Meconium-stained amniotic fluid

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Green-stained amniotic fluid, often referred to as meconium-stained amniotic fluid, is present in 5% to 20% of patients in labor and has been traditionally considered an obstetrical hazard. Discolored amniotic fluid has been attributed to the presence of heme catabolic products from the passage of fetal colonic content (meconium), intraamniotic bleeding, or both. The frequency of green-stained amniotic fluid increases as a function of gestational age, reflecting maturation of the gastrointestinal system, and reaches approximately 27% in postterm gestation.

Before the introduction of routine continuous fetal heart rate monitoring, green-stained amniotic fluid during labor was associated with fetal acidemia (umbilical artery pH $<$7.00), neonatal respiratory distress, and seizures, and was considered a risk factor for cerebral palsy. Hypoxia has been considered the main mechanism responsible for fetal defecation and meconium-stained amniotic fluid; however, most fetuses with meconium-stained amniotic fluid do not have fetal acidemia. Nonetheless, in the absence of fetal heart rate abnormalities, meconium-stained amniotic fluid is not associated with fetal acidemia. Intraamniotic infection/inflammation has emerged as an important factor in meconium-stained amniotic fluid in term and preterm gestations, and green-stained amniotic fluid is a risk factor for maternal and neonatal infections. Whether intraamniotic infection/inflammation results in discoloration of amniotic fluid via oxidative stress or the passage of meconium has not been determined. Two randomized clinical trials suggest that, in patients with meconium-stained amniotic fluid, intrapartum administration of antibiotics decreases the rate of clinical chorioamnionitis. Meconium aspiration syndrome is a severe complication typical of term newborns, which develops in 5% of cases presenting with meconium-stained amniotic fluid. Meconium aspiration syndrome is attributed to the mechanical and chemical effects of aspirated meconium coupled with local and systemic fetal inflammation. A systematic review of randomized controlled trials suggested that amnioinfusion may decrease the rate of meconium aspiration syndrome. Routine naso/oropharyngeal suctioning and tracheal intubation in cases of meconium-stained amniotic fluid have not been shown to be beneficial and are no longer recommended in obstetrical practice. Histologic staining of the membranes with meconium has been used in the context of medical legal litigation to attempt to time the occurrence of fetal injury. This has been largely based on the results of in vitro experiments. However, extrapolation of these findings to the clinical setting is unwarranted. Experimental studies in animals and observational studies in human fetuses suggest that fetal defecation is a physiological phenomenon throughout pregnancy.

Key words: discolored amniotic fluid, fetal colonic content, green-stained amniotic fluid, intraamniotic infection, intraamniotic inflammation

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Introduction

Green-stained amniotic fluid, often referred to as meconium-stained amniotic fluid (MSAF), has been considered an obstetrical hazard for centuries. It has become increasingly clear that not all green-stained amniotic fluid is attributable to meconium and that not all meconium is green. This article will review the composition of meconium, the clinical significance of MSAF and its implications for obstetrical management, and the pathophysiology of meconium aspiration syndrome (MAS), and will

TABLE 1
Meconium composition

- Water (70%–80%)
- Intestinal epithelial cells
- Squamous cells
- Vernix caseosa
- Fetal hair
- Amniotic fluid
- Bile pigments (eg, bilirubin, zinc-coproporphyrin)
- Bile acids (eg, chenodeoxycholic and cholic acids)
- Pancreatic enzymes
- Free fatty acids

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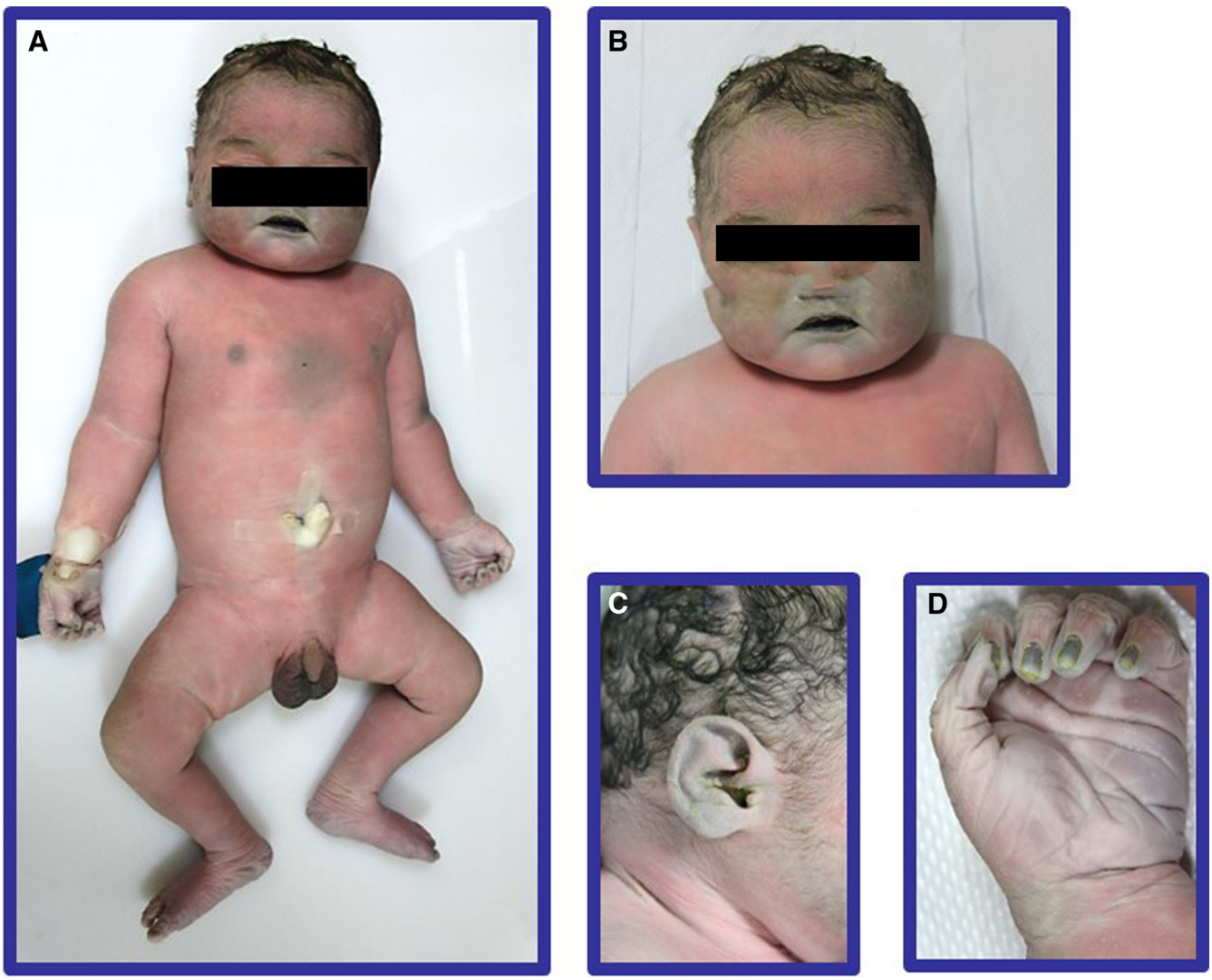
FIGURE 1
Meconium-stained neonate at 39+4 weeks of gestation



A, Yellow-greenish discoloration of fetal skin at different body sites. The evidence of peripheral cyanosis is shown in **B,** lips, **C,** ears, and **D,** fingertips. **C,** Meconium is also present in the ear canal.

Photo courtesy of Dr Sunil Jaiman.

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TABLE 2
Risk factors for meconium-stained amniotic fluid

- Postterm pregnancy
- Prolonged labor
- Clinical chorioamnionitis
- Fetal growth restriction
- Preeclampsia
- Oligohydramnios
- Vaginal breech delivery
- Maternal drugs (eg, cocaine, castor oil, bowel purgatives)
- Herbal substances (eg, "isihlambezo")
- Intrahepatic cholestasis of pregnancy

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close with some thoughts about the physiology of fetal defecation.

What is meconium?

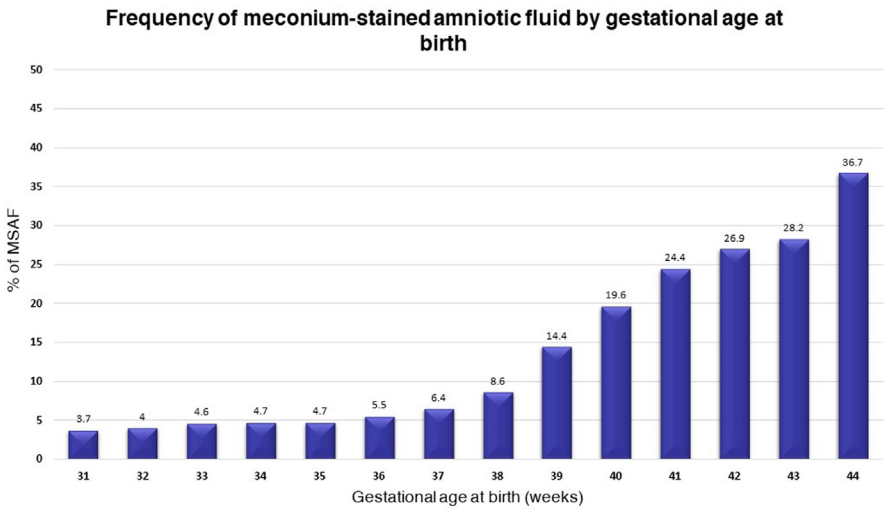
The word "meconium" is derived from the Greek word *mekoni*, which means "poppy juice" or "opium-like," referring to the belief that fetal exposure to meconium would lead to neonatal sleepiness or depression,[1–3] a concept generally attributed to Aristotle.[1,2,4]

Meconium is the fetal colonic content, which is mainly composed of water (72%–80%),[2] exfoliated skin cells, lanugo, vernix caseosa, and gastrointestinal secretions[5–16] (Table 1). The typical greenish-yellow color of meconium is attributed to bile pigments.[7–11,17,18] Bilirubin, a product of heme catabolism, is the main pigment in meconium, detectable in the fetal liver and gallbladder from 14 weeks of gestation.[19] Whereas the intestinal content of children and adults is rich in bacteria, meconium during fetal life is sterile,[20] as shown by metagenomic studies[21] that controlled for contamination of reagents and by studies in nonhuman primates and mice.[22,23]

Meconium as an obstetrical hazard

Völtern reported in 1687 that MSAF was associated with fetal death,[24] an observation subsequently confirmed by multiple authors[25–28] (Figure 1). Indeed, MSAF is considered a risk factor for neonatal hypoxic-ischemic encephalopathy,[3,29–31] neonatal sepsis,[3,32,33] neonatal seizures,[34] MAS,[35–39] and cerebral palsy.[40–43] Several clinical conditions (eg, prolonged labor, fetal growth restriction, oligohydramnios, vaginal breech delivery, etc.) have been related to the passage of meconium into the amniotic fluid,[44–51] and the risk factors for MSAF are reported in Table 2.

FIGURE 2
Frequency of MSAF as a function of gestational age



Modified from Balchin et al.[50]

MSAF, meconium-stained amniotic fluid.

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Factors associated with meconium-stained amniotic fluid

Gestational age

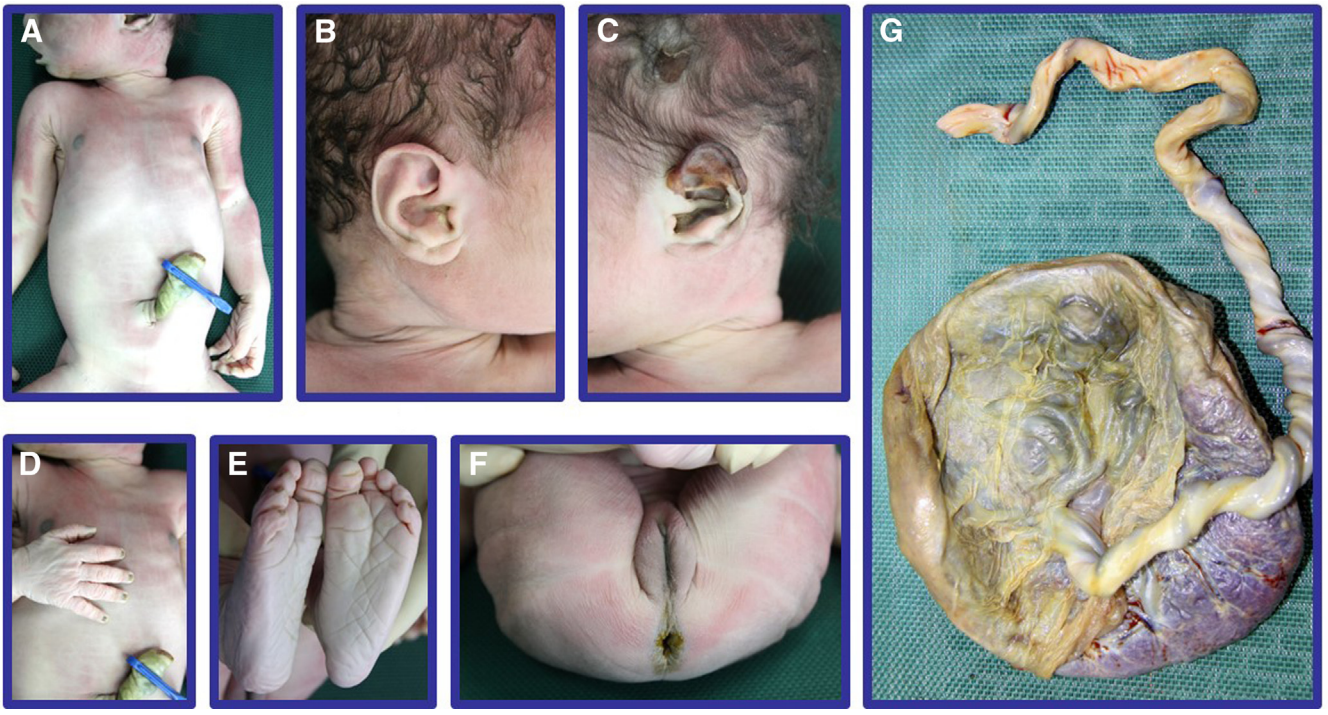
The rate of MSAF increases as a function of gestational age and can reach 27% at 42 weeks of gestation[44,45,50,52–60] (Figure 2). A subset of postterm neonates is affected by the postmaturity syndrome, defined as fetal growth restriction in a postterm gestation.[61] The presence of MSAF is a criterion of stage II postmaturity syndrome proposed by Clifford[61] (Figure 3).

The higher rate of meconium passage reported in term gestations is thought to reflect maturation of the gastrointestinal system. Observational studies in guinea pigs[62] and monkeys[63] have shown that intestinal peristalsis increases with advancing gestational age. Similar evidence in human fetuses was reported in studies documenting fetal gastrointestinal motility with amniography.[64]

The following endocrine factors have been implicated in the increased frequency of meconium passage at term:

1. Motilin, a gut hormone produced by enteroendocrine cells in the duodenum,[65] capable of inducing intestinal peristalsis.[66] The concentrations of motilin are significantly higher in umbilical cord blood from term neonates compared with preterm neonates[67] and from those with MSAF at term.[68,69]
2. Cortisol, which increases in fetal plasma at the time of parturition,[70,71] can also induce intestinal motility, as demonstrated in an observational study of pregnant monkeys where intraamniotic injection of glucocorticoids resulted in meconium passage.[72]

FIGURE 3
Meconium in postmaturity syndrome with fetal death at 40 weeks of gestation



A–E, The neonate shows the classical features of postmaturity syndrome characterized by loss of vernix caseosa, loss of subcutaneous fat and presence of macerated, wrinkled skin. Meconium passage is documented by the **F,** greenish-yellow discoloration of the anus, **A,** the discoloration of the skin, and **G,** the green-yellow staining of placental membranes.

Photo courtesy of Dr Sunil Jaiman.
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3. Corticotropin-releasing factor, a hormone that increases with gestational age,[73–76] can also accelerate fetal gut motility.[77–82]

Fetal hypoxia

MSAF has been associated with fetal acidemia[34,83,84] and chronic hypoxia (estimated by erythropoietin concentrations in umbilical cord blood).[85–87] The largest study to examine the relationship between MSAF and fetal acidemia included 42,709 term pregnancies, of which 8136 had MSAF. Meconium was associated with a significantly higher rate of umbilical artery pH ≤7.00 (7% [56/8136] vs 3% [95/34,573]; $P<.001$; odds ratio [OR], 2.5; 95% confidence interval [CI], 1.8–3.4)[34] (Table 3). However, most neonates born to mothers with MSAF do not have evidence of metabolic acidemia at birth.[56,85,88–93] Indeed, in a recent retrospective study including 3590 deliveries, MSAF was not associated with umbilical artery acidemia, and 80% of neonates with MSAF had a pH ≥7.20.[54] These conflicting results between the 2 largest studies may be explained by differences in the use of intrapartum fetal heart rate monitoring. In the first study,[34] surveillance was performed with intermittent auscultation, whereas in the second study,[54] continuous electronic fetal heart rate monitoring was used. It is possible that improved surveillance allows earlier detection of a compromised fetus, and thus the association between MSAF and umbilical artery acidemia was not observed.

It is widely accepted that fetal hypoxia leads to meconium passage.[34,83,84,94] However, experimental studies have challenged this view. For example, constricting the maternal aorta in pregnant rabbits to induce maternal acidemia and fetal distress is not associated with meconium passage,[95] and neither is acute inhalational hypoxia of pregnant sheep sufficient to drop the fetal partial pressure of oxygen.[96,97] Similarly, repeated cord occlusion leading to fetal acidemia is not associated with meconium passage in fetal sheep. However, a role for the autonomic nervous system in the regulation of pathologic fetal defecation has been proposed. Indeed, sympathetic system blockade, achieved chemically with 6-hydroxydopamine, in the same experimental paradigm of cord occlusion does lead to meconium passage.[98] It is also noteworthy that normal fetal defecation in animal studies is observed in the absence of fetal hypoxemia or acidemia.[99]

Intraamniotic infection/inflammation

MSAF is associated with microbial invasion of the amniotic cavity in term and preterm gestations.[57,58,100,101] In patients with preterm labor and intact membranes, those with green-colored amniotic fluid have a higher rate of

TABLE 3
Outcomes in neonates born with meconium-stained amniotic fluid compared with neonates born with clear amniotic fluid

Outcome	MSAF	Clear AF	*P* value	Odds ratio	95% CI
Total infants	8136[a]	34,573[a]			
Apgar score ≤3					
1-min	123 (15)	201 (6)	<.001[a]	2.6	2.1–3.2
5-min	14 (2)	19 (1)	.003[a]	3.1	1.6–6.0
Umbilical artery pH ≤7.00	56 (7)	95 (3)	<.001[a]	2.5	1.8–3.4
Apgar score ≤3 at 5 min and pH ≤7.00	9 (1)	5 (0.1)	<.001[a]	7.6	3.0–19.3
Special care nursery admission	193 (24)	248 (7)	<.001[a]	3.3	2.8–3.9
Respiratory distress[a]	223 (27)	288 (8)	<.001[a]	3.3	2.8–3.9
IVH grade III or IV	1 (0.1)	2 (0.1)	0.5	2.1	0.2–22.2
Seizures in the first 24 h	17 (2)	13 (0.4)	<.001[a]	5.6	2.9–10.5
Cesarean delivery					
Total	1170 (14)	2420 (7)	<0.001[a]	2.1	1.9–2.2
Dystocia	609 (7)	1328 (4)	<0.001[a]	1.9	1.8–2.1
Fetal distress	472 (6)	628 (2)	<0.001[a]	3.2	2.9–3.6

Modified from Nathan et al.[34]

AF, amniotic fluid; *CI*, confidence interval; *IVH*, intraventricular hemorrhage; *MSAF*, meconium-stained amniotic fluid.

[a] Halo or ventilator therapy.

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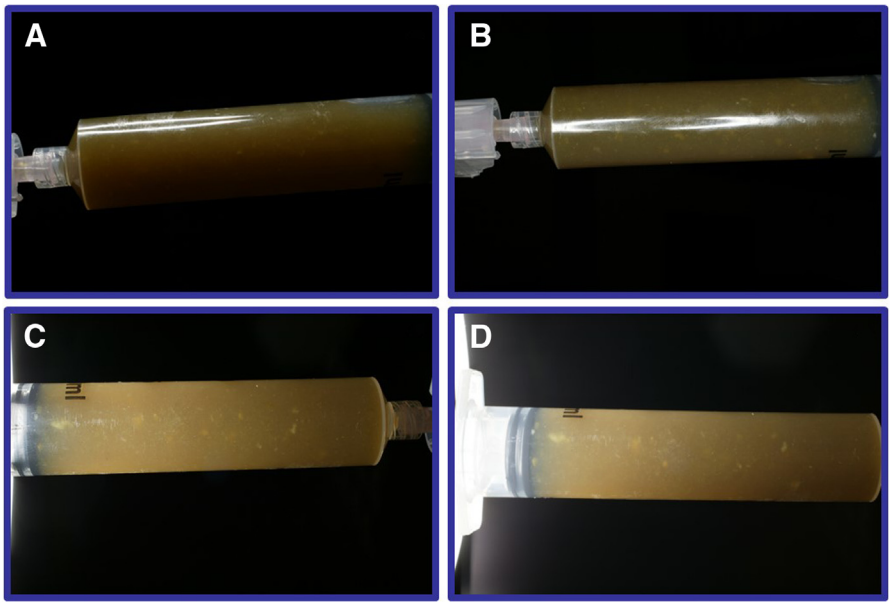
FIGURE 4
Meconium-stained amniotic fluid



A–B, Green meconium. **C–D,** "Thin," yellow meconium. The traditional concept is that meconium is green when first passed and can become yellow over time.

Gallo. Meconium-stained amniotic fluid. Am J Obstet Gynecol 2022.

positive amniotic fluid cultures for bacteria compared to those with clear amniotic fluid (33% [10/30] vs 11% [75/677]; *P*=.001).[58] Mazor et al[102] confirmed the association between MSAF and the presence of bacteria in the amniotic fluid in patients with preterm labor and intact membranes (38 [17/45] vs 11% [15/135]; *P*<.001) and also with clinical chorioamnionitis (22 [10/45] vs 6% [8/135]; *P*=.003).[102] The same association between meconium and intraamniotic infection has been reported at term. In patients with clinical chorioamnionitis, those with MSAF have a higher rate of microbial invasion of the amniotic cavity and bacterial endotoxin compared to those with clear amniotic fluid (19.6% [13/66] vs 4.7% [2/42]; *P*<.05; and 46.9% [31/66] vs 4.7% [2/42]; *P*<.001; respectively).[100] In addition, the concentrations of interleukin (IL)-6 are higher in MSAF, providing evidence of an intraamniotic inflammatory response.[100]

The green discoloration of amniotic fluid in the context of intraamniotic

TABLE 4
Adverse neonatal and maternal outcomes associated with thick meconium-stained amniotic fluid

Neonatal outcomes	Maternal outcomes
Abnormal fetal heart rate tracing	Cesarean delivery
Meconium aspiration syndrome	Puerperal endometritis
Neonatal intensive care unit admission	Clinical chorioamnionitis
Need for neonatal ventilation	Intrapartum fever
Hypoxic-ischemic encephalopathy of the neonate	Intraamniotic infection
Small for gestational age	
Low Apgar score	

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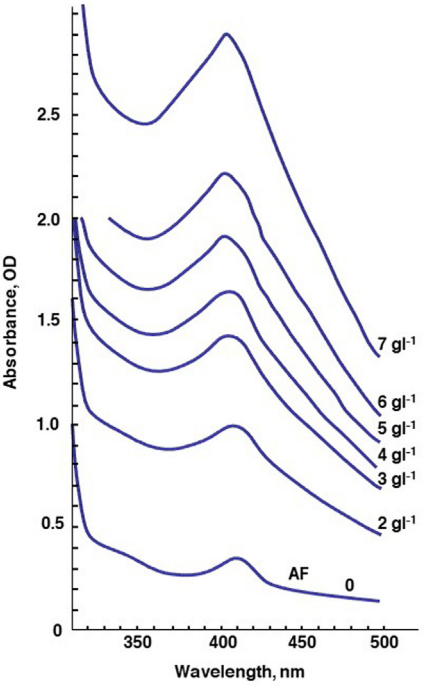
FIGURE 5
Spectrophotometric analysis of amniotic fluid



The absorption spectra of amniotic fluid (after centrifugation) with different concentrations of meconium are shown. The band height is linearly correlated with meconium concentration. Modified from Molcho et al.[131]

OD, optical density.

Gallo. Meconium-stained amniotic fluid. Am J Obstet Gynecol 2022.

infection/inflammation has been attributed either to oxidative stress in the amniotic cavity or to the passage of meconium. Bacteria can elicit intraamniotic inflammation, which in turn leads to the generation of reactive oxygen species[103,104] capable of inducing oxidation of heme catabolic products such as bilirubin.[105,106] We have previously proposed that bacteria and amniotic fluid with inflammatory products, when ingested by the fetus, may stimulate bowel peristalsis.[58,100] An alternative explanation for the association between intraamniotic infection/inflammation and MSAF is that meconium enhances bacterial proliferation by serving as a growth factor[57,58,102] and by inhibiting the bacteriostatic properties of amniotic fluid or antagonizing host defense systems,[107–109] thus increasing the risk of infection.

Prolonged gestation, fetal hypoxia, and intraamniotic infection/inflammation could explain only a subset of patients with MSAF. However, the cause for the remaining cases has yet to be elucidated. Omics analysis of amniotic fluid stained with meconium would provide an insight into the pathophysiology of MSAF and could allow the identification of biomarkers that can serve in the stratification of patients according to MSAF etiology.

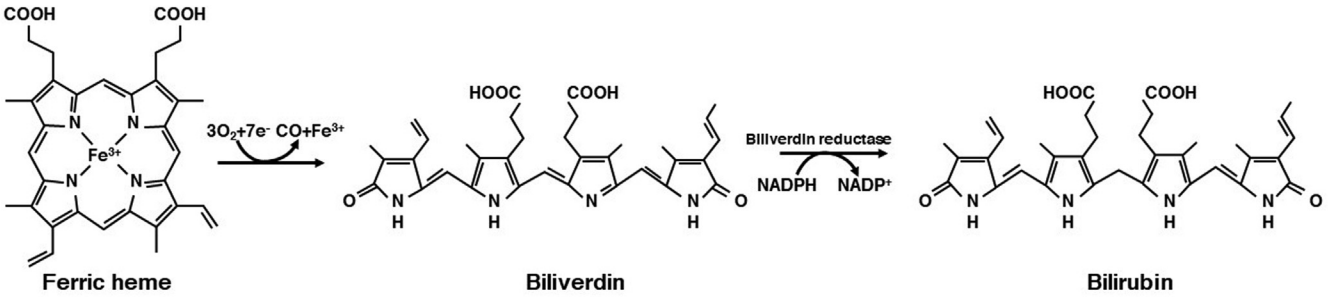
Assessment of meconium-stained amniotic fluid

Typically, MSAF is diagnosed after rupture of membranes or by amniocentesis. Occasionally, sonographic particulate matter in amniotic fluid raises the suspicion for MSAF. The ultrasound criteria originally proposed for the identification of MSAF include (1) a diffuse echogenic pattern throughout the amniotic cavity; (2) a clear contrast between the amniotic fluid and the umbilical vessels; and (3) layering in the more dependent areas.[110] However, this appearance is not specific to meconium and can be seen in the presence of vernix or even blood.[111–115] In a study of 278 patients who were scanned 24 hours before delivery, the prevalence of echogenic amniotic fluid was 3.2%, and of these patients, 44% (4/9) had MSAF, with a sensitivity of 14% and a positive predictive value of 44%.[116] Therefore, ultrasound has limited diagnostic value for identifying this condition.

Green-colored amniotic fluid has been detected at amniocentesis for genetic indications in the midtrimester or in the third trimester. In the past, serial transabdominal amniocenteses were used as a method of surveillance in women with prolonged gestations to detect postmaturity syndrome[117] or in patients with intrahepatic cholestasis of pregnancy to assess the risk of fetal death.[118] However, serial amniocenteses for these indications have been abandoned because of the lack of evidence that detection of meconium and induction of labor improve pregnancy outcomes.[119]

Meconium in amniotic fluid has been classified according to its thickness into grades 1 to 3 (grade 1: lightly stained amniotic fluid, green or yellow; grade 2: green- or yellow-stained amniotic fluid with some particulate matter; and grade 3: dense meconium with "pea-soup" consistency).[120] However, the most commonly used classification in obstetrical practice divides MSAF into "thick" and "thin" (Figure 4). Thick meconium is associated with higher rates of abnormal fetal heart tracings,[54,83,121,122] MAS,[121,123–127] neonatal intensive care unit (NICU) admission,[84,127–129] need for neonatal ventilation,[123] hypoxic-ischemic encephalopathy of the neonate,[123,130] small for gestational age,[123] and low Apgar scores.[32,83,124,125,128,129] Similarly, higher rates of cesarean delivery,[6,66,79,131–133] puerperal endometritis,[134] clinical

FIGURE 6
The catabolism of heme



Heme is first transformed into biliverdin and then to bilirubin in the reticuloendothelial system. The first reaction consists of the conversion of c to biliverdin, and it is catalyzed by the heme oxygenase system. Subsequently, biliverdin reductase reduces biliverdin to bilirubin.
Modified from Rodwell et al.[149]

COOH, carboxyl group; *$NADP^+$*, nicotinamide adenine dinucleotide phosphate; *NADPH*, nicotinamide adenine dinucleotide phosphate hydrogen.

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chorioamnionitis,[84,134] intrapartum fever,[54] and intraamniotic infection[135] have been reported in women with thick MSAF (Table 4). Obstetrical conditions, such as oligohydramnios associated with postterm pregnancies[136,137] or uteroplacental insufficiency,[122,138–141] altered fetal swallowing,[142] increased amount of meconium passage, and impaired reabsorption of meconium by amniotic membrane macrophages,[143,144] have been implicated in the genesis of thick MSAF.

A quantitative approach to assess meconium load in the amniotic fluid is the "meconium-crit."[145] This test is based on the same principle as that used to calculate the hematocrit with a capillary tube, and results correlate well with meconium concentrations[145] but may not reflect neonatal outcomes. Other methods such as spectrophotometry[131] and nuclear magnetic resonance spectroscopy have also been proposed as tools to estimate meconium concentration in amniotic fluid. The band height in the spectrophotometric tracing correlates with meconium concentrations[131] (Figure 5). Nuclear magnetic resonance spectroscopy can also be used to quantitate meconium in amniotic fluid on the basis of T1 and T2 relaxation times that decrease with increasing concentrations of meconium.[146,147] Neither spectrophotometry, nuclear magnetic resonance spectroscopy, nor meconium-crit have been implemented in clinical practice.

Green-stained amniotic fluid is not always indicative of meconium

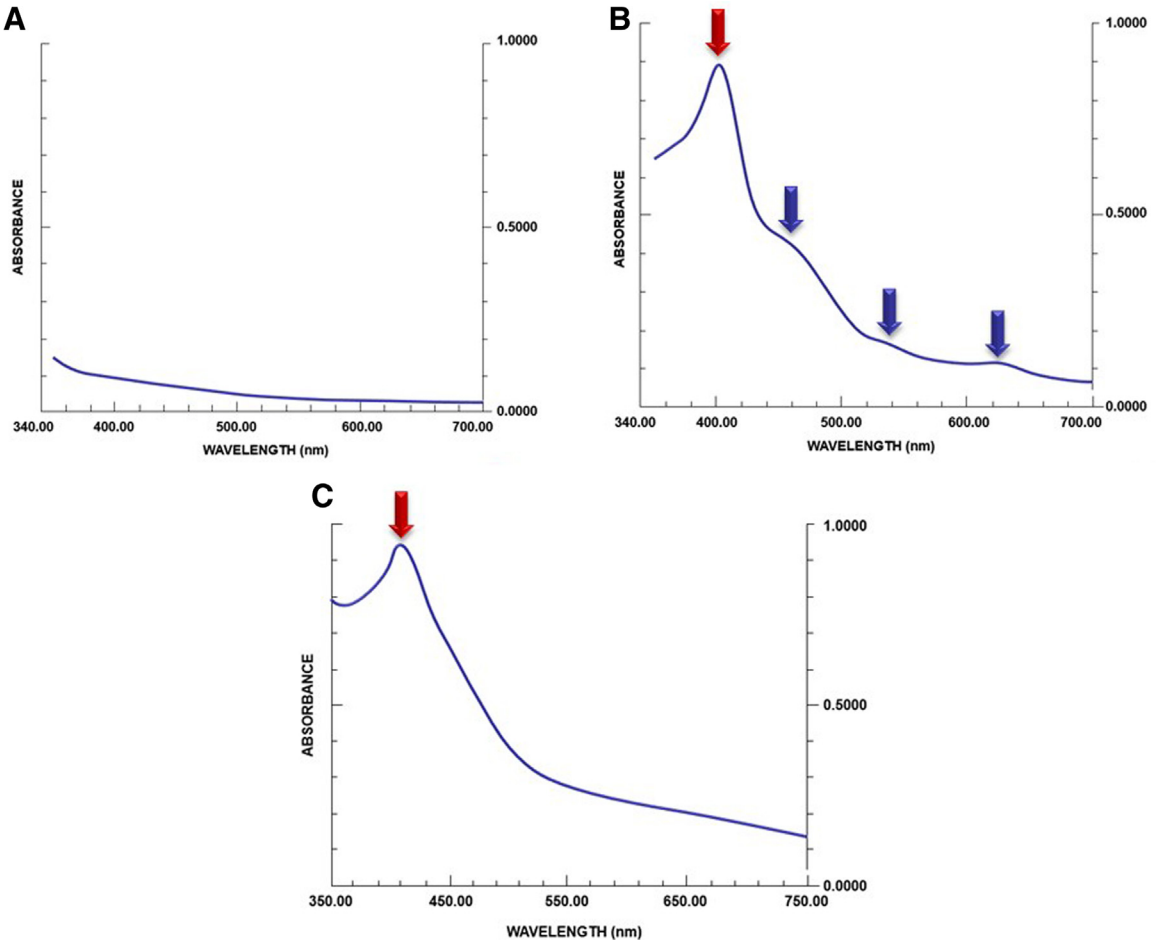
The traditional view that all green-stained amniotic fluid is due to meconium has been challenged. Meconium has been described as green, and this color is attributed to biliverdin,[148] an intermediate product of heme catabolism that can be subsequently reduced to bilirubin in the reticuloendothelial system through a reversible reaction (Figure 6). Indeed, the oxidation of bilirubin leads to biliverdin.[106,150,151] Bilirubin, a yellow pigment, is consistently detected in meconium,[17,19] whereas there is paucity of evidence that this is the case for biliverdin.[17]

Green- and brown-discolored amniotic fluid at the time of midtrimester amniocentesis has been reported in 1.2% to 8% of cases[152–161] and is associated with pregnancy loss in 9% of cases.[160] Brown amniotic fluid was considered an indicator of intraamniotic hemorrhage, whereas green fluid was attributed to the presence of meconium. Spectrophotometric analysis of midtrimester discolored amniotic fluid performed by Hankins et al[159] reported that green and brown discolorations were attributable to previous episodes of intraamniotic bleeding, as reflected by the presence of free hemoglobin. Clear amniotic fluid has a smooth absorption spectrum (Figure 7), whereas contamination with either blood or meconium typically adds an absorption peak near 400 nm (also known as "Soret band")[149,162] attributable to the presence of hemoglobin in the former and of meconium pigments (eg, bilirubin) in the latter.[149,158,159,163] However, the absorption spectrum in intraamniotic bleeding shows additional peaks (attributed to oxyhemoglobin, methemoglobin, and methemalbumin) that can be used in the differential diagnosis with MSAF.[158] Amniotic fluid contaminated with meconium at term usually tests negative for hemoglobin and shows no extra peaks at spectrophotometric analysis (Figure 7).

Maternal implications of meconium-stained amniotic fluid

MSAF is associated with intraamniotic infection,[58,100,101] clinical chorioamnionitis,[47,58,102,134,164–168] puerperal endometritis,[134,169] postcesarean infection,[170] postpartum hemorrhage,[169,171–173] and dehiscence of perineal lacerations.[174,175] The association between meconium and infection provides a rationale for exploring the role of antibiotic administration in MSAF. A systematic review of 2 randomized clinical trials[176–178] in women with MSAF allocated to intravenous ampicillin/sulbactam vs placebo showed that intrapartum antibiotic administration reduced the frequency of clinical

FIGURE 7
Spectrophotometric analysis of amniotic fluid at different gestational ages



A, Normal term amniotic fluid spectrum. The typical spectrum shows a smooth declining slope without peaks, suggesting lack of chemical compounds absorbing light. **B,** Typical spectrophotometric tracing of discolored second-trimester amniotic fluid with a maximum peak near 405 nm ("Soret band") (*red arrow*) and several secondary absorption peaks at 450 nm, 550 nm, and 620 nm (*blue arrows*). **C,** Spectrophotometric tracing of term meconium-stained amniotic fluid. There is a peak at 405 nm (*red arrow*) and a smooth declining slope without additional peaks.

Modified from Alger et al.[158]

Gallo. Meconium-stained amniotic fluid. Am J Obstet Gynecol 2022.

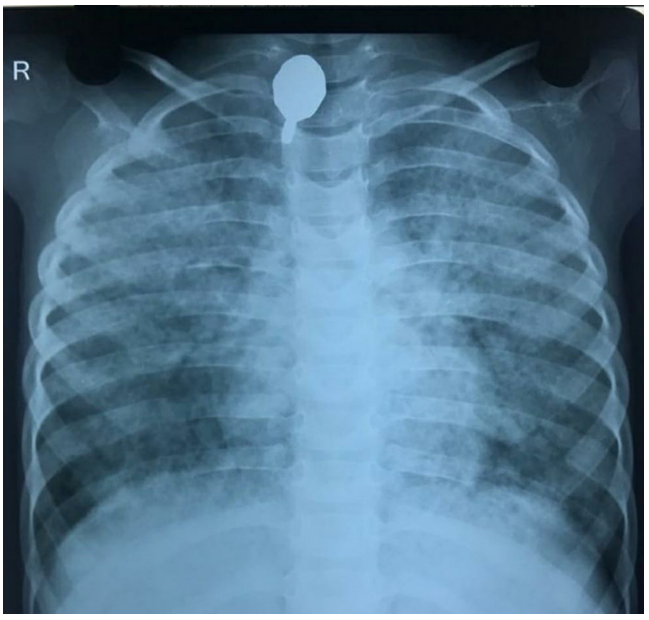
chorioamnionitis (relative risk [RR], 0.36; 95% CI, 0.21−0.62) but not the frequency of postpartum endometritis (RR, 0.50; 95% CI, 0.18−1.38), neonatal sepsis (RR, 1.00; 95% CI, 0.21−4.76), or NICU admission (RR, 0.83; 95% CI, 0.39−1.78). An alternative approach is administering antibiotics selectively to patients at particularly high risk for infection, determined by a high concentration of IL-6 or matrix metalloproteinase-8 (MMP-8) in amniotic fluid obtained through a transcervical catheter coupled with point-of-care testing.[179] The value of this approach should be the subject of prospective studies.

Neonatal implications of meconium-stained amniotic fluid: meconium aspiration syndrome

MSAF occurs in 5% to 20% of deliveries at term[3,35,84,165,180−182] and is a risk factor for neonatal complications[131,136,137,142,143,145] such as MAS,[35−39] neonatal sepsis,[3,32,33] pulmonary disease,[84,183] neonatal seizures[34,184] and long-term neurologic disability (eg, cerebral palsy).[40−42]

MAS is defined as respiratory distress in term neonates born to mothers with MSAF that cannot be otherwise explained (Figure 8). MAS is a cause of neonatal morbidity and mortality,[1,3,30,35,88,122,185] and the reason why only 5%[35−39] of infants exposed to meconium develop MAS remains an enigma (Figure 9). Typically, MAS affects neonates with an intrauterine event that causes intrapartum or antepartum fetal hypoxia[129,165,180,186−191] leading to meconium passage, fetal gasping,[192,193] and meconium aspiration before birth. However, a large fraction of neonates with MAS do not have acidemia at birth[55,88−93] (Table 5); therefore,

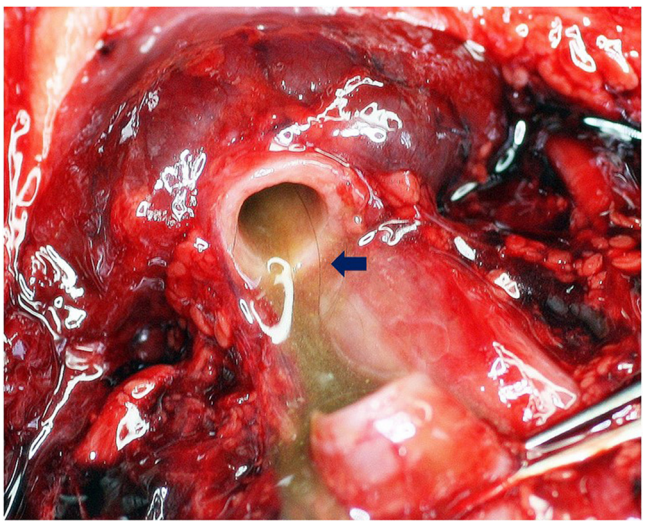
FIGURE 8
Chest X-ray showing bilateral patchy opacifications



Gallo. Meconium-stained amniotic fluid. Am J Obstet Gynecol 2022.

alternative mechanisms must be involved in the pathogenesis of this syndrome. Meconium itself can cause local damage in the fetal lungs through (1) a mechanical effect, which can cause airway obstruction leading to atelectasis or air trapping within the bronchioles and the alveoli[101,194,195] (Figure 10); (2) a chemical effect of meconium content (eg, free fatty acids, bile salts, pancreatic phospholipases) that can result in surfactant inactivation[196,197]; and (3) a local inflammatory response (pneumonitis)[194,195,198,199] that can lead to decreased pulmonary function.[200,201] Persistent pulmonary hypertension (PPHN) occurs in 20% to 40% of infants with MAS. Several mechanisms have been implicated in the development of PPHN in MAS.[202] Parenchymal lung disease with poor alveolar recruitment and decreased functional residual capacity (FRC) or hyperinflation with increased FRC contribute to elevations in pulmonary vascular resistance.[203] In addition, the release of chemically vasoactive mediators, such as endothelin-1, thromboxane-A2, and prostaglandins, has also been shown to contribute to the development of PPHN in MAS.[204] Vascular remodeling changes such as hyperplasia of the vascular media and interstitium, narrowing of the vessel lumen, tortuosity of the arteries, and muscularization of the alveolar septal arterioles have been described in MAS.[205,206]

FIGURE 9
Autopsy of a neonate with evidence of meconium in the trachea from MAS



The *arrow* points to fetal hair within the trachea.

MAS, meconium aspiration syndrome.

Gallo. Meconium-stained amniotic fluid. Am J Obstet Gynecol 2022.

In the context of intraamniotic infection/inflammation, fetal swallowing of amniotic fluid containing bacteria,[207,208] endotoxins,[58,100,101,209] alarmins,[132,207,210–215] inflammatory mediators,[100,101,133,216,217] and phospholipase A2 can induce fetal inflammatory response syndrome (FIRS) resulting in diffuse lung injury.[55,101,218] The combination of pulmonary inflammation and capillary damage/leakage occurring during FIRS could explain the association between MSAF, intraamniotic inflammation/infection, and MAS.[55] The knowledge that FIRS is a risk factor for the development of MAS has clinical implications. Indeed, umbilical cord blood concentrations of IL-6 or C-reactive protein could assist in the identification of infants who have systemic inflammation.

Prophylactic intrapartum transcervical amnioinfusion in cases with MSAF has been proposed to reduce the rate of MAS and other adverse neonatal outcomes. Subsequently, this procedure was abandoned after the publication of a meta-analysis that reported no evidence of benefit in terms of reduction of the

TABLE 5
Prevalence of neonatal acidemia in meconium aspiration syndrome

	Neonatal umbilical artery pH at birth in MAS cases			
Author, date	**pH ≥7.20**	**pH <7.20**	**pH <7.10**	**pH <7.00**
Lee et al,[55] 2016	(58.3%) (7/12)	41.7% (5/12)	16.7% (2/12)	8.3% (1/12)
Blackwell et al,[88] 2001	60.4% (29/48)	39.6% (19/48)	na	na
Yeomans et al,[90] 1989	83.3% (5/6)	16.7% (1/6)	na	na
Trimmer et al,[91] 1991	50%–100% (1–2/2)	0%–50% (0–1/2)	na	na

MAS, meconium aspiration syndrome; *na*, not applicable.

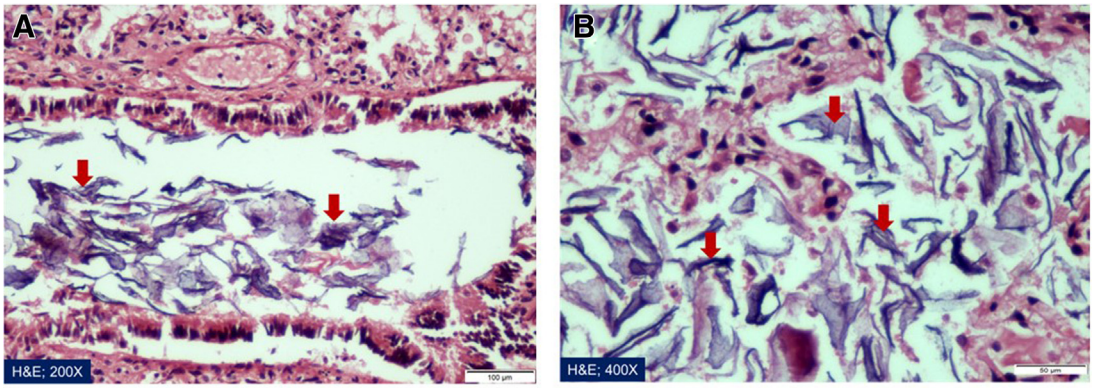
Gallo. Meconium-stained amniotic fluid. Am J Obstet Gynecol 2022.

rate of MAS (RR, 0.59; 95% CI, 0.28–1.25), 5-minute Apgar score <7 (RR, 0.90; 95% CI, 0.58–1.41), or cesarean delivery (RR, 0.89; 95% CI, 0.73–1.10). Prophylactic amnioinfusion seems to have a role only in clinical settings with limited peripartum surveillance given that it helps to reduce the risk of MAS (RR, 0.25; 95% CI, 0.13–0.47).[219] However, these results and interpretation have been a subject of debate.[220,221] Recently, a new meta-analysis that reassessed the value of prophylactic amnioinfusion in the presence of MSAF reported a reduction of MAS by 67% (pooled OR, 0.33; 95% CI, 0.21–0.51). Amnioinfusion has been proposed to dilute thick meconium, thereby diminishing the mechanical and the proinflammatory effects. This meta-analysis is reported in detail in this issue of the *American Journal of Obstetrics & Gynecology*.

Because neonatal airway obstruction by meconium is believed to be causal of MAS, several approaches have been proposed to remove meconium from the fetal and neonatal airways to prevent aspiration (reviewed in detail by Wiswell[185]). Routine endotracheal intubation was introduced in the 1970s after Burke-Strickland and Edwards[222] and Gregory[223] reported a favorable experience with this practice. Gregory et al[223] found that 56% of newborns delivered by mothers with MSAF already had meconium in the trachea at the time of birth, and that in 10% of the cases, meconium was present below the vocal cords, although not visible in the mouth or pharynx. The authors further noted a reduction in the frequency of pneumothorax, mechanical ventilation, and continuous positive airway pressure after early endotracheal intubation.[223]

Other investigators advocated complete removal of meconium from the oropharynx and nasopharynx before the delivery of the chest and onset of air breathing as a method to prevent meconium aspiration. This procedure was performed with either a bulb syringe or a suction catheter. The combined approach consisted of immediate oro/nasopharyngeal suctioning at the time of delivery of the fetal head, followed by endotracheal intubation and suctioning after birth. This management was reported to reduce the rate of MAS from 1.9% (18/947) to 0.4% (1/273) with endotracheal aspiration alone.[224] The combined approach has been used in labor and delivery units for decades[225]; however, subsequent studies questioned efficacy[226–228] and safety.[229–233] Indeed, complications such as laryngeal lesions leading to stridor and hoarseness,[229] bradycardia,[230] apnea,[230] hypoxemia, and desaturation[231–233] were reported. Observational studies also showed no benefit in the reduction of MAS.[234–238] Such findings led to the design and execution of randomized controlled trials[183,229,239–244] and subsequent meta-analyses,[226–228] which demonstrated that neither routine suctioning of the oro/nasopharynx after the delivery of

FIGURE 10
Meconium in the fetal bronchiole and alveoli

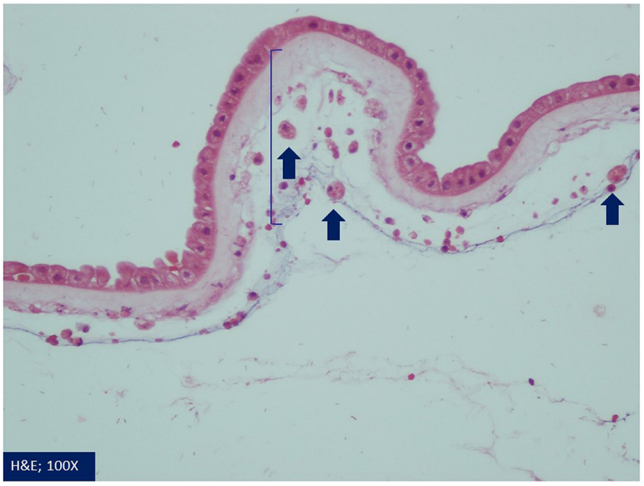


A, Bronchiole. **B,** Alveoli. The *arrows* indicate fetal anucleated squamous cells, one of the components of meconium. Stained with H&E.

H&E, hematoxylin and eosin.

Gallo. Meconium-stained amniotic fluid. Am J Obstet Gynecol 2022.

FIGURE 11
Chorioamniotic membranes stained with H&E in a case of MSAF



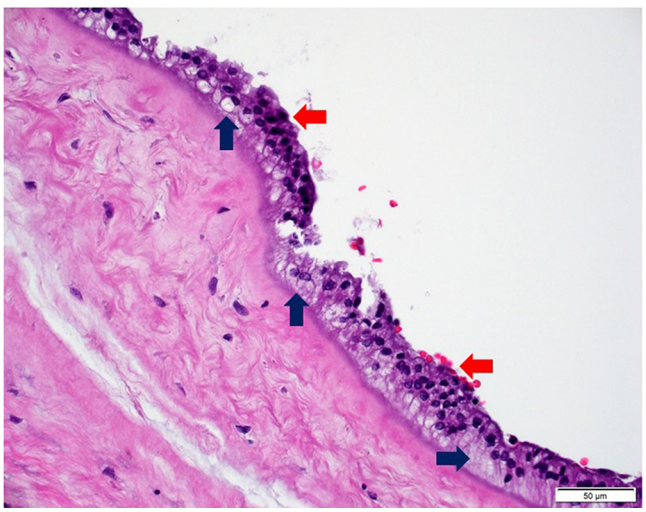
Meconium is visualized within macrophages (*blue arrows*) in the amnion and chorion stroma (*blue squared parenthesis*). Meconium-laden macrophages are recognized by the pink staining of the cytoplasm after excluding hemosiderin pigment with Prussian blue staining (not shown).

H&E, hematoxylin and eosin.; *MSAF*, meconium-stained amniotic fluid

Gallo. Meconium-stained amniotic fluid. Am J Obstet Gynecol 2022.

the head,[226] nor intubation with tracheal suctioning in both vigorous[227] and nonvigorous newborns,[228] prevented or altered the frequency and course of MAS. Consensus has emerged that infants born to mothers with MSAF should no longer routinely receive suctioning at birth, whether or not they are vigorous. The current recommendation is that management should be guided by general neonatal resuscitation principles rather than a prespecified approach.[245]

FIGURE 12
Chorioamniotic membranes stained with H&E in a case of MSAF



Reactive amnion hyperplasia (*red arrows*) and cytoplasmic vacuolation (*blue arrows*) are observed.

H&E, hematoxylin and eosin; *MSAF*, meconium-stained amniotic fluid.

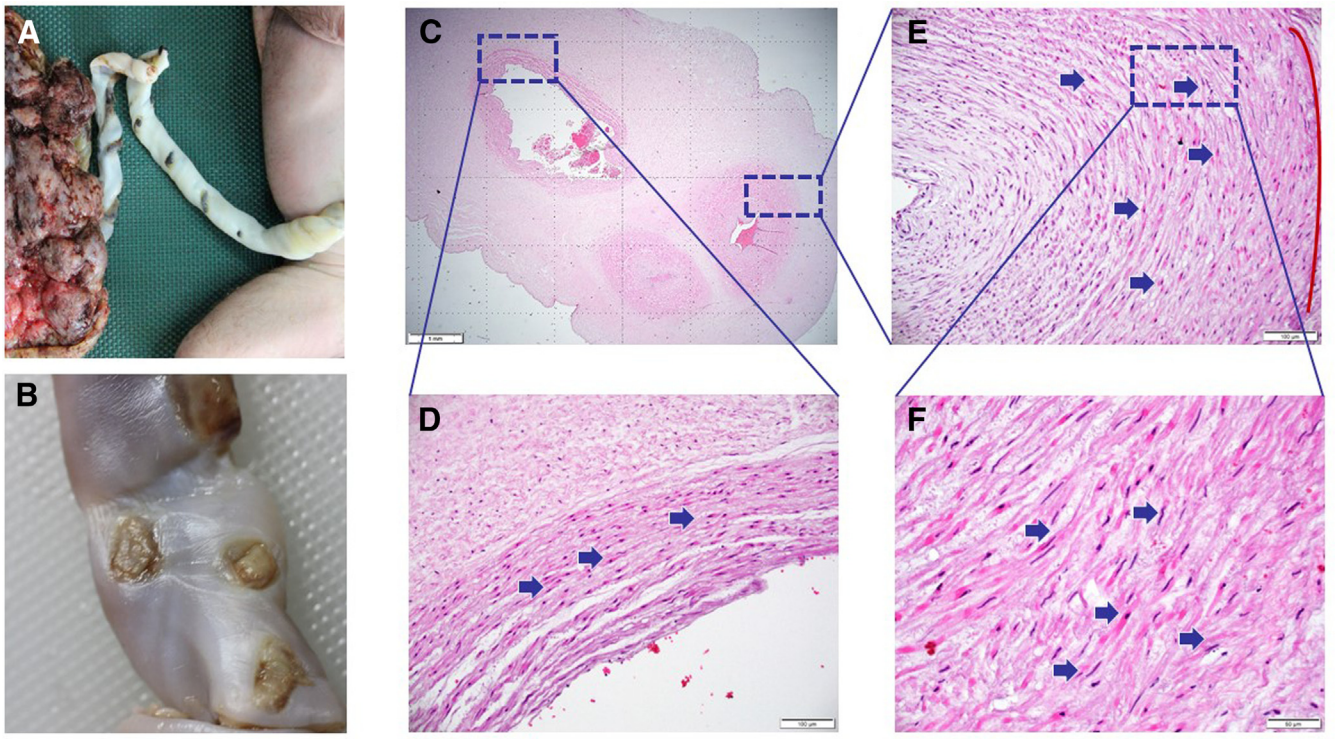
Gallo. Meconium-stained amniotic fluid. Am J Obstet Gynecol 2022.

Placental histopathologic findings in meconium-stained amniotic fluid

MSAF often results in green-yellow staining of the chorioamniotic membranes and umbilical cord at gross placental inspection (Figure 3, G). Microscopically, meconium-laden macrophages are the hallmark of this condition. These phagocytic cells can be identified by the presence of brown-yellow—colored cytoplasmic granules on hematoxylin and eosin staining[143,246] (Figure 11). However, similar granules can be observed in the placentas of patients who had intraamniotic bleeding.[246] Histochemistry staining for hemosiderin[247–249] (eg, Perls Prussian blue, Gomori or Berlin blue) can assist in the differential diagnosis of bleeding vs meconium. The rationale is that bilirubin in meconium does not contain iron, whereas hemosiderin (an iron-storage complex in cells) does. Pathologists rely on this approach to diagnose meconium staining of the fetal membranes. If iron staining is negative, the diagnosis of meconium is made. Meconium contains zinc-coproporphyrin I,[10,250] and investigators have recently developed a monoclonal antibody for this compound and identified this molecule in chorioamniotic membranes.[250] This would provide direct evidence of the presence of meconium.

Meconium in the chorioamniotic membranes has been used in medical legal litigations to time adverse events and to formulate arguments about medical negligence. Desmond et al[251] reported that immersion of the lower extremities of neonates in MSAF would cause mild yellow staining of toenails in 4 to 6 hours, whereas yellow staining of the vernix caseosa would take 12 to 14 hours. The time required to stain the chorioamniotic membranes has been a subject of debate. For example, Miller et al[252] incubated disks of chorioamniotic membranes with meconium and reported that meconium-laden macrophages were present in the amnion after 1 hour of exposure and in the chorion after 3 hours. A subsequent experiment in which the exposure to meconium was restricted to the amnion found that it took 24 to 48 hours for a substantial

FIGURE 13
Meconium-induced umbilical cord vessel myonecrosis



A, Gross image of the umbilical cord in a case of fetal death with meconium-stained amniotic fluid at term. Several areas of ulceration are observed. The Wharton's jelly is eroded and the vessels are exposed. The dark color represents the muscularis of the vessels. **B,** Shallower ulcerations of the cord. The muscularis is not eroded in this part of the cord. **C,** Hematoxylin and eosin staining of the umbilical cord. The umbilical vein is on the top and the 2 arteries below. At this magnification, myonecrosis is not evident. **D,** The wall of the umbilical vein with evidence of myonecrosis. **E,** Umbilical artery with damaged myocytes (*blue arrows*) is observed in the muscular layer closer to the amniotic cavity. Cytoplasmic hypereosinophilia with nuclear pyknosis is evident. The *red line* indicates the outer perimeter of the umbilical vessel closer to the amniotic cavity, and the *red arrow* indicates the umbilical cord artery lumen. **F,** Cytoplasmic and nuclear changes are better seen at higher magnification (*blue arrows*).

Gallo. Meconium-stained amniotic fluid. Am J Obstet Gynecol 2022.

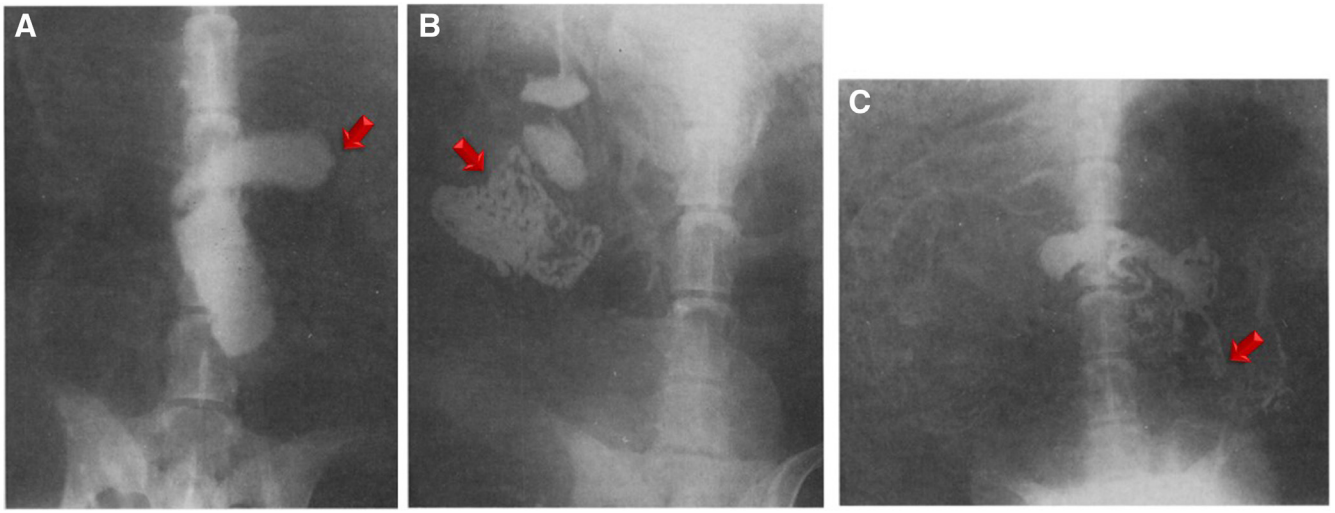
number of meconium-laden macrophages to be observed.[143] This finding has been interpreted as indicating that meconium staining of the membranes reflects fetal defecation that occurred at least 1 day before delivery of the placenta. By contrast with these in vitro experiments, an in vivo observational study documented the duration of meconium exposure (by change in the color of amniotic fluid from clear to meconium-stained). This study showed that meconium was present in most placental tissues within 10 minutes from exposure and that there was no relationship between the duration of exposure to meconium and the extent and intensity of meconium uptake by macrophages in the placental membranes.[253] We are not persuaded that examination of the placenta for meconium staining can lead to reliable inferences about the timing of fetal injury.

Placental inflammatory lesions associated with meconium exposure

A large body of evidence supports an association between inflammation and MSAF. Indeed, MSAF contains mediators such as IL-6,[217] tumor necrosis factor alpha,[133] IL-1β,[133] IL-8,[133] and phospholipase A2,[101] with inflammatory and/or chemotactic properties.[133,198,254] Meconium can have a direct effect on the amnion. Indeed, incubation of amnion with meconium resulted in reactive amnion hyperplasia and cytoplasmic vacuolation after 1 hour of meconium exposure[252] (Figure 12). Inflammatory lesions of the chorioamniotic membranes and umbilical cord are present in approximately 60% of cases of MSAF.[199,255,256] Given that MSAF is associated with intraamniotic infection in approximately 20% of cases, it is difficult to determine to what extent these lesions are attributable to MSAF or rather to intraamniotic infection/inflammation.[164,255–257]

Attempts have been made to identify specific placental lesions associated with meconium, such as necrosis of the chorionic plate and the muscular layer of the umbilical cord vessels[199,258] (Figure 13). Moreover, meconium has been proposed to cause vasoconstriction of the umbilical cord vessels[258] and cord vessel

FIGURE 14
Evidence of in utero fetal defecation in goats by serial radiographic examinations

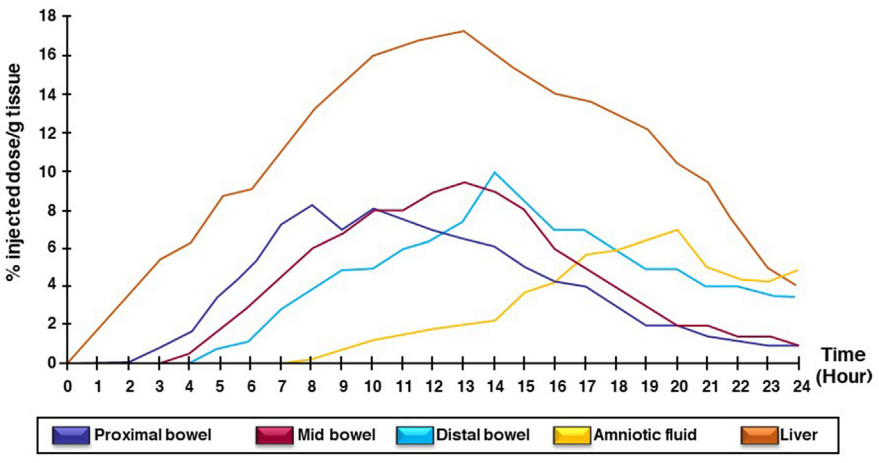


A, After intragastric injection via nasogastric tube, the nonhydrosoluble contrast medium persists in the stomach (*red arrow*) 4 hours after injection. **B,** Evidence of contrast media in the small bowel (*red arrow*) 8 hours from injection. **C,** Eventually, the contrast material is excreted in the amniotic cavity (*red arrow*) where it delineates the fetal body surface and fills the fetal airways.

Reproduced with permission from Kizilcan et al.[99]

Gallo. Meconium-stained amniotic fluid. Am J Obstet Gynecol 2022.

FIGURE 15
Experimental study in rabbits investigating excretion of a radioactive substance (^{99m}Tc-HIDA)



Analysis of radioactivity of tissues from fetuses harvested at a rate of 1 per hour demonstrates that there is physiological transit of radioactive meconium through the gastrointestinal tract (proximal bowel, mid-bowel, and distal bowel) into the amniotic fluid. The colored lines represent the magnitude of radioactivity in different tissues.

Modified from Ciftci et al.[260]

HIDA, hepatobiliary iminodiacetic acid, ^{99m}*Tc*, technetium-99m.

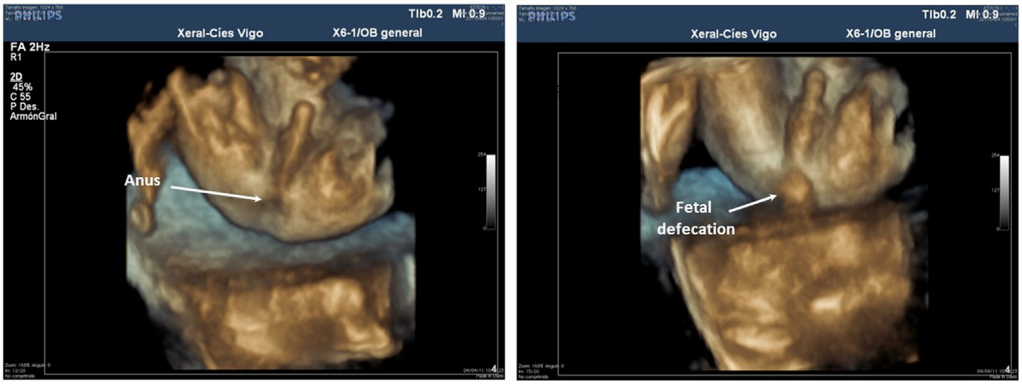
Gallo. Meconium-stained amniotic fluid. Am J Obstet Gynecol 2022.

necrosis,[199,259] hence fetal distress or death.[199]

Fetal defecation as a physiological event

The traditional view has been that the fetus does not pass meconium in the absence of a pathologic process such as hypoxia or infection. However, an accumulating body of evidence suggests that defecation in utero is a physiological phenomenon, and this is supported by the following observations: (1) when a nonhydrosoluble contrast medium is administered via nasogastric tube in fetal goats, the contrast is subsequently detected in the amniotic fluid by serial radiographic examinations[99] (Figure 14); (2) radioactive technetium-99m (^{99m}Tc- hepatobiliary iminodiacetic acid [HIDA]), injected intramuscularly in fetal rabbits, is detectable in the gastrointestinal tract and then in the amniotic cavity[260] (Figure 15); and (3) closure of the fetal anus with a purse-string suture prevents the technetium from appearing in the amniotic fluid.[261]

FIGURE 16
Evidence of fetal defecation with 4-dimensional ultrasound



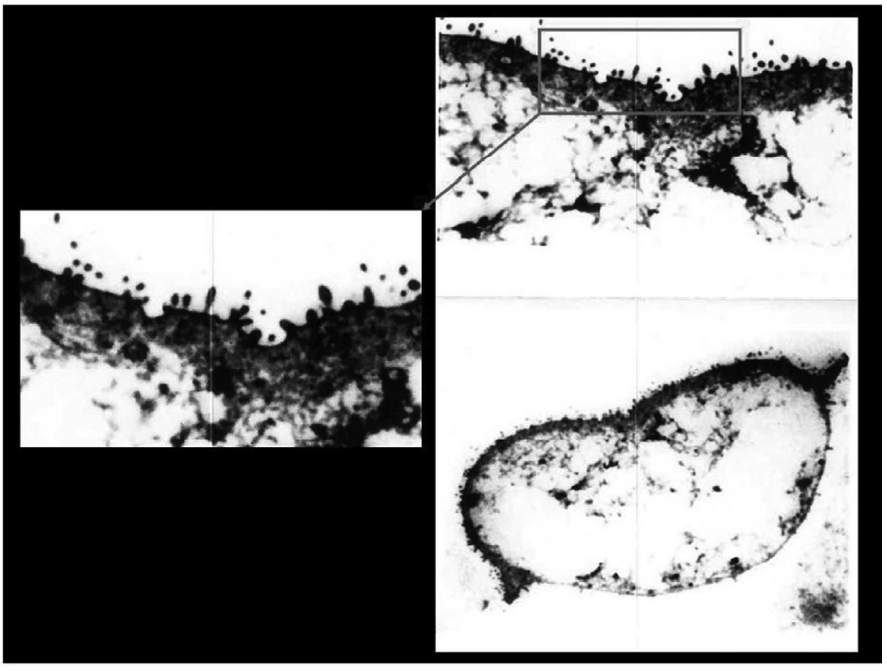
The anus was examined for 10 to 15 minutes.

Courtesy of López Ramón y Cajal et al.[264]

Gallo. Meconium-stained amniotic fluid. Am J Obstet Gynecol 2022.

Detailed high-resolution ultrasound has shown defecation by the human fetus.[262] López Ramón y Cajal and Ocampo Martínez[262,263] evaluated fetal anal sphincter behavior in pregnancies between 15 and 41 weeks of gestation. Defecation was documented in all cases, with the highest frequency between 28 and 34 weeks of gestation (Figure 16). Amniocentesis performed shortly after defecation in a subset of patients demonstrated clear amniotic fluid containing "whitish" material, which was consistent with bowel epithelium at microscopic examination[264] (Figure 17). The passage of meconium has also been observed during fetoscopy in a case of stage III twin-to-twin transfusion syndrome at 19 weeks of gestation[266] (Figure 18; Videos 1 and 2). In this case, the stool was green in color, which could be attributed to oxidative stress during the pathologic process.

FIGURE 17
Electron microscopic image of fetal cells found in fetal defecation



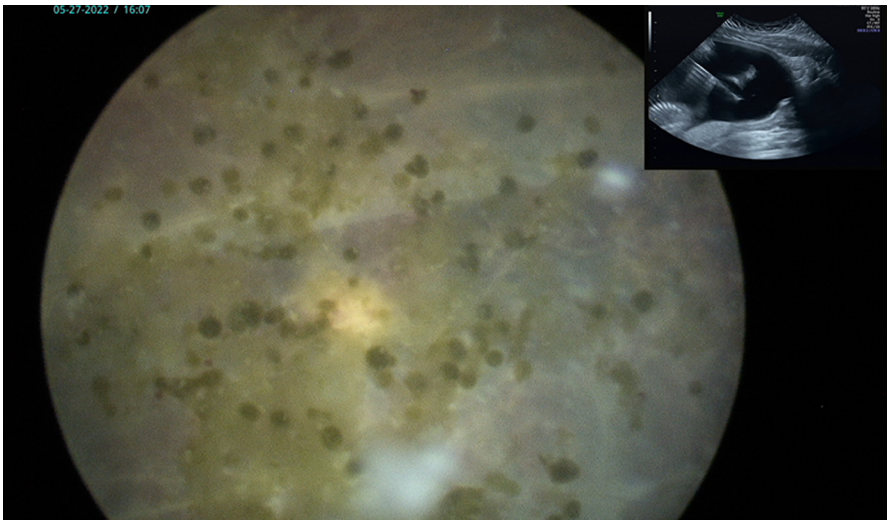
Structures are present on the cell surface that resemble primitive villi (*boxed area*).

Reproduced with permission from López Ramón y Cajal.[265]

Gallo. Meconium-stained amniotic fluid. Am J Obstet Gynecol 2022.

Further evidence supporting that defecation occurs in utero derives from a study of 31 fetal autopsies, ranging from 14 to 27 weeks of gestation. The presence of green-colored meconium at different levels of the intestinal tract was detected in 28 cases, and evidence of defecation, determined by the presence of meconium in the anus, was less frequent after 21 weeks of gestation,[267] which is the time when the external anal sphincter becomes fully developed.[268–270] These findings are consistent with the observation that the concentrations of intestinal enzymes (alkaline phosphatase and disaccharidases) in amniotic fluid peaked at 17 to 18 weeks of gestation, and decreased after 22 weeks.[271,272] Given that meconium contains colored pigment (eg, bilirubin) and that fetal defecation occurs throughout pregnancy,[262] it remains to be clarified why

FIGURE 18
Meconium debris in amniotic cavity of twin B (recipient) during fetoscopy



Courtesy of Dr Ramen Chmait.

Gallo. Meconium-stained amniotic fluid. Am J Obstet Gynecol 2022.

MSAF occurs only in up to 20% of deliveries at term.

Conclusion

MSAF occurs in up to 20% of term pregnancies and is a risk factor for adverse maternal and neonatal outcomes. Hypoxia and/or intraamniotic infection/inflammation can be found in a subset of patients. When meconium is present, continuous fetal heart rate electronic monitoring is indicated as a normal cardiotocographic tracing that effectively excludes fetal hypoxia. Assessment of intraamniotic infection/inflammation can be performed by an analysis of amniotic fluid with a rapid point-of-care test for IL-6 or MMP-8. Antibiotic treatment of mothers with MSAF can reduce the rate of clinical chorioamnionitis. Defecation in utero is a physiological phenomenon; however, hypoxia, intraamniotic infection/inflammation, and postterm pregnancies are factors associated with MSAF. In the absence of these 3 factors, the etiology remains unknown. Omics analysis of amniotic fluid with meconium could help in the understanding of the pathophysiology of MSAF and in identifying new biomarkers for risk stratification of patients according to MSAF etiology. ■

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