 2 analyzer (Radiometer) (Table 2). Venous platelet counts were performed with a Coulter counter (Coulter Electronics, Krefeld, Germany), and the hematocrit was measured with a microhematocrit centrifuge Z 230 HA (Hermle, Welingen, Germany). Venous coagulation parameters (PTT, PTI, factors II, V, VII, VIII, IX, X, XI, AT III, protein C) were measured on a Chromotimer (Behringwerke, Marburg, Germany) (24, 25). At each time, a total of 15 mL of blood was withdrawn.

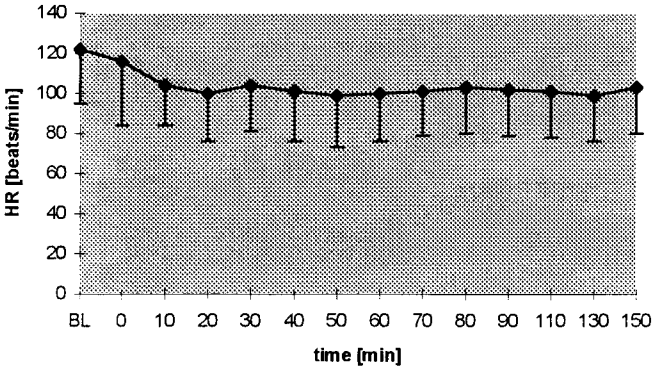

Figure 2. No statistical difference in heart rate was found between the fetal membranes group and the historical control group. This result was attributable to the therapeutic protocol described in the text.

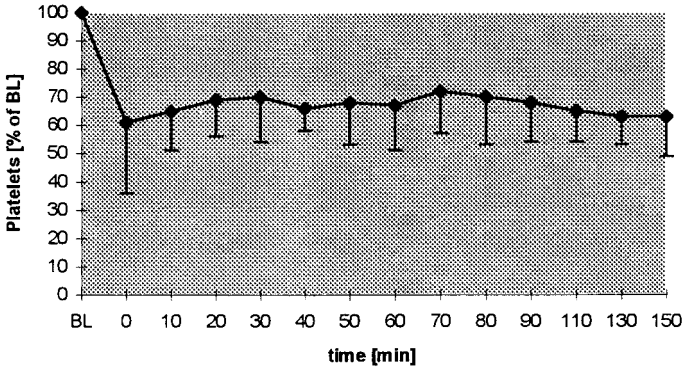

Figure 3. The platelets were significantly lower in the fetal membranes group compared with the control group ($p < .005$).

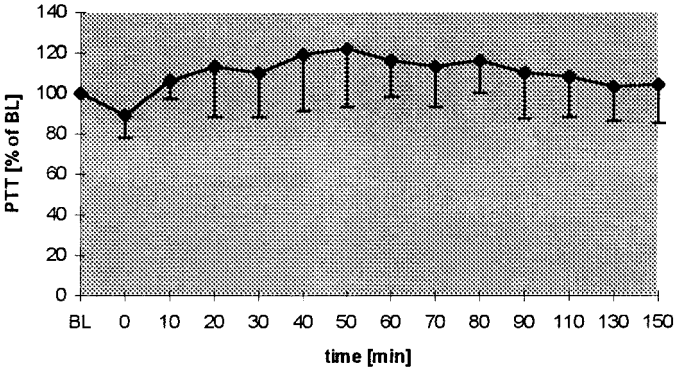

Figure 4. Partial thromboplastin time was significantly prolonged in the fetal membranes group compared with the control group ($p < .05$).

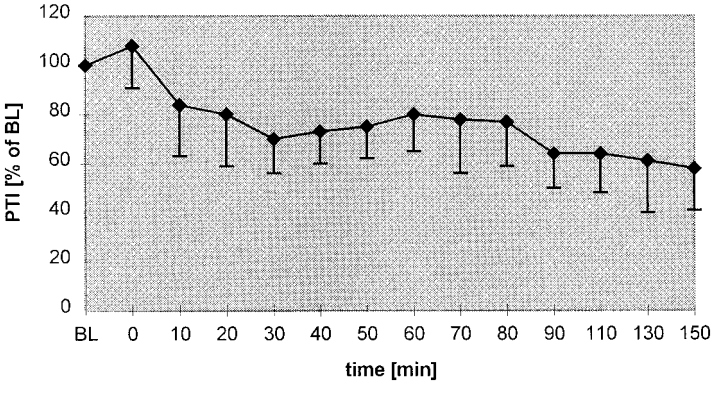

Figure 5. Prothrombin time index was significantly lower in the fetal membranes group ($p < .01$). Note that a lower prothrombin time index (European) is equivalent to a prolonged prothrombin time (American).

Fluids were replaced as an initial bolus of 500 mL lactated Ringer's followed by a regime that aimed to keep the hematocrit close to 75% of the value at t1. The mean arterial pressure (MAP) was allowed to vary between 60 mm Hg and 120 mm Hg. If MAP rose >120 mm Hg, magnesium sulfate up to 0.1 g kg^{-1} was slowly administered. If MAP fell <60 mm Hg, a commercially available (Akrinor, Asta Medica, Frankfurt/Main, Germany) mixture of theodrenalin (10 mg/Ampule) and cafedrin (200 mg/Ampule) was given, titrated to effect. If sufficient MAP was not achieved after one ampoule of Akrinor, inotropic support was instituted with dopamine 5–10 μg kg^{-1} min^{-1}. Heart rate was allowed to vary between 60 and 120 beats/min. If heart rate rose >120 beats/min, magnesium sulfate up to 0.1 g kg^{-1} was slowly administered. If heart rate fell <60 beats/min, atropin was given in 0.1 mg increments, titrated to effect.

FM were collected from each animal by cesarean section. Access to the uterus was gained via hypogastric midsection. The amniotic bags were exposed and opened by hysterotomy. The fetus was then killed by injection of potassium chloride, delivered, and discarded.

FM were shredded in a two-step process, first mechanically (Ultra-Turrax, Janke & Kunke, IKA-Werk, Staufen, Germany) and then ultrasonically (Potter-Elvejhem-Homogenisator, Braun, Melsungen, Germany).

The animals received 2 g shredded FM homogenized in 50 mL lactated Ringer's solution. Administration was done via the ear vein with a syringe pump (Perfusor, Braun, Melsungen, Germany). A historical model (26), this pump was used for the present study because it allows administration rates up to 600 mL hr^{-1}. Administration was started within 1 hr after the end of the cesarean section.

After the administration, animals were monitored and kept stable according to protocol for 150 mins. Finally, they were killed with potassium chloride.

For each animal and each coagulation parameter, the values at t4 to t16 were expressed as relative values of the value at t3 (baseline). All coagulation parameters are shown in Figures 1 – 13 as percentages of the baseline value.

For each animal and each parameter, the sum of the relative values for t4 to t16 was calculated. The six such sums for each parameter of the FM animals were compared with the six such sums for the (previous) control animals (21) by the two-sided Wilcoxon's rank order test for unpaired observations (27). Consequently, values at t1 and t2 were not subjected to statistical analysis. For parameters not included in our previous publication (fac-

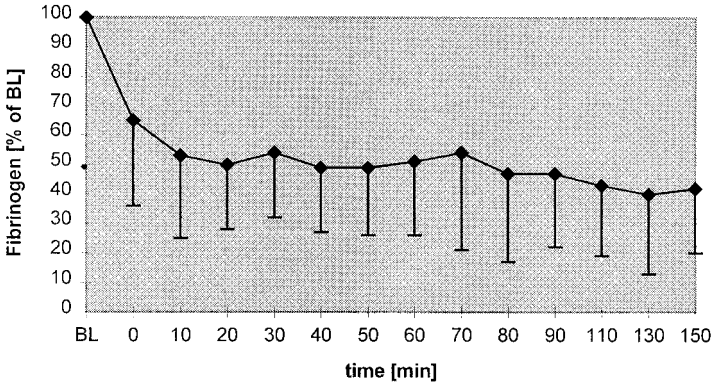

Figure 6. The fibrinogen levels decreased significantly in the fetal membranes group compared with the control group ($p < .02$).

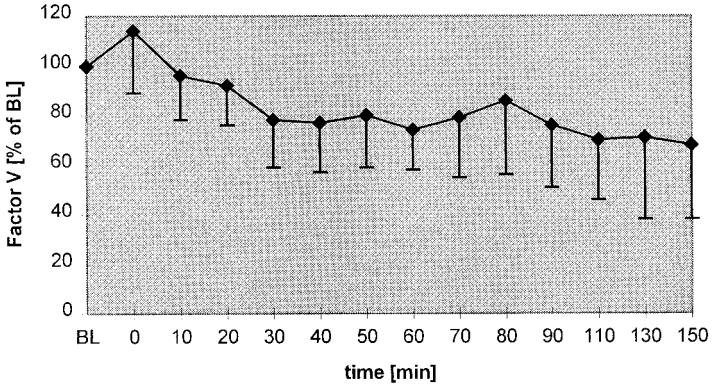

Figure 7. Factor V activity decreased significantly ($p < .01$) in the fetal membranes group as compared with the control group.

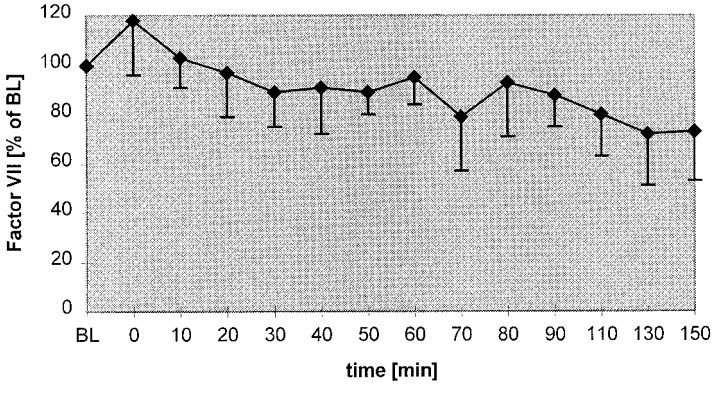

Figure 8. Factor VII activity decreased significantly ($p < .005$) in the fetal membranes group as compared with the control group.

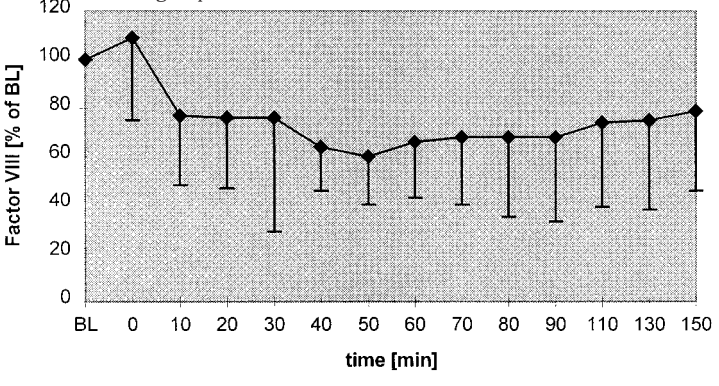

Figure 9. Factor VIII activities showed a decreasing trend in the fetal membranes group, but the difference from the control group was not statistically significant.

tors II, IX, X and XI), only descriptive statistics are given.

A Bonferroni correction was not applied. A difference of $p < .05$ was considered significant.

RESULTS

Weight of the animals was 26 ± 2 kg. Number of fetuses was 6 ± 3. All 6 animals survived until the end of the observation period. No statistical differences in blood pressure (Fig. 1), heart rate (Fig. 2), or hematocrit were found between the FM group and the historical control group. This result was because of the therapeutic protocol described above.

Coagulation Studies. The platelets were significantly lower in the FM group compared with the control group ($p < .005$) (Fig. 3). PTT was prolonged in the FM group compared with the control group ($p < .05$) (Fig. 4).

PTI was lower in the FM group ($p < .01$) (Fig. 5). Note that a lower prothrombin time index (European) is equivalent to a prolonged prothrombin time (American).

The fibrinogen levels decreased significantly in the FM group compared with the control group ($p < .02$) (Fig. 6). The factor V ($p < .01$) and VII ($p < .005$) activities decreased significantly in the FM group as compared with the control group (Figs. 7 and 8). The factor VIII activities showed a decreasing trend in the FM group, but the difference from the control group was not statistically significant (Fig. 9).

Factors II, IX, X and XI showed a decreased activity after FM administration; however, as these could not be compared with the control group from our previous study, these factor activities were not evaluated statistically (Figs. 10–13).

The coagulation inhibitors AT III ($p < .05$) and protein C ($p < .005$), decreased in the FM group as compared to the control group (Figs. 14 and 15).

DISCUSSION

The classic presentation of AFE is sudden dyspnea, cyanosis, and hypotension, often followed by cardiocirculatory arrest within minutes. In ~12% of cases, hemorrhage is the presenting sign (3). Coagulopathy is virtually always present, except in cases where the patients die before it can be assessed (2, 28, 29).

Although >50 animal studies on experimental amniotic fluid administration

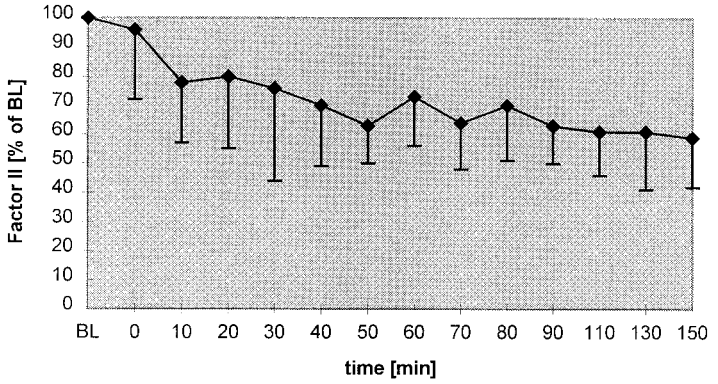

Figure 10. Factor II showed decreased activity after fetal membrane administration.

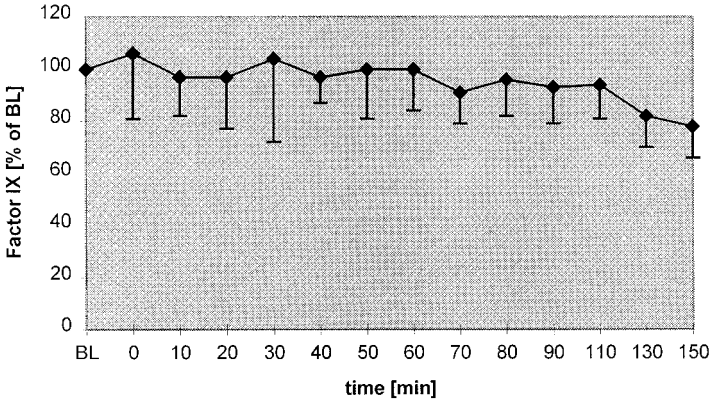

Figure 11. Factor IX showed decreased activity after fetal membrane administration.

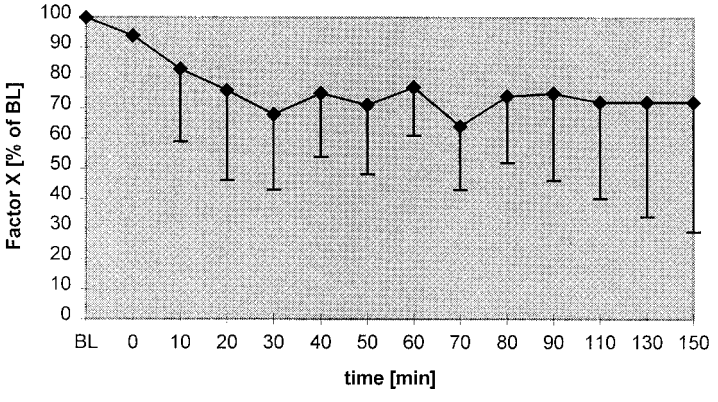

Figure 12. Factor X showed decreased activity after fetal membrane administration.

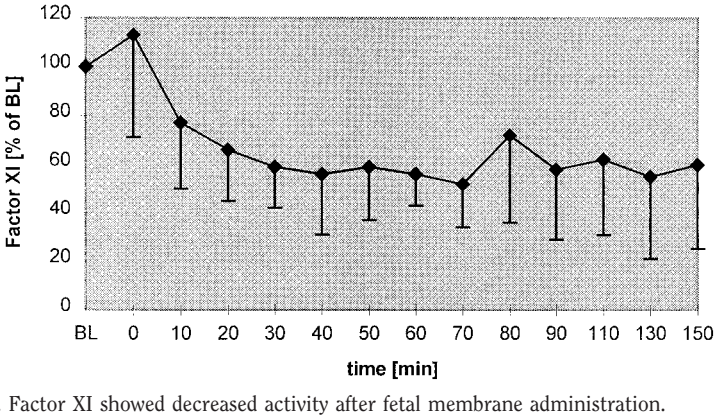

Figure 13. Factor XI showed decreased activity after fetal membrane administration.

Fetal membranes may play a role in amniotic fluid embolism, and fetal membranes may contain an agent that is released in amniotic fluid embolism and causes coagulation disorders like disseminated intravascular coagulation.

have been published (21), their results have often been conflicting. In the studies in our laboratory, the mini-pig was chosen because its coagulation system, anatomy, and physiology are more related to those of humans than are those of other experimental animals (24). However, the placentation in pigs is different from that of humans (30). Our model, like all models previously described, represents not the way in which amniotic fluid, meconium, FM, air, or other uterine content enters the circulation, but the consequences of their entry.

The design of our study included general anesthesia. Although anesthesia might influence the results, this setting assured a stress- and pain-free situation for the animals, good conditions for monitoring, and optimal treatment to achieve a high survival rate. In a recent study that used an anesthetic technique similar to ours, an influence on the coagulation was excluded (31). Furthermore, some human cases occur under anesthesia during cesarean section.

Because methods did not differ from earlier studies, and to spare animals, we did not include a prospective control group. This is a major limitation of our study.

Disseminated intravascular coagulation (DIC) is accepted as a typical feature of AFE, but little is known about its pathophysiology or the causative agents. In our previous study, which used native amniotic fluid stained with meconium and the clear supernatant after centrifugation, some coagulation disturbances similar to DIC were observed (21). However, the clinical picture of DIC, espe-

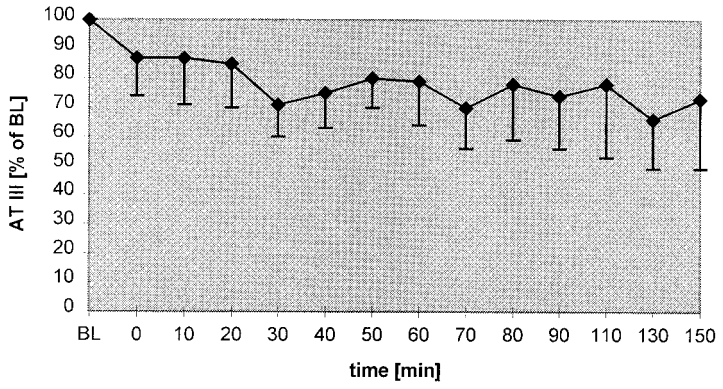

Figure 14. Coagulation inhibitor antithrombin III decreased in the fetal membranes group as compared with the control group ($p < .05$).

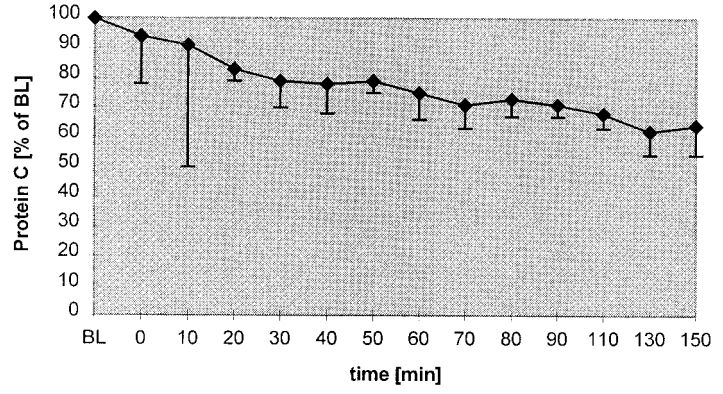

Figure 15. Coagulation inhibitor protein C decreased in the fetal membranes group as compared with the control group ($p < .005$).

cially the tendency toward bleeding from the surgical wounds in the neck and abdomen, could not be observed. Other clinical symptoms of AFE, like cyanosis, cardiovascular instability, and death, could only be observed in some of the animals administered with amniotic fluid that contained meconium. These findings support the idea that the entry of pure, autologous amniotic fluid into the circulation is an innocuous event (21, 32, 33).

We therefore reasoned that there might also be other substances involved in childbirth that trigger AFE. Pure venous air embolism as the sole pathophysiological mechanism triggering AFE can be excluded, as the coagulation disorders induced by it are much less pronounced (34). To our knowledge, FM has not been investigated as a causative agent of AFE.

To test the hypothesis that FM might have the power to induce DIC, one must be sure that the amount of FM is sufficient to provoke the disorders and one must exclude the possibility of false negative results as a result of underdosage. We therefore used the high dose of 2 g FM.

The use of pregnant pigs and fresh, autologous FM seemed preferable to us, as AFE occurring in humans always involves fresh autologous components. Homologous or even xenologous FM might induce intolerance reactions, which could have an unwanted influence on the results. Likewise, we used fresh FM (maximum storage time, 1 hr) to exclude storage effects.

The administration of FM triggered a response with the typical pattern seen in DIC: decreased platelets and fibrinogen, prolonged PTT, decreased PTI, and to some extent a consumption of coagulation factors. A significant decrease in protein C and AT III was also seen. These results are very similar to those previously reported after administration of amniotic fluid that contained meconium (21), except for a much less pronounced lengthening of the PTT after FM.

As all changes are consistent with DIC, FM may play a role in AFE, and FM may contain an agent that is released in AFE and causes coagulation disorders like DIC. However, there was no clinical bleeding or lethal outcome in any of the FM animals. Consequently, neither FM alone, nor amniotic fluid with or without meconium, nor air is sufficient to explain the full clinical picture of AFE. The clinical picture of AFE might thus be explained by a combination of pathomechanisms, an unknown factor, or a reaction in humans that is not reproducible in animals.

REFERENCES

1. Pritchard JA, Mac Donald PC (Eds): Williams Obstetrics, Sixteenth Edition. New York, Appleton-Century-Crofts, 1980, pp 519–520
2. Clark SL, Hankins GDV, Dudley DA, et al: Amniotic fluid embolism: Analysis of the national registry. *Am J Obstet Gynecol* 1995; 172:1158–1169
3. Morgan M: Amniotic fluid embolism. *Anesthesia* 1979; 34:20–32
4. Hibbard BM, Anderson MM, Drife JO, et al: Report on confidential enquiries into maternal deaths in the United Kingdom 1988–1990. London, HMSO, 1994, p 17
5. Hibbard BM, Anderson MM, Drife JO, et al: Report on confidential enquiries into maternal deaths in the United Kingdom 1991–1993. London, HMSO, 1996, p 18
6. Hankins GDV, Snyder RR, Clark SL, et al: Acute hemodynamic and respiratory effects of amniotic fluid embolism in the pregnant goat model. *Am J Obstet Gynecol* 1993; 168: 1113–1130
7. Adamsons K, Mueller-Heubach E, Myers RE: The innocuousness of amniotic fluid infusion in the pregnant rhesus monkey. *Am J Obstet Gynecol* 1971; 109:977–984
8. Attwood HD, Downing SE: Experimental amniotic fluid and meconium embolism. *Surg Gynecol Obstet* 1965; 120:255–262
9. Azegami M, Mori N: Amniotic fluid embolism and leukotrienes. *Am J Obstet Gynecol* 1986; 155:1119–1124
10. Cron RS, Kilkenny GS, Wirthwein C, et al: Amniotic fluid embolism. *Am J Obstet Gynecol* 1952; 64:1360–1363
11. Dutta D, Bhargava KC, Chakravarti RN, et al: Therapeutic studies in experimental amniotic fluid embolism in rabbits. *Am J Obstet Gynecol* 1970; 106:1201–1208
12. Halmagyi DFJ, Starzecki B, Shearman RP: Experimental amniotic fluid embolism: Mechanism and treatment. *Am J Obstet Gynecol* 1962; 84:251–256
13. Jaques WE, Hampton JW, Bird RM, et al: Pulmonary hypertension and plasma thromboplastin-antecedent deficiency in dogs: Experimental induction by infusion of amniotic fluid. *AMA Arch Pathol* 1960; 69:248–256
14. Kitzmiller JL, Lucas WE: Studies on a model of amniotic fluid embolism. *Obstet Gynecol* 1972; 39:626–627
15. MacMillan D: Experimental amniotic fluid infusion. *J Obstet Gynaecol Br Commonw* 1968; 75:849–852
16. Reeves JT, Daoud FS, Estridge M, et al: Pul-

monary pressor effects of small amounts of bovine amniotic fluid. *Respir Physiol* 1974; 20:231–237
17. Reis RL, Pierce WS, Behrendt DM: Hemodynamic effects of amniotic fluid embolism. *Surg Gynecol Obstet* 1969; 129:45–48
18. Schneider CL: Coagulation defects in obstetrical shock: Meconium embolism and heparin; fibrin embolism and defibrination. *Am J Obstet Gynecol* 1955; 69:758–775
19. Spence MR, Mason KG: Experimental amniotic fluid embolism in rabbits. *Am J Obstet Gynecol* 1974; 119:1073–1078
20. Stolte L, van Kessel H, Seelen J, et al: Failure to produce the syndrome of amniotic fluid embolism by infusion of amniotic fluid and meconium into monkeys. *Am J Obstet Gynecol* 1967; 98:694–697
21. Petroianu GA, Altmannsberger SHG, Maleck WH, et al: Meconium and amniotic fluid embolism: Effects on coagulation in pregnant mini-pigs. *Crit Care Med* 1999; 27:348–355
22. Petroianu GA, Altmannsberger SHG, Maleck W, et al: ZEro STRess Anesthesia Induction (ZESTRANI) in Göttingen minipigs. *Scand J Lab Anim Sci* 1998; 25(Suppl 1):109–115
23. Petroianu GA, Maleck W, Bergler WF, et al: Oximetry for amniotic fluid embolism detection in mini-pigs: Tail or snout ? *Zentralbl Veterinärmed A* 1996; 43:415–421
24. Petroianu G, Maleck W, Altmannsberger S, et al: Blood coagulation, platelets and haematocrit in male, female, and pregnant Göttingen minipigs. *Scand J Lab Anim Sci* 1997; 24:31–41
25. Kolde HJ: ChromoTime System-A new generation of coagulation analyses. *Behring Inst Mitt* 1985; 78:176–187
26. Schulte am Esch J, Goerig M: Anesthetic equipment in the history of German anesthesia. Dräger, Lübeck, 1997, pp 110–111
27. Dawson JD, Lagakos SW: Size and power of two-sample tests of repeated measures data. *Biometrics* 1993; 49:1022–1032
28. Chatelain SM, Quirk JG: Amniotic and thromboembolism. *Clin Obstet Gynecol* 1990; 33:473–481
29. Lau G, Chui PP: Amniotic fluid embolism: A review of 10 fatal cases. *Singapore Med J* 1994; 35:180–183
30. Lü J, Petroianu G, Widjaja B, et al: Transplacental kinetics of lead in pregnant mini-pigs. *Arch Toxicol* 1997; 71:187–192
31. Roussi J, Andre P, Samama M, et al: Platelet functions and haemostasis parameters in pigs: Absence of side effects of a procedure of general anesthesia. *Thromb Res* 1996; 81: 297–305
32. Tio AG: Contribucion al estudio y empleo del liquido amniotico. *Revista Peruana de Obstetricia* 1955; 3:84
33. Tio AG: Clinical use of amniotic fluid. *J Amer Med Ass* 1956; 161:996
34. Petroianu G, Jatzko A, Heil P et al: Massive Air Embolism (VAE) in mini-pigs: Monitoring the coagulation. *In*: Papers Presented at the Vth International Meeting on High-Pressure Biology, Bennett PB, Demchenko I, Marquis RE (Eds). Rochester, NY, University of Rochester, 1998, pp 281–287