The Journal of Maternal-Fetal & Neonatal Medicine

ISSN: 1476-7058 (Print) 1476-4954 (Online) Journal homepage: www.tandfonline.com/journals/ijmf20

Changes in adrenomedullin in bronchoalveolar lavage fluid with chorioamnionitis in a sheep-based model

Adele Fabiano, Daniele Panichi, Simonetta Picone, Giuseppe Lapergola, Gabriella Levantini, Ebe D'Adamo, Mariachiara Strozzi, Danilo AW. Gavilanes, Boris W. Kramer, Francesca Gazzolo, Ali Saber Abdelhameed & Diego Gazzolo

To cite this article: Adele Fabiano, Daniele Panichi, Simonetta Picone, Giuseppe Lapergola, Gabriella Levantini, Ebe D'Adamo, Mariachiara Strozzi, Danilo AW. Gavilanes, Boris W. Kramer, Francesca Gazzolo, Ali Saber Abdelhameed & Diego Gazzolo (2025) Changes in adrenomedullin in bronchoalveolar lavage fluid with chorioamnionitis in a sheep-based model, The Journal of Maternal-Fetal & Neonatal Medicine, 38:1, 2456502, DOI: 10.1080/14767058.2025.2456502

To link to this article: https://doi.org/10.1080/14767058.2025.2456502

Published online: 23 Jan 2025.

THE JOURNAL OF MATERNAL-FETAL & NEONATAL MEDICINE
2025, VOL. 38, NO. 1, 2456502
https://doi.org/10.1080/14767058.2025.2456502

Taylor & Francis
Taylor & Francis Group

ORIGINAL ARTICLE

Changes in adrenomedullin in bronchoalveolar lavage fluid with chorioamnionitis in a sheep-based model

Adele Fabiano[a], Daniele Panichi[b], Simonetta Picone[c], Giuseppe Lapergola[d], Gabriella Levantini[d], Ebe D'Adamo[d], Mariachiara Strozzi[e], Danilo AW. Gavilanes[f], Boris W. Kramer[g], Francesca Gazzolo[h], Ali Saber Abdelhameed[i] and Diego Gazzolo[d]

[a]Neonatal Intensive Care Unit, San Giovanni Addolorata Hospital, Rome, Italy; [b]Neonatal Intensive Care Unit, Maggiore Hospital, AUSL Bologna, Bologna, Italy; [c]Neonatology and Neonatal Intensive Care Unit, Policlinico Casilino General Hospital, Rome, Italy; [d]Neonatal Intensive Care Unit, G d'Annunzio University of Chieti, Italy; [e]Ospedale Cardinal Massaia, Asti, Italy; [f]Department of Pediatrics and Neonatology, Maastricht University, Maastricht, The Netherlands; [g]Department of Neonatology, Poznan University of Medical Sciences, Poznan, Poland; [h]Magna Graecia University, Catanzaro, Italy; [i]Department of Pharmaceutical Chemistry, College of Pharmacy, King Saud University, Riyadh, Saudi Arabia

ABSTRACT

Background: Adrenomedullin (AM) is a potent angiogenic, antioxidant and anti-inflammatory peptide protecting the developing lung from injury due to bronchopulmonary dysplasia (BPD) of the preterm infant. At this stage, no data on the potential effects of chorioamnionitis (CA) occurrence and glucocorticoids (GC) administration on AM in developing lungs are still lacking.
Objective: to investigate, in a sheep-based model, the positive/side-effects of combined exposure to CA and GC on AM concentrations measured in bronchoalveolar lavage fluid (BALF).
Methods: Time-mated ewes were randomly admitted to one of six treatment groups receiving injection: saline (controls); lipopolysaccharide (L) in intra-amniotic fluid treated alone at 7 or 14 d before delivery or associated with betamethasone (B) intramuscularly; B treated alone (7d) or associated with L (14d). Lambs were surgically delivered at 120 days gestation and euthanized. BALF was used for AM measurement in the studied groups.
Results: AM BALF levels significantly ($p < 0.05$, for all) changed both to B and L exposure in a time-dependent manner. The latter was characterized by AM levels at short term superimposable to controls, whilst significantly ($p > 0.05$) decreased at long-term. The former showed increased AM at short and decreased at long-term ($p < 0.05$, for all), respectively.
Conclusions: the present results showing AM BALF changes in a sheep-based model support the AM role in the hemodynamic patterns due to CA and BPD occurrence and open the way to further studies investigating the role of vasoactive agents as trustable markers of lung development/damage.

ARTICLE HISTORY
Received 11 October 2024
Revised 12 December 2024
Accepted 13 January 2025

KEYWORDS
Adrenomedullin; BALF; chorioamnionitis; BPD; sheep

Introduction

Adrenomedullin (AM) is an endogenous peptide with potent angiogenic, antioxidant, and anti-inflammatory properties first isolated from human pheochromocytoma cells [1]. AM is expressed in several tissues of the body, including the blood vessels, the kidneys, and the lungs, playing a protective role in the cardiovascular, cerebral, and respiratory systems [2–8].

Studies on humans and experimental models have shown that AM plays a crucial role in endothelial growth, in vascular development and in the maintenance of the endothelial barrier function after acute hypo/hyperoxia insult causing multiorgan/lung injury [5,9–11]. Notably, in the human fetal lung the increased expression of AM is gestational age (GA) dependent, suggesting a role for the peptide in the mechanisms of fetal lung differentiation, maturation and in the transitional changes in human pulmonary blood flow at birth [10–12]. More recently, in a rat model of induced bronchopulmonary dysplasia (BPD), it has been shown that AM: i) promotes lung angiogenesis and alveolar development [13,14], and ii) protects against acute lung injury due to mechanical ventilation, ischemia-reperfusion and hyperoxia injury, and following administration of inflammation-inducing

CONTACT Diego Gazzolo dgazzolo@hotmail.com Neonatal Intensive Care Unit, G. d'Annunzio University of Chieti, 65100 Chieti, Italy.

agents (i.e. lipopolysaccharide, L) [15]. Moreover, it has been suggested that AM might protect the developing lung from alveolar injury occurring in BPD.

Despite recent technological advances in diagnosis and treatment, BPD still constitutes the most dramatic complication in infants born extremely preterm [16]. In preterm infants, BPD accounts from 48 to 68% and is inversely proportional with GA [17]. The etiology of BPD is multifactorial: genetic susceptibility, pregnancy disorders (hypertension, smoking), prematurity, mechanical ventilation, sepsis and chorioamnionitis (CA) are the major factors currently involved [16,17]. In this regard, an understanding of the timing of lung vascular and alveolar developments/damage is of the utmost importance for successful therapeutic strategies. At this stage, the standard of care treatment to improve fetal lung maturation consists of a single course of glucocorticoids (GC) administered prior to delivery [18]. Results from an experimental model showed that GC can inhibit both functional lung maturation and lung development.[19]. This means that a large number of preterm infants are exposed *in-utero* to pro/anti-inflammatory stimuli, both of which can alter lung development, thus predisposing to BPD. In this regard, data on the potential effects of CA and GC on changes in AM in developing lungs are still lacking.

Therefore, in the present sheep-based model we investigated the positive/side-effects of combined exposure to CA and GC on AM concentrations measured in bronchoalveolar lavage fluid (BALF).

Materials and methods

The study was approved by Animal Ethics Committees at the University of Maastricht, The Netherlands.

Time-mated ewes carrying singleton fetuses were randomly admitted to one of the six groups receiving injection of: i) L in intra-amniotic fluid (10 mg Escherichia coli 055:B5, Sigma Chemical, St. Louis, MO, USA), ii) betamethasone (B) intramuscularly (Celestone Soluspan, Schering-Plough, North Ryde, New South Wales, NSW, Australia) at a dose of 0.5 mg/kg maternal weight, or iii) saline (controls) at 107 days and/or 114 days GA in different permutations and combinations (Figure 1). A single intramuscular injection of 150 mg medroxyprogesterone acetate (Depo-Provera, Kenral, NSW, Australia) at 100 days GA preventing prematurity due to betamethasone treatment was administered in all ewes. At 120 days GA (term = 150 days) surgical delivery and euthanize with 100 mg/kg pentobarbital was performed in all lambs. Each fetus was weighed, and fetal cord blood was collected. The lungs were removed, separated, and weighed prior to a bronchoalveolar lavage with 0.9% NaCl. BALF was collected for AM measurement.

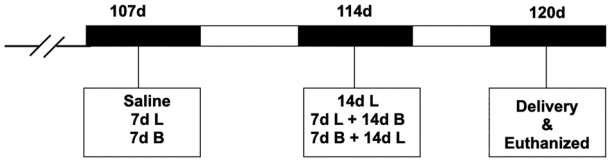

Figure 1. Study design. Pregnant ewes received an intra-amniotic (IA) injection of lipopolysaccharide (L) and/or an intra-muscular injection of betamethasone (B) and/or an equivalent injection of saline for control animal at 107 days and/or 114 days gestation (GA). Lambs were delivered preterm by cesarean section at 120 days GA (term = 150 days GA).
Abbreviations: 7d, 7-day exposure; 14d, 14-day exposure.

AM measurement

AM in BALF was measured after extraction and purification in 500 µL of samples collected at animal sacrifice and stored at −70 °C. A specific RIA kit (Phoenix Pharmaceuticals, Mountain View CA, USA) with rabbit polyclonal antibody raised against human AM was used. Each measurement was performed in duplicate, and the averages were reported. The sensitivity of the assay was 0.34 ng/mL. The antibody cross-reacts 100% with human AM and no cross-reactivity was shown with rat AM, amylin, CGRP, endothelin-1, α-atrial natriuretic peptide, or brain natriuretic peptide. The intra- and inter-assay coefficients of variance were 5.1% and 12.0%, respectively.

Monitoring parameters

In all animals studied, birthweight (BW), gender (male/female), cord blood pH, partial pressure of arterial oxygen (pO_2), and partial pressure of arterial carbon dioxide (pCO_2) were recorded.

Statistical analysis

Data is reported as mean and standard deviation. The Kolmogorov–Smirnov test was performed for assessing a Gaussian distribution. ANOVA 1-way was used for repeated measurements (followed by the *post hoc* Tukey test for multiple comparisons) and t- test for two groups only. Linear regression analysis was performed for correlations between AM and clinical parameters. A $p < 0.05$ was considered significant.

Results

In Table 1 descriptive data of the studied groups are reported. No significant differences among groups ($p > 0.05$, for all) have been found regarding BW, gender, and analysis of blood gas in the fetal cord (pH, pCO_2, pO_2).

Table 1. Animals' general characteristics in the studied groups: controls; lipopolysaccharide (L) treated alone at 7 or 14 days (d) before delivery or associated with betamethasone (B); B treated alone or associated with L. Data is given as mean and SD.

Group	BW (Kg)	Gender (M/F)	Cord Blood pH	pO_2 (mmHg)	pCO_2 (mmHg)
Controls	2.61 ± 0.37	1/4	7.24 ± 0.07	9.38 ± 3.77	72.50 ± 10.10
7d L	2.34 ± 0.54	4/4	7.26 ± 0.07	7.74 ± 3.02	72.41 ± 9.28
14d L	2.53 ± 0.31	5/3	7.26 ± 0.06	7.66 ± 3.61	66.56 ± 9.30
7d B	2.67 ± 0.39	4/3	7.20 ± 0.10	8.77 ± 4.65	77.26 ± 15.45
14d B + 7d L	2.67 ± 0.27	1/5	7.25 ± 0.11	9.82 ± 4.60	71.77 ± 15.56
14d L + 7d B	2.09 ± 0.16	3/5	7.25 ± 0.08	8.60 ± 2.58	73.62 ± 9.65

Abbreviations: BW, birthweight; M, male; F, female; pO_2 arterial oxygen partial pressure; pCO_2, arterial carbon dioxide partial pressure.

Concentrations of AM in BALF were measurable in all samples collected. Results are reported in Table 2.

In the control group, AM levels were significantly higher ($p < 0.05$, for all) than those detected in 14d L alone, in 7d B alone and in the 14d L/B groups. No significant differences ($p > 0.05$, for both) were observed between controls and between 7d L and 14d B/L groups.

AM concentrations, in the 7d L group, were significantly higher ($p < 0.05$, for all) than those of the 14d L, 7d B and 14 L/B groups. AM levels in the 7d L group did not differ ($p > 0.05$, for both) when compared with those of the control and 14d B/L group.

In the 14d L group, no differences in AM levels were observed when compared with 7d B, whilst it was lower ($p < 0.05$, for all) than that detected in the control, 14d B/L and 7d L groups.

Lower ($p < 0.05$, for all) AM levels were observed in 7d B than the control, 7d L and 14d B/L groups. No differences in AM ($p > 0.05$, for all) were detectable between 7d B and 14d L and 14d L/B, respectively.

AM was higher ($p < 0.05$, for all) in the 14d B/L group than the 14d L, 7d B and 14d L/B groups. Furthermore, no differences in AM were observed with the control and 7d L groups.

In the 14d L/B group, AM was lower ($p < 0.05$, for all) than the control, 7d L and 14d B/L groups. No differences in AM ($p > 0.05$, for both) were shown when compared with the 14d L and 7d B groups.

Finally, no correlations ($p > 0.05$, for all) between AM, measured in all studied groups, and fetal cord blood pH, pCO_2 and pO_2 were found.

Discussion

Despite technological improvement in perinatal diagnosis and treatment, CA still constitutes a leading cause of about 70% of preterm deliveries [20]. The main dramatic pattern of CA is characterized by fetal systemic and lung inflammation and injury finally resulting in BPD [21]. Knowledge of the main pathophysiological

Table 2. Adrenomedullin (AM) bronchoalveolar lavage fluid levels expressed in ng/Kg in the studied animals: controls; lipopolysaccharide (L) treated alone at 7 or 14 days (d) before delivery or associated with betamethasone (B); B treated alone or associated with L. Data is given as mean and SD. AM levels were higher ($p < 0.05$, for all) in: i) controls than 14d L alone, 7d B alone and 14d L/B groups, ii) 7d L than 14d L, 7d B and 14 L/B groups, and iii) 14d B/L than 14d L, 7d B, and 14d L/B groups. Moreover, AM levels were lower ($p < 0.05$, for all) in: i) 14d L than controls, 14d B/L and 7d L groups, ii) 7d B than controls, 7d L and 14d B/L groups, and iii) 14d L/B than controls, 7d L and 14d B/L groups, respectively.

Group	AM (ng/Kg)
Controls	2910.89 ± 435.53
7d L	2763.88 ± 612.64
14d L	2147.46 ± 284.49
7d B	2158.84 ± 334.82
14d B + 7d L	2971.67 ± 431.29
14d L + 7d B	2065.24 ± 316.62

mechanisms leading to BPD is still incomplete and a matter of debate. In this regard, the increase in the number of studies on animal models, particularly sheep-based, offers additional and useful information [22]. Notably, clinical and experimental studies have evidenced that antenatal corticosteroids are also effective in the context of CA [23]. Combined exposure to antenatal GC and CA is common for preterm fetuses, but the timing of exposure can vary from case to case [24,25]. Thus, measurement of biomarkers involved in fetal-neonatal lung and cardiovascular development could be especially useful [26].

In the present study we tested the interactions of antenatal exposure to B and intra-amniotic L on the concentrations of a well-known vasoactive and angiogenic agent, namely AM, in BALF. Results provide evidence that exposure to both B and L significantly affected concentrations of AM in BALF in a time-dependent manner. Furthermore, AM levels significantly changed depending on the combined administration of B-L or L-B. To our knowledge, the present findings constitute the first observation in a population selected in such way.

The pattern of AM in BALF of the fetuses exposed to L warrants further consideration. In particular, we found that AM in BALF is superimposable to controls in the short term (i.e. 7d), whilst decreasing in the long-term (i.e. 14 d) from the occurrence of CA. The latter confirms, in part, previous observations in animal models where an AM deficiency was found after L administration [15]. Discrepancies regarded the animal model (murine vs. sheep), and design of the study protocol [27]. AM deficiency has been found to empower and impair the resolution of the administration of L and its induced lung injury [15]. The main

known mechanism regarded the activation of STAT1 and STAT3 in saccular lungs and pulmonary vessels [28]. Transcription factors are involved in the pathogenesis of BPD regulating a cascade of events such as cell proliferation and development, inflammation, injury repair and vascularization [29]. Conversely, these findings, characterized by an increase in AM in the first phases from administration of L, constitute the first observation in a sheep-based model and are backed up by a previous observation in a murine lung model [30]. The explanation lies in the pathophysiological steps leading to sepsis characterized by an early vasodilation process, from administration of L, in which AM together with other vasoactive agents play a significant role [31]. However, this phenomenon is transient as confirmed by the decreased AM levels in BALF at 14d supporting the idea that the fetal lung is exposed to inflammatory injury leading to impaired lung and pulmonary vasculature development [32].

In the present series we also found that administration of B alone and combined early and/or late administration of L/B (7-14d) affected AM levels in BALF. Results on administration of B alone showing decreased AM levels offer support to the controversial and debated relationship between AM and steroid administration [33]. Discrepancies with previous observations, in humans and animal models, lie in different sites of AM assessment (placenta, blood vs. BALF), choice of steroid to be administered (dexamethasone vs. betamethasone), study-design and endpoints [34]. Another explanation may reside in the interval between administration of GC and timing of delivery, as shown in the 14d B/7d L group [35].

The findings on the different effects of the administration of L/B and vice versa on AM deserve further consideration. In particular AM reached its highest peak in early L (7d) and late B (14d) treated animals. Results suggestive of increased AM release due to early occurrence of CA as for the 7d L alone group offer additional support to the phenomenon of sepsis-related transient vasodilation [36]. Another explanation for increased AM regards the effects of B, suggesting the time-dependent effects of GC on the prevention of lung immaturity [37,38]. Studies in human and animal models showed the longer the interval between administration of GC and delivery, the lower the effects of GC on lung maturation [39]. This is corroborated by lower AM levels in 7d B/14d L animals. Altogether, it is reasonable to argue for the consolidated beneficial effects of GC on lung maturation once, in high-risk cases for CA, administered at the due time before delivery. Further studies in humans and animal models aimed at elucidating the best time for GC administration and the timing of delivery are therefore justified. In this respect, the concomitants side-effects of acute/chronic hypoxia on AM in the perinatal period have to be taken into the due account [40–42].

In the present study we identified the following limitations: i) no recordings of hemodynamic parameters in fetal/placental districts have been performed, and ii) no AM levels in fetal-maternal blood were measured in order to explore differences in AM content among different districts.

In conclusion, the present results on changes in AM in BALF due to different scenarios occurring in a sheep-based model complicated by CA and BPD pave the way to further studies aimed at elucidating the role of vasoactive agents such as AM as reliable markers of lung development/damage.

Author contributions

AF, DP, SP, GL, GL, EDA, MS, DAWG, BWK, FG, ASA, contributed to the conceptualization, investigation and writing of the original draft. DG contributed to the project administration, conceptualization, investigation, supervision and writing, review and editing. All the authors have accepted responsibility for the entire content of this submitted manuscript and approved submission. All authors have read and agreed to the published version of the manuscript.

Disclosure statement

The funding organizations played no role in the study design; in the collection, analysis, and interpretation of data; in the writing of the report or in the decision to submit the report for publication.

Data availability statements

Due to infrastructure limitations authors cannot provide data availability.

Funding

Research funding: This work is part of the I.O. PhD International Program under the auspices of the Italian Society of Neonatology and was partially supported by grants to DG from "I Colori della Vita Foundation," Italy. Authors extend their sincere appreciation to Researchers Supporting Project number (RSPD2025R750), King Saud University, Riyadh, Saudi Arabia.

References

[1] Kitamura K, Kangawa K, Kawamoto M, et al. Adrenomedullin: a novel hypotensive peptide isolated from human pheochromocytoma. Biochem Biophys Res Commun. 1993; 192(2):553–560. doi:10.1006/bbrc.1993.1451.

[2] Totsune K, Takahashi K, Mackenzie HS, et al. Increased gene expression of adrenomedullin and adrenomedullin-receptor complexes, receptor-activity modifying protein (RAMP) 2 and calcitonin-receptor-like receptor (CRLR) in the hearts of rats with congestive heart failure. Clin Sci (Lond). 2000;99(6):541–546. doi:10.1042/cs0990541.

[3] Nagata D, Hirata Y, Suzuki E, et al. Hypoxia-induced adrenomedullin production in the kidney. Kidney Int. 1999;55(4):1259–1267. doi:10.1046/j.1523-1755.1999.00361.x.

[4] Hirata Y, Hayakawa H, Suzuki Y, et al. Mechanisms of adrenomedullin-induced vasodilation in the rat kidney. Hypertension. 1995;25(4 Pt 2):790–795. doi:10.1161/01.hyp.25.4.790.

[5] Marinoni E, Di Iorio R, Alò P, et al. Immunohistochemical localization of adrenomedullin in fetal and neonatal lung. Pediatr Res. 1999;45(2):282–285. doi:10.1203/00006450-199902000-00021.

[6] Satoh F, Takahashi K, Murakami O, et al. Immunocytochemical localization of adrenomedullin like immunoreactivity in the human hypothalamus and the adrenal gland. Neurosci Lett. 1996;203(3):207–210. doi:10.1016/0304-3940(95)12294-x.

[7] Kis B, Abrahám CS, Deli MA, et al. Adrenomedullin in the cerebral circulation. Peptides. 2001;22(11):1825–1834. doi:10.1016/s0196-9781(01)00533-2.

[8] Florio P, Abella R, Marinoni E, et al. Adrenomedullin blood concentrations in infants subjected to cardiopulmonary bypass: correlation with monitoring parameters and prediction of poor neurological outcome. Clin Chem. 2008;54(1):202–206. doi:10.1373/clinchem.2007.087700.

[9] Kamata S, Kamiyama M, Usui N, et al. Is adrenomedullin involved in the pathophysiology of persistent pulmonary hypertension of the newborn? Pediatr Surg Int. 2004;20(1):24–26. doi:10.1007/s00383-003-1071-9.

[10] Gazzolo D, Marinoni E, Giovannini L, et al. Circulating adrenomedullin is increased in preterm newborns developing intraventricular hemorrhage. Pediatr Res. 2001;50(4):544–547. doi:10.1203/00006450-200110000-00020.

[11] Boldt T, Luukkainen P, Fyhrquist F, et al. Birth stress increases adrenomedullin in the newborn. Acta Paediatr. 1998;87(1):93–94. doi:10.1111/j.1651-2227.1998.tb01393.x.

[12] Rudolph AM. Adrenomedullin: its role in perinatal adaptation. Acta Paediatr. 1998;87(3):235–236. doi:10.1111/j.1651-2227.1998.tb01430.x.

[13] Vadivel A, Abozaid S, van Haaften T, et al. Adrenomedullin promotes lung angiogenesis, alveolar development, and repair. Am J Respir Cell Mol Biol. 2010;43(2):152–160. doi:10.1165/rcmb.2009-0004OC.

[14] Menon RT, Shrestha AK, Shivanna B. Hyperoxia exposure disrupts adrenomedullin signaling in newborn mice: implications for lung development in premature infants. Biochem Biophys Res Commun. 2017;487(3):666–671. doi:10.1016/j.bbrc.2017.04.112.

[15] Shrestha AM, Menon RT, Yallampalli C, et al. Adrenomedullin deficiency potentiates lipopolysaccharide-induced experimental bronchopulmonary dysplasia in neonatal mice. Am J Pathol. 2021;191(12):2080–2090. doi:10.1016/j.ajpath.2021.09.001.

[16] Jobe AH, Bancalari E. Bronchopulmonary dysplasia. Am J Respir Crit Care Med. 2001;163(7):1723–1729. doi:10.1164/ajrccm.163.7.2011060.

[17] Kalikkot Thekkeveedu R, Guaman MC, Shivanna B. Bronchopulmonary dysplasia: a review of pathogenesis and pathophysiology. Respir Med. 2017;132:170–177. doi:10.1016/j.rmed.2017.10.014.

[18] Fee EL, Stock SJ, Kemp MW. Antenatal steroids: benefits, risks, and new insights. J Endocrinol. 2023;258(2):e220306. doi:10.1530/JOE-22-0306.

[19] Schmidt AF, Kemp MW, Rittenschober-Böhm J, et al. Low-dose betamethasone-acetate for fetal lung maturation in preterm sheep. Am J Obstet Gynecol. 2018;218(1):132.e1–132.e9. doi:10.1016/j.ajog.2017.11.560.

[20] Hall M, Hutter J, Suff N, et al. Antenatal diagnosis of chorioamnionitis: a review of the potential role of fetal and placental imaging. Prenat Diagn. 2022;42(8):1049–1058. doi:10.1002/pd.6188.

[21] Panneflek TJR, Kuypers KLAM, Polglase GR, et al. The influence of chorioamnionitis on respiratory drive and spontaneous breathing of premature infants at birth: a narrative review. Eur J Pediatr. 2024;183(6):2539–2547. doi:10.1007/s00431-024-05508-4.

[22] Stojanovska V, Atta J, Kelly SB, et al. Increased prostaglandin e2 in brainstem respiratory centers is associated with inhibition of breathing movements in fetal sheep exposed to progressive systemic inflammation. Front Physiol. 2022;13:841229. doi:10.3389/fphys.2022.841229.

[23] Collins JJP, Kuypers E, Nitsos I, et al. LPS-induced chorioamnionitis and antenatal corticosteroids modulate Shh signaling in the ovine fetal lung. Am J Physiol Lung Cell Mol Physiol. 2012;303(9):L778–787.

[24] Jobe AH. Antenatal factors and the development of bronchopulmonary dysplasia. Semin Neonatol. 2003;8(1):9–17. doi:10.1016/s1084-2756(02)00188-4.

[25] Pereira-Fantini PM, Tingay D, Lakshminrusimha S. A complex inflammatory mix: chorioamnionitis, antenatal steroids and early postnatal budesonide. Pediatr Res. 2024;96(3):560–562. doi:10.1038/s41390-024-03219-y.

[26] Hooper SB, Harding R. Fetal lung liquid: a major determinant of the growth and functional development of the fetal lung. Clin Exp Pharmacol Physiol. 1995;22(4):235–247. doi:10.1111/j.1440-1681.1995.tb01988.x.

[27] Mannel DN. Advances in sepsis research derived from animal models. Int J Med Microbiol. 2007;297(5):393–400. doi:10.1016/j.ijmm.2007.03.005.

[28] Pradhan A, Dunn A, Ustiyan V, et al. The S52F FOXF1 mutation inhibits STAT3 signaling and causes alveolar capillary dysplasia. Am J Respir Crit Care Med. 2019;200(8):1045–1056. doi:10.1164/rccm.201810-1897OC.

[29] Thébaud B, Abman SH. Bronchopulmonary Dysplasia: where have all the vessels gone? Roles of angiogenic growth factors in chronic lung disease. Am J Respir Crit Care Med. 2007;175(10):978–985. doi:10.1164/rccm.200611-1660PP.

[30] Agorreta J, Zulueta JJ, Montuenga LM, et al. Adrenomedullin expression in a rat model of acute lung injury induced by hypoxia and LPS. Am J Physiol Lung Cell Mol Physiol. 2005;288:536–545.

[31] Dutzmann J, Daniel JM, Bauersachs J, et al. Emerging translational approaches to target STAT3 signalling and its impact on vascular disease. Cardiovasc Res. 2015;106(3):365–374. doi:10.1093/cvr/cvv103.

[32] Zhang S, Patel A, Moorthy B, et al. Adrenomedullin deficiency potentiates hyperoxic injury in fetal human pulmonary microvascular endothelial cells. Biochem Biophys Res Commun. 2015;464(4):1048–1053. doi:10.1016/j.bbrc.2015.07.067.

[33] Marinoni E, Sambuchini A, Pacioni K, et al. Effects of corticosteroids and progesterone on adrenomedullin output and expression in human fetal membranes and placenta trophoblasts. Am J Obstet Gynecol. 2010;202(2):196.e1–6. doi:10.1016/j.ajog.2009.10.870.

[34] Marinoni E, Zacharopoulou C, Di Rocco A, et al. Effect of betamethasone in vivo on placental adrenomedullin in human pregnancy. J Soc Gynecol Investig. 2006; 13(6):418–424. doi:10.1016/j.jsgi.2006.05.003.

[35] Duan W, Ming L, Zhu Y. Antenatal corticosteroid administration to birth interval and neonatal outcomes in very preterm infants: a secondary analysis based on a prospective cohort study. PLoS One. 2023;18(2):e0281509. doi:10.1371/journal.pone.0281509.

[36] Makino I, Honda K, Makino Y, et al. Phenotypic changes of adrenomedullin receptor components, RAMP2, and CRLR mRNA expression in cultured rat vascular smooth muscle cells. Biochem Biophys Res Commun. 2001;288(3):515–520. doi:10.1006/bbrc.2001.5805.

[37] Dalziel SR, Lim VK, Lambert A, et al. Antenatal exposure to betamethasone: psychological functioning and health related quality of life 31 years after inclusion in randomized controlled trial. BMJ. 2005;331(7518):665–677. doi:10.1136/bmj.38576.494363.E0.

[38] Kuypers E, Collins JJP, Kramer BW, et al. Intra-amniotic LPS and antenatal betamethasone: inflammation and maturation in pre-term lamb lungs. American Journal of Physiology-Lung Cellular and Molecular Physiology. 2012;302(4):L380–L389. doi:10.1152/ajplung.00338.2011.

[39] Norman M, Piedvache A, Børch K, et al. Association of short antenatal corticosteroid administration-to-birth intervals with survival and morbidity among very preterm infants: results from the EPICE cohort. JAMA Pediatr. 2017;171(7):678–686. doi:10.1001/jamapediatrics.2017.0602.

[40] Abella R, Satriano A, Frigiola A, et al. Adrenomedullin alterations related to cardiopulmonary bypass in infants with low cardiac output syndrome. J Matern Fetal Neonatal Med. 2012;25(12):2756–2761. doi:10.3109/14767058.2012.718393.

[41] Di Iorio R, Marinoni E, Lituania M, et al. Adrenomedullin increases in term asphyxiated newborns developing intraventricular hemorrhage. Clin Biochem. 2004;37(12):1112–1116. doi:10.1016/j.clinbiochem.2004.04.011.

[42] Di Iorio R, Marinoni E, Gazzolo D, et al. Maternal nitric oxide supplementation increases adrenomedullin concentrations in growth retarded fetuses. Gynecol Endocrinol. 2002;16(3):187–192. doi:10.1080/gye.16.3.187.192.