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Morphological changes in the lungs of meconium-stained piglets

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Abstract. Meconium staining of the skin is a common event associated with fetal hypoxia, stillbirths, weak-born piglets, and neonatal mortality. Aspiration of meconium leads to meconium aspiration syndrome (MAS). This study was undertaken to assess the relationship between the degree of meconium staining of the skin at birth, meconium aspiration, and pulmonary changes in porcine neonates. A total of 353 farrowing sows and 3,693 born piglets were monitored during parturition and for 15 days after delivery. Umbilical cords were classified as normal or ruptured. Meconium staining in the skin was graded as nonstained, mildly, moderately, and severely stained. Mortality from birth to 15 days of age was 8.4%. The lungs from 60 meconium-stained piglets and 60 lungs from nonstained piglets were collected and microscopically examined for meconium aspiration and inflammation. Rupture of the umbilical cord was significantly higher ($P < 0.01$)

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in meconium-stained piglets. Microscopically, 32% and 40% of the lungs had evidence of meconium for the stained and nonstained groups, respectively. The microscopic grade of meconium aspiration and inflammatory cells was not different between nonstained and meconium-stained piglets. Aspiration of meconium induced a granulomatous response in the lungs. It was concluded that the grade of meconium staining is a good indicator of fetal hypoxia, but not a good predictor for meconium aspiration and MAS in piglets.

Key words: Meconium; neonatal mortality; umbilical cord.

Asphyxia during parturition is one of the leading causes of intrapartum stillbirths and also contributes substantially to neonatal mortality.^{5,6} The main culprit for fetal hypoxia in near-term fetuses is a decrease in placental blood flow, which can be the result of uterine contractions, umbilical cord compression or rupture, and placental detachment.^{4,12} During intrauterine hypoxia there is visceral redistribution of fetal blood, increased intestinal peristalsis, and relaxation of the anal sphincter that eventually lead to defecation and contamination of the amniotic fluid with meconium. In persistent or severe hypoxia there are also violent inspiratory movements with opened glottis causing aspiration of meconium-contaminated amniotic fluid.² Affected fetuses can die at birth or survive and develop a postnatal condition known as meconium aspiration syndrome (MAS).^{2,15} This syndrome can also occur when babies or animals are born with meconium lodged in the hypopharynx and subsequently aspirate this material at birth with the first few breaths of air.² In either case, aspirated meconium causes airway obstruction, aeration problems, and pulmonary inflammation.

Although experimental models using laboratory and domestic animals are frequently used in the study of human MAS, there are only sporadic reports in calves and foals.⁷ As reported in pediatric medicine, meconium staining of the skin is a reliable indicator of fetal anoxia in piglets.^{12,14} Stained stillbirths and piglets dying at birth or during the first few hours of life may also have gross evidence of meconium in the respiratory tracts.¹³ However, preliminary observations in this laboratory suggest that meconium does not elicit pulmonary inflammatory response in intrapartum stillbirths, perhaps because the time elapsed between aspiration and death is so short. What remains to be elucidated is whether meconium-staining and meconium aspiration results in pulmonary inflammation in piglets born alive. The aim of this study was first to determine if the grade of meconium staining of the skin at birth relates to the degree of meconium aspiration and, second, to investigate if pulmonary inflammation typical of MAS occurs in piglets born alive with meconium aspiration.

The study was conducted in a semi-intensively managed pig farm in Central Mexico. Three hundred and fifty-three hybrid Landrace, Yorkshire, and Large White sows of mixed parity were monitored during 4 months before parturition. Five to six days before the expected date of parturition, sows were allocated individually in plastic-coated floor farrowing pens where they stayed for 15 days. Farrowing was induced in all sows by administration of prostaglandins on day 113 of gestation. Sows were continuously monitored starting at 72 hours before the expected farrowing date and during parturition. At birth,

piglets were classified as liveborn or stillborn. Piglet mortality was classified as stillbirth or neonatal mortality if the piglet died between the time of parturition to day 15, when animals were removed from the sow. According to the degree of meconium staining on the skin, piglets were classified as nonstained, mildly, moderately, and severely stained (Fig. 1). Based on the time of death, piglets were stratified into 3 groups: Group 1 included piglets that died at birth or before day 4; Group 2 included piglets that died 4 to 7 days after birth; and Group 3 included those piglets dying between 8 and 15 days after birth.

At the time of birth umbilical cords were examined and classified as adhered (normal) or ruptured. Adhered cords included those that remained attached or in close contact to the placenta after expulsion or those that detached a few minutes later as a result of piglet movement. Umbilical cords with a loss of continuity along their length at the time of birth were classified as ruptured.

The lungs of 120 piglets that were born alive but died after birth were removed from the thoracic cavity en toto and immersed in 10% neutral-buffered formalin. Fixed lungs were dissected, and the main bronchi were inspected for meconium. Lung samples for histopathology were systematically taken, making a single longitudinal section (2-mm thick) of the entire left lung and 4 transverse sections along the major axes of the right apical, cardiac, intermediate, and diaphragmatic lung lobes. Tissues were embedded in paraffin, sectioned at 4 microns, and stained with hematoxylin and eosin. Periodic acid-Schiff counterstained with hematoxylin was used to identify meconium in cases where the recognition of meconium using hematoxylin and eosin was difficult. The presence and distribution of amniotic fluid cells, meconium, and inflammatory cells were evaluated and scored for bronchi, bronchioles, and alveoli. The scoring system was defined as follows: absent (–) when alveoli and airways were free of epidermal epithelial cells, meconium, or inflammatory cells; minimal (±) when there were sporadic pieces of meconium or epidermal or inflammatory cells; mild (+) when epithelial cells, meconium, or inflammatory cells were present in low frequency; moderate (++) when epithelial cells, inflammatory cells, and meconium were partially filling the bronchoalveolar spaces; and severe (+++) when the airways and alveoli were largely filled with epithelial cells, meconium, or inflammatory cells (Fig. 2).

Data were expressed as percentages or means \pm SD. The associations between meconium staining on the skin, umbilical cord rupture, and time of death were evaluated using 1-way analysis of variance. The distribution and grade of meconium and histological findings in the lungs of neonates born stained with meconium-stained versus

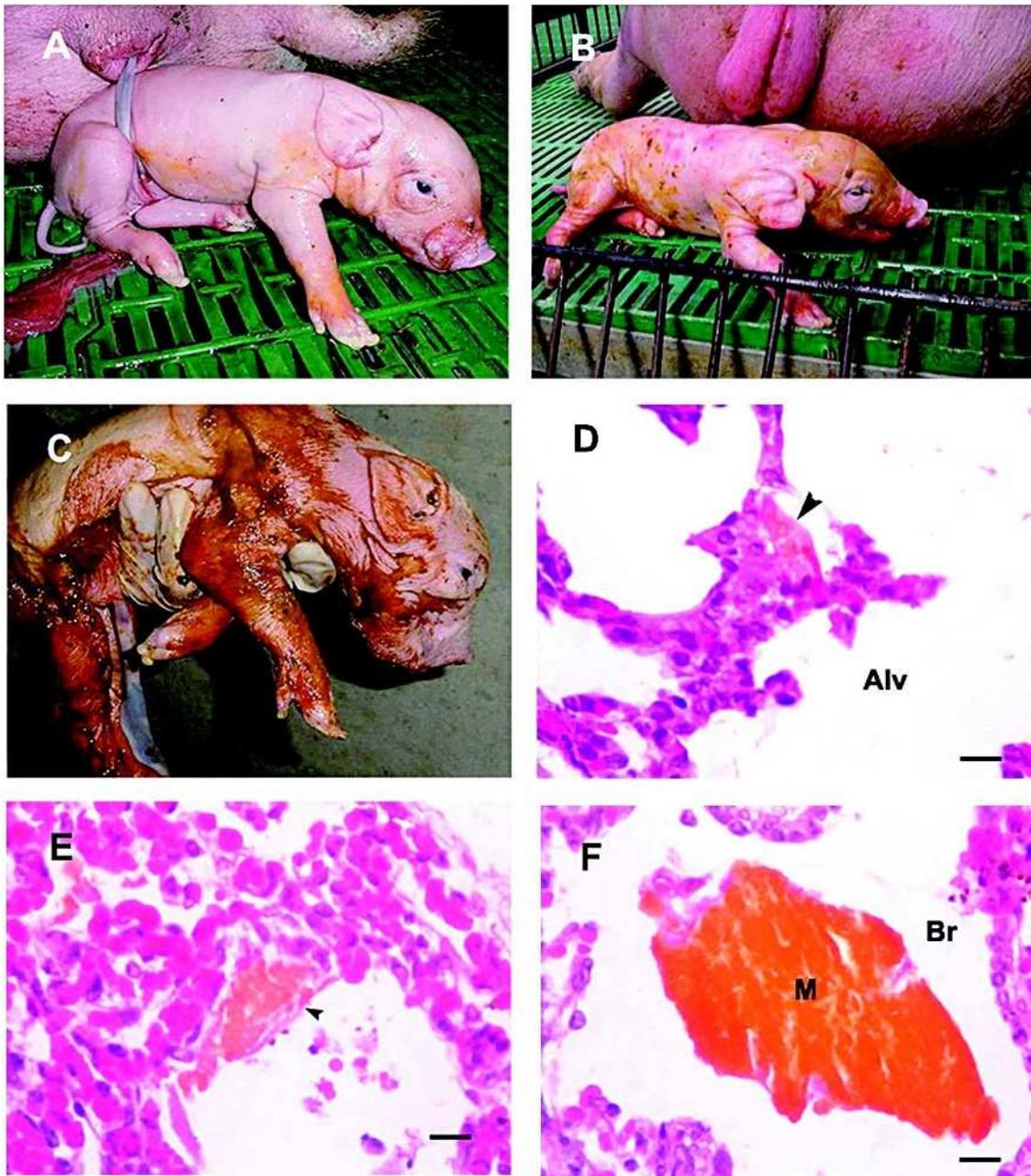


Figure 1. Grades of meconium staining in the skin of piglets. **A**, mild; **B**, moderate; **C**, severe. Microscopic grades of meconium aspiration in the lungs of piglets. **D** = minimal; **E** = mild; **F** = moderate. Hematoxylin and eosin stain. Bar = 10 μ m.

nonstained were compared using the Student's *t*-test, analysis of variance, and chi-square test. $P \leq 0.05$ was considered statistically significant.³

From the 353 parturitions monitored in this study, 3,342 (90.49%) piglets were liveborn and 351 (9.51%) were stillborns. Meconium staining of the skin was present in 1,301 (38.9%) liveborn piglets and in 127 stillborns (58.2%). Of all meconium-stained liveborn piglets, 63% had mild staining, 24% moderate staining, and 13% severe staining (Fig. 1). For the stillborn piglets that had stained skin, the percentages were 58%, 22%, and 20%, respectively. There were no significant differences ($P > 0.05$) in the grades of

meconium staining (mild, moderate, or severe) between stillborn and liveborn piglets.

Neonatal mortality occurred in 282 (8.4%) liveborn piglets. Neonatal mortality in the nonstained group (9.6%) was significantly higher ($P < 0.05$) than in the meconium-stained liveborns (6.7%). According to the degree of meconium staining, the neonatal mortality rate was significantly higher ($P < 0.05$) in piglets born with mild meconium staining as compared to those with moderate or severe staining. The level of meconium staining at birth was not associated ($P > 0.05$) to time of death. Ruptured umbilical cords were most commonly found ($P < 0.05$) in

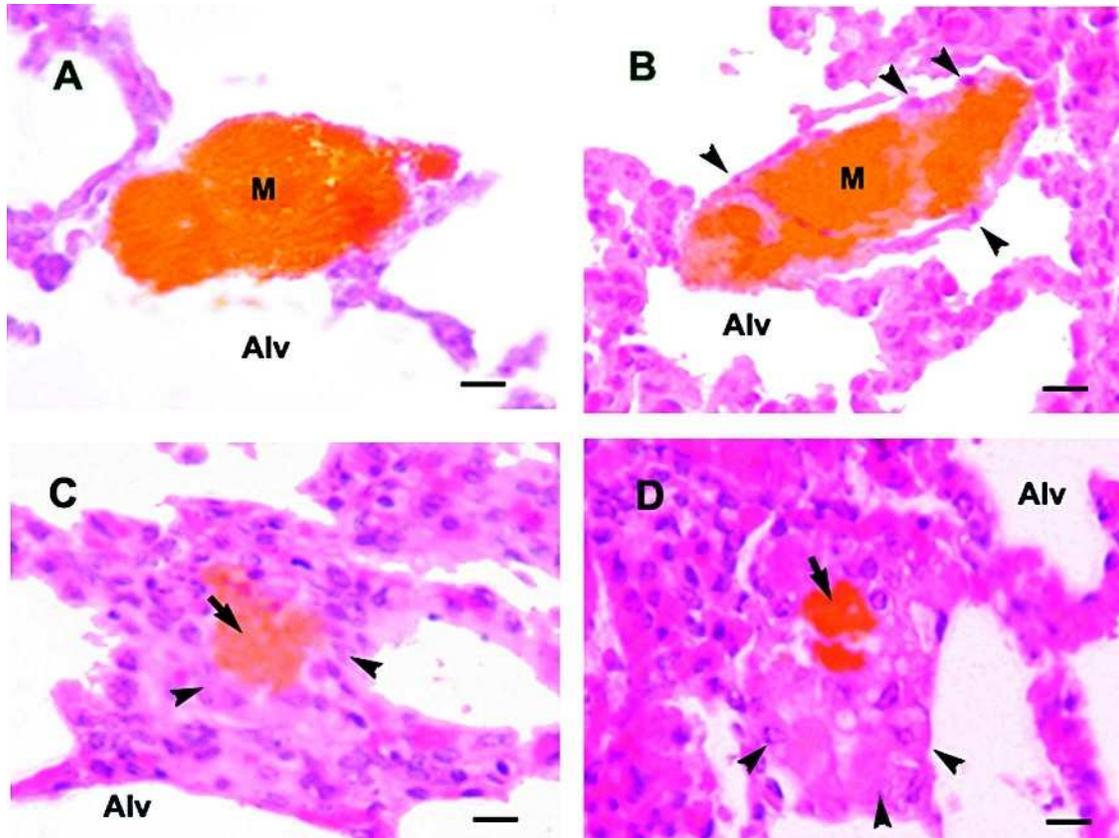


Figure 2. Chronology of the pulmonary response to meconium in neonatal piglets. **A**, meconium (M) free in alveoli; **B**, meconium (M) in alveolar space surrounded by inflammatory cells (arrowheads); **C**, sequestered meconium (arrows) in alveolar walls surrounded by macrophages (arrowheads); **D**, granulomatous response showing meconium (arrow) completely surrounded by macrophages (arrowheads); Alv = alveoli. Hematoxylin and eosin stain. Bar = 10 μ m.

meconium-stained piglets (33%) as compared to those of the nonstained group (10%).

Meconium was not grossly observed in the bronchi or trachea of piglets for both groups, and no gross lesions were observed in the lungs. Meconium was microscopically observed in alveolar and bronchiolar lumens of piglets in both the stained and nonstained groups. Meconium appeared on hematoxylin and eosin stain as a yellow-gold granular aggregate containing abundant keratin and epithelial squames (Fig. 2). Meconium matrix stained intensely red on period acid–Schiff stain. The frequency of meconium in the lung decreased with the age of the piglet in both groups. Paradoxically, the percentage of lungs with meconium at birth or within the first 3 days of life was lower (55%) in piglets born stained as compared to those not stained (80%). However, there were no significant differences ($P > 0.05$) in the frequency of meconium in the lungs of piglets at any time of postnatal death in either group.

The amount of meconium in the lungs was only minimal, mild, or moderate. There were no lungs showing severe degree of meconium aspiration as defined previously. Because of the minimal degree of meconium in the lung, it was not possible to perform a meaningful statistical

comparison between the degree of meconium in the lung and meconium staining of the skin.

Meconium was not associated with inflammatory cells in the lungs of piglets that were stillborn or died within the first 3 days of birth (Fig. 2A). In contrast, piglets that died 4 to 7 days after birth had meconium surrounded by pulmonary alveolar macrophages and polymorphonuclear leukocytes. This inflammatory reaction also involved the bronchiolar and alveolar epithelia (Fig. 2B). In some instances, meconium appeared attached to the alveolar walls (Fig. 2C). The presence of meconium in the lungs of 8–15-day-old piglets was also surrounded by alveolar macrophages and few multinucleated cells that formed focal granulomas (Fig. 2D). The distribution of meconium in the lungs was multifocal, but involving all pulmonary lobes. There were no significant differences ($P > 0.05$) in the topographical distribution of meconium in the lung.

Epidermal epithelial cells and keratin spicules consistent with amniotic aspiration were frequently observed in the bronchioles and alveoli of both the stained and nonstained groups. In both groups, these epithelial cells were only observed in piglets younger than 7 days. Polymorphonuclear leukocytes were sporadically seen in the bronchoalveolar spaces, and these cells were not always associated

with meconium particles. The frequency and severity of pulmonary edema was slightly higher in meconium-stained piglets, particularly in those that died after 3 days of life. Free red blood cells were commonly observed in bronchoalveolar spaces regardless of whether meconium was present in the lungs. There were no significant differences ($P > 0.05$) in the microscopic findings between meconium-stained and nonstained piglets.

Neonatal mortality in this study was lower than the rates of 10% to 30% reported by others.⁹ In addition, the percentage of intrapartum stillborns stained with meconium was also notably lower (58.2% vs. 86.5%) than the percentage reported in another study.¹³ This discrepancy likely reflects different husbandry practices and herd health status between farms. However, meconium staining was higher in liveborn piglets than in stillborn piglets. The mild to moderate meconium staining in piglets is consistent with other studies and suggests that expulsion of meconium into the amniotic fluid is presumably a common event in porcine perinatology.^{12,14} Whether this meconium expulsion results from true anoxia or from an uncomplicated delivery remains to be elucidated. The increased rate of umbilical cord rupture in meconium-stained piglets supports the view that premature rupture of the umbilical cord during parturition leads to anoxia and meconium expulsion.¹⁴ It also supports the view that violent uterine contractions can lead to umbilical cord rupture and intrauterine hypoxia.¹¹

An unexpected finding was the lower mortality rate in piglets born with meconium-stained skin. This lack of a cause-effect relationship suggests that at least in the porcine species, meconium staining at birth is not a good predictor for neonatal viability. It was interesting that gross evidence of meconium was never found in the trachea or bronchi despite the fact that many piglets had microscopic evidence of meconium in the lung. It is likely that during intrauterine aspiration meconium is passed rapidly into the distal airways or that the amount of meconium in airways is insufficient to be grossly visible. This lack of relationship between gross and microscopic findings also occurs in fetal abortion, where meconium is rarely seen grossly in airways but appears microscopically during routine histopathological examination of lungs.⁸

This study showed that the grade of meconium staining of the skin did not correlate with the microscopic grade of meconium aspiration into the lungs. This lack of association has also been reported in human perinatology.¹ Therefore, meconium discoloration of the skin should be considered a sign of preceding intrauterine distress and fetal hypoxia rather than an indicator of the severity of aspiration.

As reported in humans and animals, during the first few days of life meconium remains free in bronchioles and alveoli, and it is not associated with a vigorous pulmonary inflammation. In time, meconium becomes surrounded by pulmonary alveolar macrophages and polymorphonuclear leukocytes and eventually forms microscopic granulomas.¹⁰ Although the bronchoalveolar inflammation in response to meconium aspiration is consistent with autopsy findings in babies succumbing to MAS, the lungs of piglets in this study did not show the typical epithelial necrosis and interstitial edema reported in rats and rabbits experimen-

tally inoculated with meconium.¹⁰ This difference between natural and experimental studies is likely because of the concentration of meconium in the lung. It is possible that meconium-induced alveolar injury is dose-dependent, and the concentration of meconium aspirated by piglets was insufficient to cause severe injury. Also, a low concentration of aspirated meconium in piglets could explain the lack of aeration problems such as atelectasis and hyperinflation commonly described in babies and animal models of MAS.^{15,16}

As reported in experimental studies, with time meconium is completely surrounded by pulmonary alveolar macrophages and eventually becomes sequestered into alveolar walls. At the later stages of aspiration meconium is no longer visible but becomes part of a focal granulomatous response. To the authors' knowledge, this is the first time that microscopic granulomas are reported in the lungs of pigs or other domestic animals as a long-term effect of meconium aspiration. This multifocal granulomatous response in the piglets was similar to that described 7 days after the inoculation of meconium in neonatal rats. Mineralization of meconium reported in rats after 14 days of experimental inoculation was not observed in the lungs of piglets.¹⁰

The multifocal distribution of meconium in all pulmonary lobes of piglets was similar to that described in natural cases and experimental models of MAS in calves, piglets, and laboratory animals.^{7,10,16} In contrast to mature animals in which aspiration and inflammation tend to be severe and cranioventral, intrauterine aspiration of meconium and amniotic fluid results in a mild but diffuse inflammatory response.⁸ Aspiration of amniotic fluid in piglets was corroborated by the presence of epidermal epithelial cells and keratin derived from the amniotic fluid. It is widely accepted that these cells and keratin desquamate from the fetal epidermis into the amniotic fluid. This fluid is normally ingested by the fetus along with other exfoliated cells from the digestive tract.² Epithelial cells and keratin were free in bronchoalveolar spaces in piglets from birth to 3 postnatal days, and after this time epithelial cells become attached to alveolar walls eventually leading to microscopic granulomas.

It was concluded that meconium staining of the skin occurs frequently in stillborn and liveborn piglets. In piglets, meconium staining at birth is a good indicator of premature rupture of the umbilical cord. However, meconium staining of the skin is not a good predictor of the amount of meconium aspiration into the lungs in liveborn piglets. Meconium staining of the skin likely relates to the duration of fetal hypoxia during parturition but it does not relate to neonatal mortality. Meconium aspiration in liveborn piglets induces a mild multifocal granulomatous inflammation involving all pulmonary lobes. Microscopically, lung lesions are similar to those described in experimental MAS but the long-term sequel of this syndrome in piglets remains to be investigated.

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