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Obstetrics and Gynaecology Research

doi:10.1111/jog.12349 J. Obstet. Gynaecol. Res. Vol. 40, No. 2: 336–337, February 2014

# Annual report of Subcommittee for Examination of Causes of Maternal Death and their Prevention in Perinatology Committee, Japan Society of Obstetrics and Gynecology, 2013

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## Introduction

Hemorrhage in the third stage of labor is the most frequent cause of maternal death. A national survey conducted by the subcommittee last year revealed the following bleeding-related factors during the third stage of labor: (i) atonic bleeding; (ii) abnormal placental adherence; (iii) abnormal placental adherence plus atonic bleeding; and (iv) placental abruption. In short, atonic bleeding is the most important factor associated with massive bleeding during the third stage of labor. In addition to this, the following two studies have been conducted this year:

## Study 1

A secondary investigation to clarify the pathology of frequently occurring atonic bleeding, involving the same patients as those studied last year.

## Study 2

To examine the relationship between the type of amniotic fluid embolism and autopsy findings, in order to clarify the pathology of amniotic fluid embolism and improve the survival rate.

## Discussion

In study 1, the results demonstrated that the fibrinogen level decreases earlier than the platelet count and antithrombin III (AT III) activity when atonic bleeding occurs; however, the fibrinogen level was measured immediately after occurrence in only 33% of all patients. Considering that the fibrinogen level was not correlated with the platelet count or AT III activity, it may be important to measure fibrinogen levels in early stages, in order to determine the pathological condition and severity of atonic bleeding. While myometrial fatigue due to prolonged labor and weak pains generally regarded as the main cause of atonic bleeding, in this study, its occurrence was not associated with prolonged labor, weak pains or the use uterotonic agents. On the other hand, with an increase in the volume of bleeding and obstetrical disseminated intravascular coagulation (DIC) scores, packed red blood cells and fresh frozen plasma (FFP) were administrated. As the fibrinogen level decreases early in atonic bleeding, the early administration of FFP may be important as an initial approach to treat the disease.

In study 2, amniotic fluid embolism was classified into two types: that involving cardiopulmonary collapse; and that following DIC. Pathologically, the former type is conventional, in which fetal and amniotic fluid

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components are observed in pulmonary blood vessels. The pathological characteristics of the latter type include uterine atony, and the presence of fetal and amniotic fluid components in uterine blood vessels. In this type, fetal and amniotic fluid components are occasionally absent in the lungs. Among cases of clinical amniotic fluid embolism without fetal and amniotic fluid components in the lungs (or pulmonary examination findings are unavailable in life-saving settings), those involving uterine atony in the presence of fetal and amniotic fluid components in uterine blood vessels may be called uterus-type amniotic fluid embolism.

## Disclosure

The authors have no conflict of interest to declare.

THE JOURNAL OF
Obstetrics and Gynaecology Research

doi:10.1111/jog.12278 J. Obstet. Gynaecol. Res. Vol. 40, No. 4: 941–945, April 2014

# Predictor of mortality in patients with amniotic fluid embolism

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## Abstract

***Aim:*** The purpose of this study was to evaluate the possibility of establishing predictors of mortality in women with amniotic fluid embolism.

***Methods:*** Our previous report identified eight factors associated with amniotic fluid embolism (AFE) fatality: dyspnea, cardiac arrest, loss of consciousness, serum sialyl Tn greater than 47 U/mL, serum interleukin-8 greater than 100 pg/mL, vaginal delivery, multiparity and term delivery. The ratio of the number of positive fatal factors to the number of possible fatal factors in the same case was calculated as the abundance ratio, which was used because information regarding all eight factors was not retrievable for all the patients at the time of registration. The patient group was divided into four quartiles based on this abundance ratio, and the mortality rate in each quartile was compared with the overall mortality rate among the 130 patients with AFE enrolled between 1992 and 2006. The validity of this approach was confirmed in another dataset from a cohort of 38 patients with AFE in 2007.

***Results:*** A statistically significant positive correlation was observed between the abundance ratio and the mortality in each quartile ($P < 0.01$) for the patients with AFE enrolled between 1992 and 2006. This result was also found in the AFE patients enrolled in 2007 ($P < 0.05$). Thus, an increased in the abundance ratio of the eight fatal factors resulted in an increased case fatality rate.

***Conclusion:*** These data suggested that the abundance ratio of fatal factors may be a useful predictor of mortality and therefore may be expected to improve prognostic accuracy in the future.

**Key words:** abundance ratio, amniotic fluid embolism, fatal factor, obstetrics, predictor of mortality.

## Introduction

Amniotic fluid embolism (AFE) syndrome is a devastating complication of pregnancy with an abrupt and fulminant onset and is one of the main causes of maternal mortality. Autopsy studies have demonstrated that AFE occurs following the contamination of the maternal circulation by fetal materials such as amniotic fluid and meconium.[1,2] Although this condition has been suggested to be caused by an uncharacterized immune reaction rather than an embolic phenomenon,[3,4] the causes and mechanisms responsible for AFE remain enigmatic. This syndrome is particularly alarming due to its unpredictability: it is likely unpreventable, and patients deteriorate quickly with high mortality. Accordingly, mortality due to AFE is often difficult to diagnose correctly. Nonetheless, risk factors for AFE have been reported. Three large population-based

Received: April 2 2013.
Accepted: August 6 2013.
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Conflict of interest: The authors declare that no competing interests exist.

retrospective cohort studies have identified age over 35 years, cesarean section, forceps- or vacuum-assisted vaginal delivery, placenta previa, placental abruption and eclampsia as possible risk factors.[5–7] It may be possible to reduce the incidence of AFE through the management of these risk factors but not the case fatality rate. Although recent population-based studies indicate a decrease in case fatality rates for AFE, data regarding the factors leading to the reduction in mortality remain scarce. Eight factors associated with mortality in AFE were previously identified by our group; to the best of our knowledge, no other studies have reported factors associated with mortality due to AFE.[8] The aim of this study was to investigate whether mortality in parturient women with AFE could be predicted using these fatal factors. Such information may establish useful predictors for mortality in patients with AFE and therefore may be expected to improve prognostic accuracy in the future.

## Methods

### AFE definition

The diagnosis of AFE was based on the clinical features listed in the Japanese Consensus Criteria for the Diagnosis of AFE.[8] The enrollment criteria were as follows: (i) at least one of the following symptoms: cardiac arrest (acute hypoxia and hypotension), respiratory arrest (dyspnea) or consumptive coagulopathy (severe obstetric hemorrhage); (ii) the onset of all signs and symptoms occurring during pregnancy, labor, cesarean section or within 12 h post-partum; and (iii) the absence of other illnesses that could explain the observed signs and symptoms.

### Medical record survey

The study subjects were recruited from the Japan AFE Registration Center in Hamamatsu University School of Medicine, Shizuoka, which is closely linked to the AFE Association of Japan, Nara Medical University. Patients who met the criteria for AFE were enrolled directly and voluntarily to this center by the chief physician and provided informed consent. The identification of AFE at the center was based on the clinical diagnosis as recorded in the medical report, without other verifying evidence. Our study was limited by its dependence on voluntary self-reporting. The study was reviewed and approved by the institutional review board of Hamamatsu University School of Medicine, which also allowed us to contact the patients or their families.

### Fatal AFE factors

Eight factors were identified as correlated with fatal AFE in our previous report[8] and included dyspnea, cardiac arrest, loss of consciousness, serum sialyl Tn (STN) greater than 47 U/mL, serum interleukin (IL)-8 greater than 100 pg/mL, vaginal delivery, multiparity and term delivery.

### Abundance ratio definition

Information regarding all eight of the fatal AFE factors was not always retrievable at the time of registration; therefore, the abundance ratio of the factors that were available was analyzed. The abundance ratio is defined as the ratio of the numbers of positive fatal factors to the number of possible fatal factors in the same case (Table 1). Because this analysis focused on increasing prognostic accuracy in patients presenting fewer than

**Table 1** Abundance ratio for fatal case calculation

| Enrollment | Denominator | | Numerator | |
|---|---|---|---|---|
| | Item | Point | Clinical manifestation | Point |
| Present | Dyspnea | 1 | Yes | 1 |
| | Cardiac arrest | 1 | No | 0 |
| | STN ≥ 47 U/mL | 1 | Yes (78 U/mL) | 1 |
| | Vaginal delivery | 1 | No (C/S) | 0 |
| | Term delivery | 1 | Yes (38 weeks) | 1 |
| | Multiparity | 1 | Yes (2-para) | 1 |
| Absent | Loss of consciousness<br>IL-8 ≥100 pg/mL | | | |
| Total | | 6 | | 4 |

Abundance ratio (%) = the number of positive fatal factors / the number of possible fatal factors reported = 4/6 = 67% This result was assigned to the 50–74% abundance ratio group.
C/S, cesarean section; IL, interleukin; STN, serum sialyl Tn.

half of these factors, cases presenting between four and seven of the fatal factors were excluded. This exclusion applied to the data for both the 2007 and 1992–2006 cohorts.

### Populations

*Data for 1992–2006 inclusion*

A total of 135 patients met the inclusion criteria, comprising both fatal AFE ($n = 65$) and non-fatal AFE ($n = 70$). Of the 135 patients, 114 (84.4%) were registered voluntarily in Japan and the other 21 (15.6%) were from other countries. This cohort was the same as that in our previous report.[8] Five (four fatal, one non-fatal) of the 135 cases were excluded based on the definition of the abundance ratio (Table 2).

*Data from 2007 (for comparison with the 1992–2006 data)*

In 2007, 38 Japanese patients met the inclusion criteria, comprising both fatal AFE ($n = 29$) and non-fatal AFE ($n = 9$). All 38 patients were registered voluntarily through the same system used in 1992–2006. No patient was excluded based on the definition of the abundance ratio.

### Statistical analysis

Information regarding certain items (i.e. fatal factors) was missing from some of the registration documents of the patients in the 1992–2006 cohort, and if there was a statistical bias in the missing data between the fatal and non-fatal cases, it would be inappropriate to compare these two groups. Thus, the frequency of missing data in both groups was compared using the $\chi^2$-test.

**Table 2** The eight fatal factors for AFE

| No. of missing items | Fatal | Non-fatal |
|---|---|---|
| 0 | 10 | 16 |
| 1 | 8 | 18 |
| 2 | 36 | 29 |
| 3 | 7 | 6 |
| 4 | 1 | 1 |
| 5 | 2 | 0 |
| 6 | 0 | 0 |
| 7 | 1 | 0 |

Cases presenting between 4 and 7 factors (the gray background sites) were excluded from the analysis. Some data were missing in the registration documents of the 1992–2006 cohort, though there was no statistical bias in the available information between fatal and non-fatal cases. $\chi^2$-Test, $P = 0.1333$.

The patient group was divided into four quartiles according to the abundance ratio results: 0–24%, 25–49%, 50–74% and 75–100%. Differences in the case fatality rates of each of the four groups were investigated for both the 1992–2006 and 2007 cohorts using Fisher's post-hoc test to confirm the relationship between the case fatality rate and the abundance ratio.

The statistical analyses were performed using SPSS version 16.0 and Statview4.1.

## Results

The $P$-value of the $\chi^2$-test was 0.1333. Therefore, we observed no statistical bias in the amount of information provided in the registration documents between the fatal and non-fatal cases (Table 2).

A correlation was observed between the abundance ratio and the case fatality rate. Statistically significant differences in mortality between each abundance ratio group were demonstrated for the 1992–2006 cohort ($P < 0.01$), and significant differences in the case fatality rate between each quartile were also demonstrated for the 2007 cohort ($P < 0.05$) (Fig. 1). Thus, an increase in the abundance ratio of the eight fatal factors resulted in an increased case fatality rate. These data suggest that the abundance ratio of fatal factors may be a useful predictor of mortality.

## Discussion

Amniotic fluid embolism is a perinatal disease with a high case fatality rate. This high mortality is a result of the difficulty that the patient's body has in responding

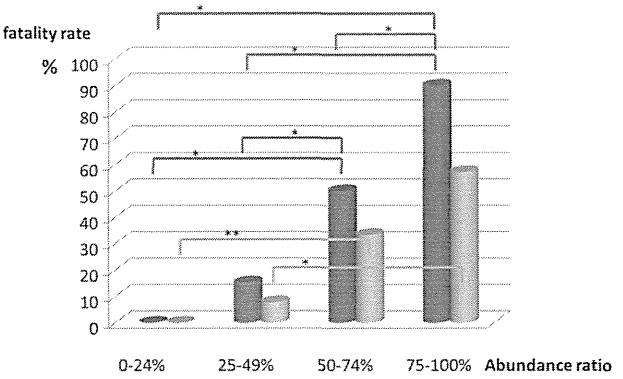


**Figure 1** Statistically significant relationships between the case fatality rate and the abundance ratio were present in both cohorts. Post-hoc test, Fisher's exact test. *$P < 0.01$; **$P < 0.05$. ▮, 1992–2006; ▮, 2007.

to a sudden onset of severe shock and hemorrhage. Moreover, it is not currently possible to predict AFE or treat it prophylactically, and simply extracting the risk factors reported elsewhere[5–7] may not solve the problem of high mortality. Conversely, understanding the risk factors associated with AFE may contribute to the prevention. Therefore, we considered the abundance ratio, whereby a high abundance ratio corresponds to a high case fatality rate, as a tool to reduce this rate. In Japan, approximately 50% of pregnant women deliver at private clinics, and patients with such conditions as AFE, which exhibits sudden, unpredictable and severe onset, would be difficult to resuscitate in these settings because of the lack of medical equipment and staff. If parturient women who have AFE or who may be at risk of mortality due to AFE can be identified, then the medical response may become sufficiently rapid enough to ensure the survival of the mother. Indeed, the early recognition of AFE with prompt intervention is paramount to a successful outcome and to decreasing the associated mortality.[9] Therefore, it is necessary to transport a patient from a private clinic to a higher level medical facility as soon as possible. In addition, the abundance ratio may be a useful index for identifying the appropriate emergency level for the requested transport, and reduced maternal mortality in AFE cases may be expected when predictors of mortality have been identified using this ratio. From an alternative point of view, this ratio offers two merits: (i) a more accurate prognosis may be achieved by analyzing the survival of patients with high abundance ratios; and (ii) this ratio becomes a useful piece of information to explain the patient's condition, which can lead to an improved relationship with the patient's family.

However, this ratio is not consistent with current medical information because the two serum markers included as fatal factors, STN[10,11] and IL-8, cannot be detected immediately. These two serum markers are crucial predictors of mortality when compared to other clinical manifestations for two reasons. First, STN is recognized as NeuAc-α-2,6-GalNAc and is present in high concentrations in meconium. The correlation between the fatality and turbid amniotic fluid has been described previously.[3] In patients with AFE who presented AF containing thick meconium, there was a shorter time from the initial presentation to cardiac arrest and an increased risk of neurological damage or death. As mentioned above, the detection of meconium passage into the maternal circulation may be a crucial factor for accurate prognosis. Second, IL-8 in the bronchoalveolar lavage fluid is the most significant predictor of mortality in patients with acute respiratory distress syndrome.[12] IL-8, a major chemoattractant for neutrophils, promotes the secretion of the proteolytic enzyme neutrophil elastase, which results in poor patient outcomes due to multiple organ failure. Patients with AFE exhibit a high frequency of adult respiratory distress syndrome (ARDS)[3] suggesting that high serum IL-8 concentrations have a critical impact on the outcomes of patients with AFE complicated by ARDS. Unfortunately, in our study, no registration data indicated a correlation between ARDS and fatal AFE. Furthermore, measurements of these two serum markers will not be available to most clinicians around the world. However, these fatal factors for AFE patients could not be substituted by other clinical symptoms associated with serum STN and serum IL-8, such as turbid amniotic fluid and elevated fever, respectively, because turbid amniotic fluid ($P = 0.289$) and elevated fever ($P = 0.514$, data not shown) were not correlated with fatal AFE in our enrolled patients.[8] We suggest that an easy-to-use kit to detect these two serum markers using monoclonal antibodies should be considered to avoid the limiting value of the abundance ratio or diagnostic criteria based on these markers.

In our study, an increase in the abundance ratio of the eight fatal factors was associated with a high case fatality rate. Because the analysis used the same population as our previous report,[8] it was expected that the abundant ratio and the case fatality rate would vary simultaneously. To validate our result, 38 patients with AFE were enrolled in 2007 and analyzed using the same abundance ratio. A significant difference was also confirmed in the 2007 cohort, though the correlation was not as strong as in the 1992–2006 cohort, likely because of the small study population and the case fatality rate. Furthermore, the data suggest that the abundance ratio is an effective predictor of mortality because the same tendency was indicated in both cohorts.

It is possible that different results could be obtained using other databases. For example, the reported incidence of AFE varies among countries. The cause of this difference has been ascribed to methodological differences in the collection and analysis of the data.[13] Our data were not population-based or national data but rather voluntarily provided data, and the population was both highly homogeneous and limited in number. Moreover, the AFE entry criteria are slightly different in Japan compared to the USA[3] and the UK,[14] though it is assumed that the interpretations of the Japanese

criteria are similar to those of the USA and UK criteria. Our entire population exhibited a case fatality rate of 56%. Because recent population-based studies have consistently reported case fatality rates ranging 11–43% (six out of eight studies reported a mortality rate of under 21%),[13] it appears that a substantial ascertainment bias toward more serious and fatal cases was present in our study. Indeed, differing results may be observed in different AFE populations. Thus, our study had several limitations. It would be interesting if other investigators evaluated the predictors of mortality using other databases and compared the results with those of our study. Any disparities in the results would likely be due to the enrollment system or differences in racial background. The evaluation of this ratio worldwide would, at the very least, show whether the AFE enrollment system should be standardized internationally, as it is unlikely that the patient AFE databases in each area or country are accurate.

## Acknowledgments

The authors are grateful to the Japan Association of Obstetricians and Gynecologists for their support of this study. This study was supported by a grant (Grant-in-Aid for Scientific Research) from the Ministry of Education.

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doi:10.1111/jog.12200 J. Obstet. Gynaecol. Res. Vol. 40, No. 2: 465–472, February 2014

# Comparison between placental gene expression of 11β-hydroxysteroid dehydrogenases and infantile growth at 10 months of age

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## Abstract

***Aim:*** The local expression of two isoenzymes of 11β-hydroxysteroid dehydrogenase, type 1 (11βHSD-1) and type 2 (11βHSD-2), regulates the access of glucocorticoid hormones to their target cells. Reports on the association between the placental expression of 11βHSD and infantile growth are limited. The aim of the present study was to investigate if the placental gene expression of 11βHSD affects infantile growth at 10 months of age.

***Methods:*** Placentas and umbilical venous cord blood were obtained from 42 singleton cases of cesarean deliveries between 31 and 40 weeks of gestation at Hamamatsu University Hospital between March 2009 and June 2010. The gene expression of both 11βHSD-1 and 11βHSD-2 was measured by quantitative reverse transcription polymerase chain reaction. Adiponectin and leptin levels in umbilical cord blood were measured using enzyme-linked immunoassay.

***Results:*** 11βHSD-1 and 11βHSD-2 gene expression in human placentas did not correlate with bodyweight or the ponderal index (PI) at 10 months of age, whereas the gene expression of 11βHSD-1, but not 11βHSD-2, correlated with birthweight as well as PI at birth. Adiponectin levels in umbilical cord blood significantly correlated with the placental gene expression of 11βHSD-1 as well as bodyweight and PI at 10 months of age, although no direct correlation was observed between them.

***Conclusion:*** No direct correlation was observed between the placental gene expression of 11βHSD and infantile growth at 10 months of age. However, the placental gene expression of 11βHSD-1 may be indirectly connected with infantile growth via adiponectin-associated metabolic regulation represented by adiponectin levels in umbilical cord blood.

**Key words:** glucocorticoid, growth, infant, placenta, pregnancy, programming.

## Introduction

Glucocorticoids exert their biological action by interacting with their receptors (glucocorticoid receptors and/or mineralocorticoid receptors) in target cells.[1,2] The access of glucocorticoid hormones to their receptors within target cells was shown to be regulated by the local expression of the enzyme 11β-hydroxysteroid

Received: April 12 2013.
Accepted: June 1 2013.
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dehydrogenase.[3,4] There are two isoenzymes of 11β-hydroxysteroid dehydrogenase, type 1 (11βHSD-1) and type 2 (11βHSD-2), both of which have been identified in the human placenta.[4] 11βHSD-1 generates active glucocorticoids from cortisone/11-dehydrocorticosterone.[4] In contrast, 11βHSD-2 inactivates glucocorticoids and protects mineralocorticoid receptors from illicit occupation by glucocorticoids in aldosterone target cells.[4]

It has been argued that the placental inactivation of cortisol by 11βHSD may protect the developing fetus from the deleterious effects of maternally derived cortisol[5] because circulating cortisol levels are 5–10-times higher in the mother than in the fetus.[6] McMullen *et al.* demonstrated that both isoforms of 11βHSD in the placentas appeared to fulfill important roles in controlling the passage of glucocorticoids between the maternal and fetal circulations in consideration of many previous reports.[7]

Changes in these enzymes in placentas have been suggested to play important roles in regulating fetal bodyweight.[7] Meta-analysis showed that prenatal treatment with placental permeable glucocorticoids was associated with a reduction in size at birth among infants born at term.[8] The enzymatic activity or gene expression of both 11βHSD-1[9,10] and 11βHSD-2[5,9,11–13] was reported to be downregulated if infants were born small. Regarding general fetal growth, placental 11βHSD-2 activity was shown to be positively correlated with birthweight,[14,15] although this was not a consistent observation.[16] These intensive studies suggest the marked contribution of placental 11βHSD expression in the regulation of fetal growth.

Moreover, intrauterine alterations in adrenocortical function and/or the metabolism of its products, which occur in fetal organs as well as in placental tissues, may have long-lasting effects leading to derangements of these systems in later life.[17,18] Nevertheless, few reports have investigated the association between the placental expression of 11βHSD and infantile growth after birth in humans.

In the present study, we hypothesized that the placental gene expression of 11βHSD affects bodyweight and/or the ponderal index (PI) at 10 months of age. We first retrospectively investigated associations between the placental gene expression of 11βHSD, cortisol levels in umbilical cord blood, and bodyweight and/or PI at 10 months of age, in 42 mothers with singleton pregnancies who were recruited to the Hamamatsu Birth Cohort (HBC)[19] and delivered neonates by elective cesarean section. We also assessed the association between the placental gene expression of 11βHSD and adipocytokine levels (e.g. adiponectin and leptin), because glucocorticoids play important roles in regulating adipocytokines,[20–23] and adipocytokines are also suggested to play important roles in fetal and/or neonatal growth.[24–27] We further investigated the associations between the levels of these substances in umbilical cord blood, birthweight, PI at birth, and weight and PI at 10 months of age.

## Methods

### Placental tissue and umbilical cord blood sampling

Placental tissues and umbilical venous cord blood were obtained from 42 cases of elective cesarean deliveries between 31 and 40 weeks of gestation due to obstetrical complications, including breech presentation, uterine scar due to previous cesarean section and/or myomectomy, at Hamamatsu University Hospital between March 2009 and June 2010. Preterm cases were preterm rupture of the membrane in addition to the indications above, and post-term cases were sudden changes in fetal presentation to breech or unfavorable cervical ripening. Mothers were singleton pregnancies among those who were recruited to the HBC.[19] We collected placentas only in cases of elective cesarean section because labor affects the expression of 11βHSD in placentas.[28,29] Informed consent was obtained after a full explanation of the study. Exclusion criteria were maternal hypertension, diabetes, gestational diabetes and glucocorticoid treatment. The backgrounds of mothers and babies are summarized in Table 1.

Each placenta was inspected by one of the researchers (Y. K.-K.) immediately after the delivery for any viable abnormalities and were excluded if found to have macroscopic alterations. Placental tissues were

**Table 1** Background of the mothers and infants

| Characteristics | Mean ± SD | Range |
|---|---|---|
| Maternal age | 33.4 ± 4.7 | 21–45 |
| Gestational week at delivery | 37.5 ± 1.2 | 32–40 |
| Pre-pregnancy BMI (kg/m$^2$) | 22.9 ± 5.33 | 17.0–41.9 |
| BMI at delivery (kg/m$^2$) | 26.5 ± 4.6 | 18.5–38.8 |
| Birthweight (g) | 2935 ± 525 | 946–3854 |
| Placental weight (g) | 540 ± 116 | 230–750 |
| Bodyweight of 10-month-old (kg) | 8.4 ± 0.9 | 6.0–10.4 |
| Number of mothers | 42 | |

BMI, body mass index.

then sampled according to the report by Wyatt *et al.*[30] and Mericq *et al.*[10] In brief, each placenta was sectioned transversely using a sterile scalpel near the cord insertion site and placental tissue of the chorionic plate was obtained by removing the basal (maternal side) as well as surface (fetal side) tissues of the placenta. Placental tissues of the chorionic plate, thus obtained, were rinsed in cold sterile saline, snap frozen in liquid nitrogen, and stored at –80°C for mRNA extraction.

Within several minutes of the delivery, umbilical venous cord blood was obtained and centrifuged at 1200 *g* for 15 min at 4°C. The plasma thus obtained was aliquoted and stored at –80°C until assayed.

### Measurement of bioactive substances in umbilical cord blood

Cortisol levels were measured using commercial radioimmunoassay cortisol kits (Immuno Tech, Osaka, Japan). Adiponectin and leptin levels were measured using commercial enzyme-linked immunoassay kits (Otsuka Pharmaceutical [Tokyo, Japan] and R&D Systems [Minneapolis, MN, USA], respectively).

### Quantitative RT–PCR analysis of placental tissue

Total RNA from subcutaneous adipose tissue was extracted as described.[31] The gene expression of human 11βHSD-1, 11βHSD-2 and β-actin was determined by quantitative reverse transcription polymerase chain reaction using High Capacity RNA to cDNA Master Mix (catalog no. 4390777; Applied Biosystems, Foster City, CA, USA) and SYBR Green PCR Master Mix (catalog no. 4309115; Applied Biosystems), according to the manufacturer's recommendations. β-Actin mRNA expression was used as an internal control. The primers used were: 11βHSD-1, forward 5′-TCCAG GGTCAATGTATCAATCACT-3′, reverse 5′-CCTTCA TGGCTGTTTCTGTGTCT-3′; 11βHSD-2, forward 5′-GGCCAAGGTTTCCCAGTGA-3′, reverse 5′-GAG GGTGTTTGGGCTCATGA-3′; and β-actin, forward 5′-AGTACTCCGTGTGGATCGGC-3′, reverse 5′-GCTGATCCACATCTGCTGGA-3′.

### Statistics

Data are expressed as means ± standard deviations (SD). Z-scores were calculated by using the formula ([data – mean of the population]/[standard deviation of the population]).[32] Infantile ages were adjusted according to their gestational age at delivery. Regarding birthweight and weight at 10 months of age, each z-score was calculated using means and SD described in the Japanese gestational age-specific reference for birthweight[33] and Japanese age-specific reference for bodyweight (http://jspe.umin.jp/pdf/zu1_a.pdf, http://jspe.umin.jp/pdf/zu1_b.pdf), respectively. Z-scores for other parameters were calculated using means and SD of the study population. The values for body mass index (BMI) and PI were directly compared to the z-scores of other parameters because they were relative parameters, that is, bodyweight (kg) was divided by (height [m])$^2$ and (height [m]),$^3$ respectively. Spearman's rank correlation coefficient was calculated between parameters. A *P*-value of less than 0.05 was regarded as significant.

### Approval

The Ethics Committee of the Hamamatsu University School of Medicine approved all the procedures of this study.

## Results

### Placental gene expression of 11βHSD, birthweight, PI at birth, and weight and PI at 10 months of age

Gene expression of 11βHSD-1 in placentas did not correlate with bodyweight or PI at 10 months of age (Fig. 1a,b), although significant positive correlations were observed with birthweight (Fig. 1c; $r = 0.30$, $P < 0.05$) and PI at birth (Fig. 1d; $r = 0.46$, $P < 0.01$). 11βHSD-1 gene expression did not correlate with height or weight increases during the first 10 months (Table 2C).

Gene expression of 11βHSD-2 in placentas did not correlate with bodyweight or PI at 10 months of age (Fig. 2a,b). They also did not correlate with birthweight (Fig. 2c) or PI at birth (Fig. 2d). 11βHSD-2 gene expression did not correlate with height or weight increases at 10 months of age (Table 2C).

### Placental gene expression of 11βHSDs, and cortisol and adipocytokine levels in umbilical cord blood

Gene expression of 11βHSD-1 and 11βHSD-2 in placentas did not correlate with cortisol levels in umbilical cord blood (Table 2A).

Gene expression of 11βHSD-1 ($r = 0.46$, $P < 0.01$), but not 11βHSD-2, positively correlated with adiponectin levels in umbilical cord blood (Fig. 4a, Table 2B). No

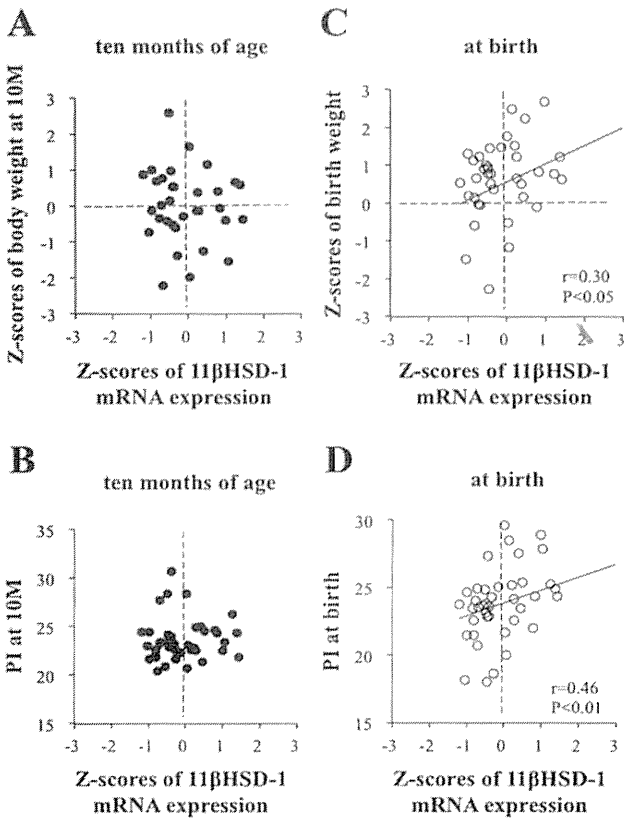


**Figure 1** Associations between placental 11β-hydroxysteroid dehydrogenase, type 1 (11βHSD-1) gene expression, (a) bodyweight and (b) PI at 10 months of age, and (c) birthweight and (d) PI at birth. Black and white circles indicate data at 10 months of age and at birth, respectively. M, months; PI, ponderal index.

significant correlation was observed between the expression of 11βHSD and leptin levels (Table 2B).

### Cortisol and adipocytokine levels in umbilical cord blood, birthweight, PI at birth, and weight and PI at 10 months of age

Cortisol levels in umbilical cord blood did not correlate with bodyweight or PI at 10 months of age (Fig. 3a,b, Table 3A), although positive correlations were observed with birthweight ($r = 0.39$, $P < 0.05$) (Fig. 3c, Table 3A) and PI at birth ($r = 0.36$, $P < 0.05$) (Fig. 3d, Table 3A).

Adiponectin levels in umbilical cord blood positively correlated with PI, but not weight, at 10 months of age ($r = 0.35$, $P < 0.05$, Fig. 4a, Table 3B). Similar correlations were observed with birthweight ($r = 0.32$, $P < 0.05$) (Table 3B) and PI at birth ($r = 0.56$, $P < 0.001$) (Fig. 4d, Table 3B). In contrast, leptin levels in umbilical cord blood did not correlate with weight or PI at 10 months of age (Table 3B), although positive correlations were observed with birthweight ($r = 0.54$, $P < 0.001$) and PI at birth ($r = 0.65$, $P < 0.001$) (Table 3B).

### Association with maternal background

Gestational weeks at delivery correlated with 11βHSD-1, but not 11βHSD-2 gene expression (Table 2D). Gestational weeks at delivery did not correlate with weight or PI at 10 months of age, although they correlated with PI at birth (Table 3C). Maternal BMI soon before delivery did not correlate with weight or PI at 10 months of

**Table 2** Association of z-scores of gene expression of 11βHSD in placentas with those of (A) cortisol levels, (B) adipocytokines levels, (C) parameters of infantile growth, (D) gestational weeks at delivery and (E) maternal parameters

| | Gene expression in placentas | |
|---|---|---|
| | 11βHSD-1 | 11βHSD-2 |
| (A) | | |
| Cortisol | NSC | NSC |
| (B) | | |
| Adiponectin levels (μg/mL) | $r = 0.46$** | NSC |
| Leptin levels (ng/mL) | NSC | NSC |
| (C) | | |
| Height increase during first 10 months | NSC | NSC |
| Weight increase during first 10 months | NSC | NSC |
| (D) | | |
| Gestational weeks at delivery | $r = 0.34$* | NSC |
| (E) | | |
| Maternal age at delivery | NSC | NSC |
| Maternal pre-pregnancy BMI (kg/m$^2$) | NSC | NSC |
| Maternal BMI soon before delivery (kg/m$^2$) | NSC | NSC |

*$P < 0.05$ and **$P < 0.01$ in correlation of z-scores. 11βHSD-1, 11β-hydroxysteroid dehydrogenase, type 1; 11βHSD-2, 11β-hydroxysteroid dehydrogenase, type 2; NSC, no statistical correlation.

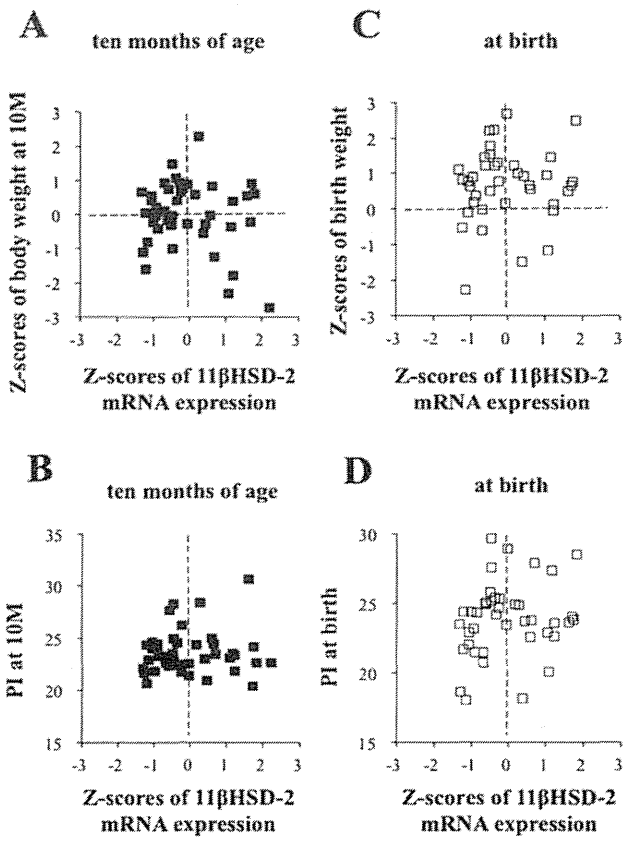


**Figure 2** Associations between placental 11β-hydroxysteroid dehydrogenase, type 2 (11βHSD-2) gene expression, (a) bodyweight and (b) PI at 10 months of age, and (c) birthweight and (d) PI at birth. Black and white squares indicate data at 10 months of age and at birth, respectively. M, months; PI, ponderal index.

age (Table 2E), although it correlated with birthweight ($r = 0.49$, $P < 0.01$) and PI at birth ($r = 0.35$, $P < 0.01$) (Table 3D).

## Discussion

Accumulating evidence has shown that changes in 11βHSD in placentas are involved in regulating fetal bodyweight.[5,7,9–15] On the other hand, there was a concern that glucocorticoids and/or their metabolism in the fetoplacental unit may have long-lasting effects.[17,18] Therefore, glucocorticoids and/or their metabolism in the fetoplacental unit affect not only fetal growth, but also glucocorticoid action throughout life after birth. However, few reports have investigated the association between the placental metabolism of glucocorticoids by 11βHSD and infantile grow.

The present study revealed that cortisol levels (Fig. 3a,b; Table 3A) as well as the placental gene expression of 11βHSD (Figs 1a,b,2a,b) did not correlate with weight or PI at 10 months of age, although significant positive correlations were observed with birthweight as well as PI at birth (Figs 1c,d,3c,d, Table 3A). These results suggest that the placental metabolism of glucocorticoids affects fetal bodyweight *in utero*, but not during the early infantile period in humans. Given the possible long-lasting effect of prenatal changes in glucocorticoids on metabolism after birth,[17,34] the influence of glucocorticoids on developing fetal organs may markedly change around the time of delivery in humans, presumably due to complete separation from the placental and/or maternal contribution. Further investigation is necessary to confirm this.

Gestational weeks at delivery correlated with placental 11βHSD-1 gene expression (Table 2D), which suggested that gestational age may affect 11βHSD-1 gene expression in addition to fetal body composition represented by PI. However, no significant correlation was observed between placental 11βHSD and weight and PI at 10 months of age, even after calculating partial correlation coefficients holding gestational weeks at delivery adjusted (data not shown). A large-scale study is necessary.

Both adiponectin and leptin levels in umbilical cord blood positively correlated with birthweight and PI at birth (Fig. 4d, Table 3B), which is consistent with previous reports.[24–26] Nakano *et al.* reported that umbilical cord adiponectin levels correlated with BMI gain from birth to 3 years of age in Japanese infants.[27] Adiponectin levels in umbilical cord blood correlated with PI at 10 months of age (Fig. 4c, Table 3B), which indicated that adiponectin-associated metabolic regulation in the fetoplacental unit was, at least partly, related to the regulation of growth during the first 10 months. Interestingly, the placental gene expression of 11βHSD-1 positively correlated with adiponectin levels in umbilical cord blood in this study population (Fig. 4a, Table 2B). It is interesting to speculate that placental 11βHSD-1 may be indirectly related to the regulation of growth during the first 10 months via adiponectin-associated metabolic regulation in the fetoplacental unit. Research is ongoing.

The metabolism of glucocorticoids in the fetoplacental unit plays a pivotal role in the regulation of fetal growth. Moreover, increasing evidence suggests that the metabolism of glucocorticoids in the fetoplacental unit has long-lasting effects after birth. However, the

**Table 3** Association of z-scores of weight and PI at 10 months old, birthweight and PI at birth with those of (A) cortisol levels, (B) adipocytokines levels, (C) gestational weeks at delivery and (D) maternal parameters

| | At 10 months old | | At birth | |
|---|---|---|---|---|
| | Weight (kg) | PI (kg/m$^3$) | Birthweight (kg) | PI (kg/m$^3$) |
| (A) | | | | |
| Cortisol | NSC | NSC | $r = 0.39$* | $r = 0.36$* |
| (B) | | | | |
| Adiponectin levels (μg/mL) | NSC | $r = 0.35$* | $r = 0.32$* | $r = 0.56$*** |
| Leptin levels (ng/mL) | NSC | NSC | $r = 0.54$*** | $r = 0.65$*** |
| (C) | | | | |
| Gestational weeks at delivery | NSC | NSC | NSC | $r = 0.41$** |
| (D) | | | | |
| Maternal age at delivery | NSC | NSC | NSC | NSC |
| Maternal pre-pregnancy BMI (kg/m$^2$) | NSC | NSC | NSC | NSC |
| Maternal BMI soon before delivery (kg/m$^2$) | NSC | NSC | $r = 0.49$** | $r = 0.35$* |

*$P < 0.05$, **$P < 0.01$ and ***$P < 0.001$ in correlation of z-scores. BMI, body mass index; NSC, no statistical correlation; PI, ponderal index.

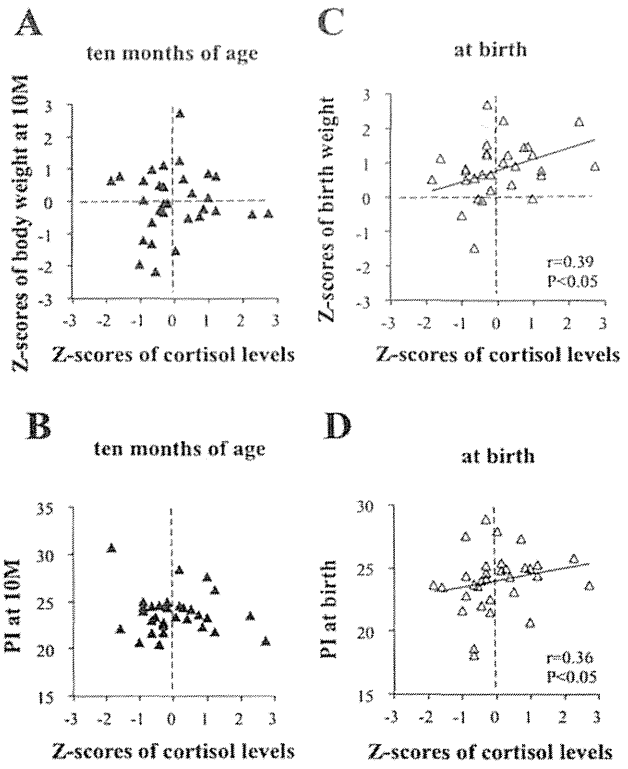


**Figure 3** Associations between cortisol levels in umbilical cord blood, (a) bodyweight and (b) PI at 10 months of age, and (c) birthweight and (d) PI at birth. Black and white triangles indicate data at 10 months of age and at birth, respectively. M, months; PI, ponderal index.

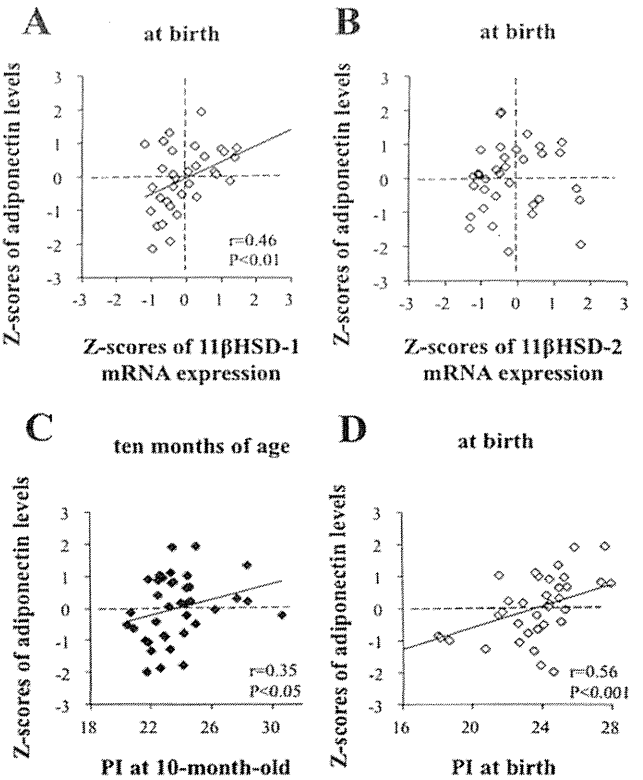


**Figure 4** Association between adiponectin levels in umbilical cord blood, placental gene expression of (a) 11β-hydroxysteroid dehydrogenase, type 1 (11βHSD-1) and (b) 11β-hydroxysteroid dehydrogenase, type 1 (11βHSD-2), (c) PI at 10 months of age and (d) PI at birth. Black and white lozenges indicate data at 10 months of age and at birth, respectively. PI, ponderal index.

present study revealed that the placental gene expression of 11βHSD-1 significantly correlated with birthweight and PI at birth (Fig. 1c,d), but not with those at 10 months of age (Fig. 1a,b), which indicated that there was no direct connection between them. Interestingly, adiponectin levels in umbilical cord blood significantly correlated with the placental gene expression of 11βHSD-1 as well as bodyweight and PI at 10 months of age, although no direct correlation was observed between the placental gene expression of 11βHSD-1 and bodyweight and PI at 10 months of age. It was suggested that the placental gene expression of 11βHSD-1 may be linked to infantile growth at 10 months of age at least partly via adiponectin-associated metabolism represented by adiponectin levels in umbilical cord blood.

The present results should be interpreted with caution because there were some limitations as follows. The number of placentas was small because most of the placental tissues were dropped in 10% formaldehyde in the HBC study and only limited numbers were sampled for mRNA expression, although there was no intentional selection. We measured the mRNA expression of 11βHSD, but not their enzyme activities or protein expression due to technical limitations. Assessing protein expression and/or enzyme activities is the aim of a future study. We assessed bodyweight and PI at 10 months of age during the infantile period because the HBC is an ongoing study and data analysis has not yet been completed; therefore, results of infantile biometry were available up to 10 months of age.

In summary, the gene expression of 11βHSD in human placentas did not correlate with infantile growth at 10 months of age. However, adiponectin levels in umbilical cord blood suggested that there may be an indirect association between the placental gene expression of 11βHSD-1 and infantile growth at 10 months old.

## Acknowledgments

The authors thank Mrs Naoko Hakamada, Mrs Yumiko Yamamoto, Mrs Naoko Kondo, Mrs Miuta Sawai, Mrs Kazuko Sugiyama, Dr Yoriko Morishima, Dr Kaori Matsumoto, Dr Ayyappan Anitha, Dr Chie Shimmura, Ms Makiko Narumiya, Ms Maiko Honda and Mr Hiroshi Tsutsumi for their secretarial or technical assistance. This work was supported in part by Grants-in-Aid for Scientific Research from the Ministry of Education, Science, Culture and Sports, Japan (no. 24390273 and no. 25670490) and Hamamatsu Foundation for Science and Technology Promotion.

## Disclosure

None declared.

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特集　周産期における出血対策と輸血

母体出血対策

# 出血をきたす疾患―治療のコツ
## ―子宮型羊水塞栓症

金山 尚裕

### 子宮型羊水塞栓症とは

臨床的羊水塞栓症，羊水塞栓症，子宮型羊水塞栓症との関係を図1に示した。なお，臨床的羊水塞栓症は救命を目的に設定された臨床所見からみた診断基準で下記の3項目を満たすものをいう。

1）妊娠中または分娩後12時間以内に発症した場合

2）下記に示した症状・疾患(一つまたはそれ以上でも可)に対して集中的な医学治療が行われた場合

A）心停止

B）分娩後2時間以内の原因不明の大量出血(1,500 mL以上)

C）播種性血管内凝固症候群

D）呼吸不全

3）観察された所見や症状がほかの疾患で説明できない場合

臨床的羊水塞栓症で剖検され肺に羊水成分を認める時，従来の羊水塞栓症であり，臨床的羊水塞栓症で肺に羊水成分を認めず，子宮に子宮弛緩症と子宮血管に羊水成分を認める症例については従来の羊水塞栓症とは区別し，子宮型羊水塞栓症と呼ぶ(図1)[1]。また救命等の理由で肺の所見はないが，子宮に子宮弛緩症と子宮血管に羊水成分を認めればこれも子宮型羊水塞栓症と呼ぶ。種々の検討からDIC先行の臨床的羊水塞栓症の多くは子宮型羊水塞栓症と同じ疾患であることが明らかに

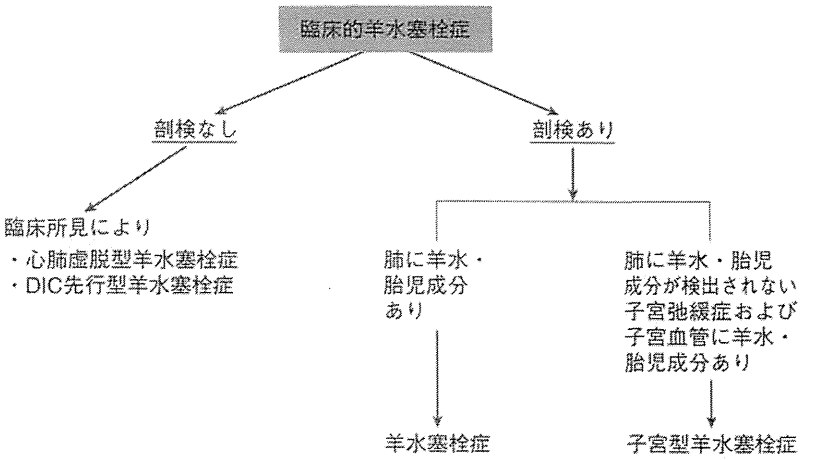


図1　羊水塞栓症の診断分類

なお，摘出子宮がある場合，子宮病理所見と臨床所見により子宮型羊水塞栓症と診断できる場合がある。

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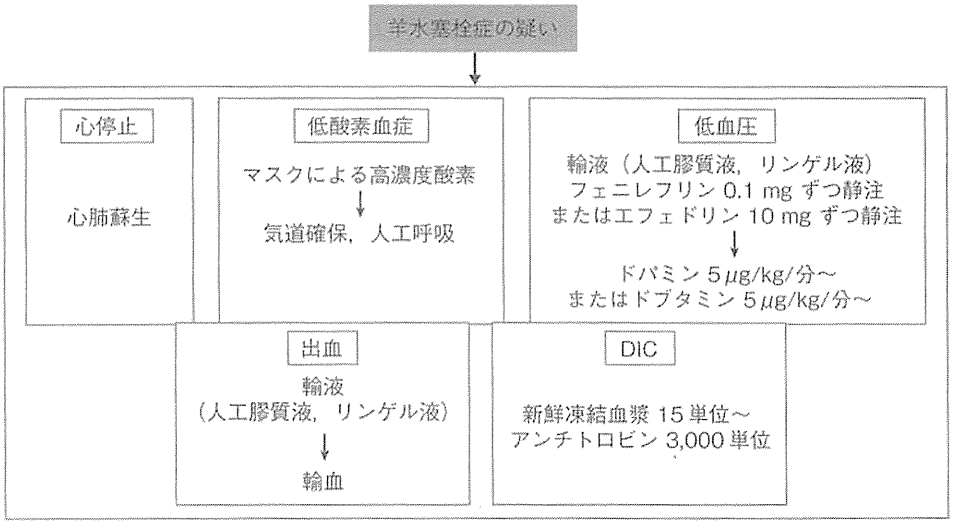


図 2　羊水塞栓症の初期対応（妊産婦死亡症例検討評価委員会，日本産婦人科医会編，2012）[4]

なってきている。

## 羊水塞栓症と子宮型羊水塞栓症の病因，病態

羊水塞栓症は羊水中の胎児成分（胎便，扁平上皮細胞，産毛，胎脂，ムチンなど）と液性成分（胎便中のプロテアーゼ，組織因子など）が母体循環に比較的大量に流入することにより発症すると考えられている[2]。羊水の流入経路は，卵膜の断裂部位より羊水成分が卵膜外漏出し，子宮筋の裂傷部位や子宮内腔に露出した破綻血管から母体循環系へ入るとされている。流入した羊水成分は，胎児成分が肺をはじめとした母体血管の小血管に物理的閉塞をきたす場合と羊水の液性成分が，アナフィラクトイド反応を起こし肺血管の攣縮，血小板・白血球・補体の活性化をきたす[3]。臨床的には心肺虚脱症状，DICを発症初期から示すのが特徴である。

一方，子宮型羊水塞栓症は羊水が子宮へ流入し，補体の活性化，キニンの大量産生が子宮を中心に発生する。その結果，子宮は強く浮腫状となり子宮弛緩症となり，重症の弛緩出血・DICとなる。

## 子宮型羊水塞栓症の臨床症状

子宮型羊水塞栓症の臨床的特徴として，分娩後に「凝固しないさらさらした血液」から始まりその後弛緩出血→大量出血→ショックになるパターンである。また初発症状として弛緩出血に先立って下腹痛を伴う原因不明の胎児機能不全があることがある。

## 治　療

### 1．初期管理

妊産婦死亡症例検討評価委員会，日本産婦人科医会から発刊されている母体安全への提言において羊水塞栓症の初期対応を図2に示す。初期のショック対応（気道確保，血管確保，補液，抗ショック薬剤投与）とDIC対策（アンチトロンビン投与，可能ならばFFP投与）後速やかに高次施設に搬送する。子宮型羊水塞栓症においては出血とDIC対策を早期から行うことが肝要である[4]。

大量出血時は異型出血をためらわない。急ぐ時には具体的にはO型RCC，AB型FFPを投与する。またFFPの早期からの大量投与が重要である。

1次施設は上記初期対応できる範囲のことを迅速に行い2次施設に搬送する。2次施設では早期よりICUで集中管理するのが望ましい。

1）重症DICが発症することが多いので早期にアンチトロンビン（3,000単位）投与する。
2）新鮮凍結血漿10～15単位以上を投与する。赤血球製剤よりも新鮮凍結血漿を優先する。赤血球製剤はあくまで出血量を見ながら投与する。FFP：RCC比 1.0以上とする。

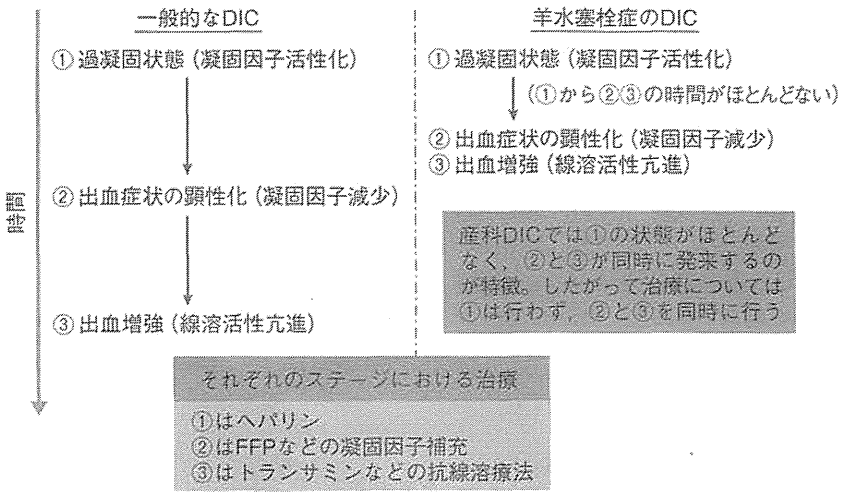


図3　羊水塞栓症によるDICの特徴

子宮型羊水塞栓症ではヘパリンを投与するタイミングがほとんどないと考えることも重要である。羊水塞栓症では胎盤のtissue factorや羊水により主にフィブリン血栓が血管内（微小血管内血栓）に出現する。この時凝固，線溶因子が消費され通常出血しないような軽微な血管の多数の損傷部位から出血しそれが大量出血となる。このようなタイプのDICは短時間で凝固と線溶が亢進するのが特徴である（図3）。したがってDICの前段である過凝固状態の時期は時間的に短く，ヘパリンを使用するタイミングはほとんどない。治療の主体は凝固因子の補充と凝固抑制，線溶抑制を同時に行うことである（図3）。したがって凝固因子補充としてFFP大量投与，凝固阻止としてアンチトロンビン，抗線溶としてウリナスタチン，トランサミンの大量投与（2～4 g/時間）をFDP，D-dimerが下降局面に入るまで行う。FDP，D-dimerが下降しても大量投与すると血栓リスクが高まるのでFDP，D-dimerをモニターしながら行うことが重要である。ウリナスタチンは30万単位を発症初期に投与する。

血小板濃厚液の投与はDICの状態を見ながら考えるが，血小板数は5万/μL以上あれば必ずしも投与を急がなくてもよい。上記治療にても改善されない重症DICでは保険適用外ではあるが国内外で実績のあるノボセブンの使用を考慮してもよい。

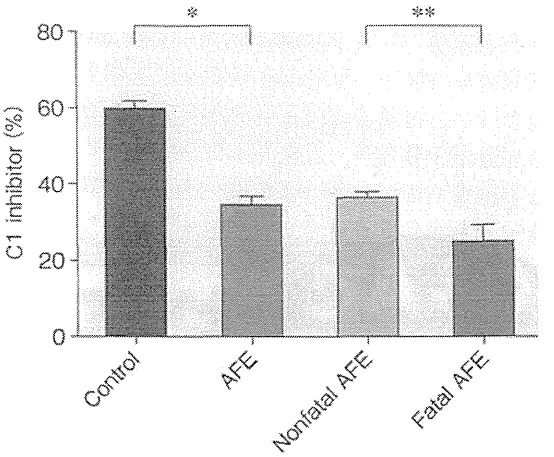


図4　C1インヒビター値と羊水塞栓症
AFE：羊水塞栓症，Nonfatal AFE：羊水塞栓症救命例，Fatal AFE：羊水塞栓症死亡例

## C1インヒビターと羊水塞栓症

最近我々はC1エステラーゼインヒビター（C1インヒビター）が羊水塞栓症で減少していることを報告した[5]。死亡例では特にC1インヒビターの低下が著しく25％を切る症例も多数存在していた（図4）。

C1インヒビターは補体系の抑制のみならず，キニン系，線溶系にも直接作用する。羊水塞栓症の子宮弛緩症（子宮浮腫），DIC，アナフィラキシー様反応はすべてC1インヒビターの低下症から発

生していることを見いだした。さらにその後の検討から，子宮型羊水塞栓症はほとんどの例でC1インヒビターの極端な低下を伴っていた(投稿中)。羊水塞栓症の治療に早期よりの新鮮凍結血漿(FFP)が有効であることは知られていたが，FFPに含まれているC1インヒビターも病態改善に寄与していることが考えられる。またC1インヒビター(ベリナート®)は遺伝性血管浮腫の治療薬として保険採用されており，C1インヒビター製剤の羊水塞栓症への応用も期待される。

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