previa before 32 GW using *trans*-vaginal ultrasonography. (B)
2 Refer the patient to an appropriate facility before 33 GW if contingency plans are considered to be insufficient. (C)
3 Prepare treatments, including the possibility of a midnight emergency cesarean section, at around 34 GW once a decision has been made to continue treating the patient at your facility. (C)
4 Be cautious of placenta accreta, especially in women with a previous cesarean section and in women with a placental site close to the previous uterine scar. (B)
5 Carefully assess the possibility of placenta accreta in women with a placental site that covers the previous uterine scar. (B)
6 Perform an elective cesarean section before 38 GW. (B)
7 Prepare for the possible need for a blood transfusion before elective cesarean section or during an emergency cesarean section. (A)
8 Inform women and their families of the risks of blood transfusion and an emergency hysterectomy in advance. (A)

CQ306: How should women with a low-lying placenta be treated?
Answer

1 Consider an elective cesarean section in women with a placental edge within 2.0 cm from the internal orifice of the uterus at 36–37 GW. (C)
2 Be cautious of placenta accreta in women with repeat cesarean sections and a placental site on the anterior uterine wall. (B)
3 Be cautious of postpartum hemorrhage. (A)

CQ307: How should women with polyhydramnios be treated?
Answer

1 Suspect polyhydramnios in women with an extraordinarily large uterus. (C)
2 Diagnose as polyhydramnios based on the amniotic fluid index and/or the single deepest pocket measured using ultrasonography. (B)
3 Investigate the cause after the diagnosis of polyhydramnios. (A)
4 Consider amnioreduction in patients with symptoms derived from a large uterus or signs of premature labor. (C)

CQ308: How should women with oligohydramnios be treated?
Answer

1 Suspect oligohydramnios in women with an extraordinarily small uterus. (C)
2 Diagnose as oligohydramnios based on the amniotic fluid index and/or the single deepest pocket measured using ultrasonography. (B)
3 Investigate the cause after the diagnosis of oligohydramnios at mid-gestation. (A)
4 Be cautious of the fetal well-being. (B)

CQ309: How should one screen for patients with FGR?
Answer

1 Measure the fundal height of the uterus at each antenatal visit to detect patients with FGR. (C)
2 Estimate the fetal size in all women around 30 GW using ultrasonography and repeat examinations if necessary. (B)
3 Be cautious of FGR, especially in women with risk factors for FGR, such as hypertension, hyperglycemia, kidney diseases, inflammatory intestinal diseases, anti-phospholipid antibody syndrome, autoimmune diseases, cardiac diseases, smoking, alcohol/caffeine abuse, previous FGR, a lean body, and inadequate weight gain during the current pregnancy. Try to remove risk factors and to treat them appropriately. (C)
4 Diagnose as FGR when the estimated fetal weight (EFW) is below the −1.5 standard deviation value of the mean EFW but not the mean birthweight according to the GW. Refer to the abdominal circumference of the fetus and serial changes in the EFW when diagnosing FGR. (C)

CQ310: How should women with FGR be managed?
Answer

1 Investigate the cause of FGR, focusing on the following points:
   1) Presence or absence of risk factors for FGR (see CQ309). (B)
   2) Malformation of the fetus and abnormal cord insertion based on an ultrasonography examination. (B)
   3) Changes in blood pressure, protein in the urine, and laboratory parameters, such as the platelet count, antithrombin activity, aspartate transaminase (AST), lactate dehydrogenase (LDH), and uric acid levels. (C)
   4) Hyperglycemia, thyroid dysfunction, and antiphospholipid antibodies. (C)

5) Congenital infections, such as rubella, cytomegalovirus, and toxoplasma. (C)

2 Suspect chromosomal aberration in a case with multiple malformations, characteristic malformations suggestive of chromosomal aberration, and/or severe FGR. A chromosomal analysis for a definite diagnosis should only be performed with informed consent. (B)

3 Consider terminating the pregnancy after taking the results of the following examinations into account:
1) NST, contraction stress test, and biophysical profile score. (B)
2) Umbilical artery flow velocity waveform using Doppler ultrasonography. (B)
3) Serial changes in measurements of the fetus. (C)

4 Monitor intrapartum FHR patterns continuously. (B)

CQ311: How should women with placental abruption be treated?

Answer

1 Remember that pregnancy-induced hypertension, previous placental abruption, premature labor, including premature rupture of the membranes, and trauma to the abdomen, such as a car accident, are risk factors for placental abruption. (B)

2 Suspect placental abruption and perform the following tests when a woman shows abnormal FHR patterns concomitant with clinical signs of premature labor, such as genital bleeding, increased uterine activity, and/or abdominal pain.
1) Ultrasonography. (B)
2) Blood test, including platelet count, antithrombin (III) activity, FDP or D-dimer, AST, and LDH. (B)

3 Monitor the FHR patterns continuously in women with an increased uterine activity after trauma to the abdomen. (C)

4 Plan for prompt delivery, in principle, after the diagnosis of a placental abruption. (A)

5 Initiate treatment for disseminated intravascular coagulation (DIC) promptly using packed red blood cells, fresh frozen plasma, and antithrombin products when a patient/blood test shows signs of DIC. (A)

6 Choose either of the following two modalities in a patient with a fetal death caused by placental abruption after considering the patient's condition and the capacity of the facility while simultaneously assessing and treating the DIC. (B)
1) Facilitated vaginal delivery with the aid of an amniotomy and/or oxytocin.
2) Emergency cesarean section.

7 Consider expectant management in a patient with a placental hematoma if the patient shows all of the following signs: unchanged size of the hematoma, normal FHR patterns, no uterine contractions, and no exacerbation of laboratory parameters for hemostasis and coagulation. (C)

CQ312: How should women with pre-eclampsia be treated?

Answer

1 Recommend admission to the hospital. (C)

2 Collaborate with facilities that have an NICU when treating pre-eclamptic patients at <32 GW. (B)

3 Determine an appropriate timing for pregnancy termination through repeated assessments of physical and blood chemistry findings in the mother and fetal development and well-being. (B)

4 If a patient complains of epigastric pain and/or headache, measure the blood pressure and perform blood tests, a non-stress test, and an ultrasonography examination to diagnose the patient as having eclampsia, HELLP syndrome, or placental abruption. Take preventive measures for an eclamptic fit if indicated. (B)

5 Consider the induction of labor in patients with mild pre-eclampsia at ≥36 GW. (C)

6 Measure the blood pressure regularly and prepare for an emergency cesarean section during a trial vaginal delivery. (B)

7 Monitor the intrapartum FHR patterns continuously. (B)

8 Refer to Table 1 in choosing an appropriate drug for the control of hypertension. (C)

CQ313: How should women with presumptive fetal macrosomia be treated?

Answer

1 Consider possible macrosomia in a woman with glucose intolerance, an infant with a presumed heavy-for-date weight, and previous macrosomia and/or shoulder dystocia. (C)

2 Inform the patient of the difficulty in making an accurate antenatal diagnosis of macrosomia. Determine the delivery mode after discussing the issue with the patient. (C)

3 Consider an emergency cesarean delivery in women with prolonged or arrested labor. (C)

4 In treating women with shoulder dystocia, call the medical staff and use a suprapubic pressure with a combination of McRoberts' maneuver and an episiotomy. Do not use Kristeller's maneuver. (C)

5 Recommend a 75-g OGTT at 6–12 weeks postpartum in women with macrosomia/shoulder dystocia and an unknown status of glucose tolerance or non-gestational diabetes mellitus in the current pregnancy. (C)

CQ314: How should women with GDM, overt diabetes in pregnancy, or diabetes mellitus be treated?

Answer

1 Control the blood glucose levels to a fasting morning level ≤95 mg/dL, a pre-meal level ≤100 mg/dL, and a 2-h post-meal level ≤120 mg/dL. (C)
2 First, instruct the patient with regard to diet therapy; initiate insulin treatment in cases with uncontrolled blood glucose levels. (B)
3 Assess fetal well-being using an NST and/or the biophysical profile score at ≥32 GW at an adequate interval. Recommend hospital admission if indicated. (C)
4 In women without problems of blood glucose control, fetal well-being, or fetal development, treat with either of the following two modalities. (B)
   1) Expectant management, including waiting for labor onset, until the end of 40 GW and subsequent labor induction at ≥41 GW.
   2) Active management with induction of labor at ≥37 GW, taking cervical maturation into account.
5 Be cautious of shoulder dystocia in cases with prolonged labor, augmentation of labor, and/or vacuum delivery. (C)
6 Determine the timing and mode of delivery individually for each case with uncontrolled blood glucose levels, exacerbated complications derived from glucose intolerance, and/or presumed macrosomia. (B)
7 Be cautious of respiratory distress syndrome in neonates born by elective cesarean section in women with <39 GW, uncontrolled blood glucose levels, or an unknown due date. (C)
8 Monitor intrapartum FHR patterns continuously in women with diabetes mellitus. (B)
9 Maintain a blood glucose level between 70–120 mg/dL during parturition. (C)
10 Be cautious of hypoglycemia and monitor blood glucose levels with changing doses of insulin during parturition, as insulin demand decreases abruptly after delivery. (B)

CQ315: How should women with an increased risk of eclampsia be treated?

Answer

1 Measure the blood pressure (BP) and semiquantify protein in the urine in all women who are admitted for delivery. (B)
2 Measure the BP regularly at an appropriate interval in parturient women diagnosed as having pregnancy-induced hypertension, a positive urine protein result at admission, or the presence of hypertension at admission. (B)
3 Measure the BP if parturient women complain of headaches, blurred vision, or epigastric pain. (B)
4 Administer $MgSO_4$ with or without antihypertensive drugs to parturient women with severe hypertension (systolic BP ≥ 160 mmHg and/or diastolic BP ≥ 110 mmHg). Try to maintain a BP in the range of 140–159/90–109 mmHg. (C)
5 When women experience a convulsive fit, take all of the following measures: (B)
   1) Measure the BP.
   2) Administer diazepam (5–10 mg, i.v., in a bolus) or $MgSO_4$ (4.0 g, i.v., over 10 min).
   3) Maintain the airway and administer oxygen after the resolution of the convulsion.
   4) Initiate a continuous 24-h i.v. of $MgSO_4$ (1.0–2.0 g/h) for the prevention of recurrence
6 When women are found to be unconscious (or convulsive), perform all three of the following tests to rule out HELLP syndrome, brain hemorrhage, and brain infarct, initiate treatment for an eclamptic fit, and consider the possibility of hysteria, epilepsy, hypoglycemia, hyperventilation syndrome, and/or local anesthetics intoxication as differential diagnoses.
   1) Physical examinations for the detection of palsy of the extremities, presence of abnormal reflex, and anisocoria. (B)
   2) Blood test including a complete blood count (CBC), antithrombin activity, AST level, alanine transaminase level, LDH, FDP or D-dimer level, and an arterial blood gas analysis. (B)
   3) Brain computed tomography/magnetic resonance imaging, if necessary. (B)
7 Plan for an early delivery after stabilizing the condition of the patient, with careful attention to the fetal well-being. (B)

CQ316: How should women with peripartum massive bleeding be treated?

Answer

1 Assess deficiency in the circulating blood volume based on both the shock index (SI) value and

the measured blood loss volume as follows: (B)

SI = pulse rate (per minute) ÷ systolic blood pressure (mmHg).

2 When women exhibit an SI of ≥1.0 or an estimated blood loss of ≥1.0 L during vaginal delivery (≥2.0 L for cesarean delivery), treat as follows, while simultaneously clarifying and removing the cause of bleeding:
   1) Insert an i.v. catheter with a large gauge and replace a sufficient volume of fluid. (A)
   2) Consider a blood transfusion and the transportation of the patient to a secondary or tertiary hospital. (B)
   3) Monitor blood pressure, pulse rate, bleeding amount, and urine output. (A)
   4) Monitor the saturation of peripheral oxygen (SpO_2) level. (C)
3 When women exhibit continuous bleeding, a frequent SI of ≥1.5, an obstetrical DIC score of ≥8, or abnormal vital signs (oliguria, coldness of peripheral skin, or decreased SpO_2), treat as follows, while simultaneously clarifying and removing the cause of bleeding:
   1) Declare an 'obstetrical critical hemorrhage'. (A)
   2) Initiate blood transfusion with packed red blood cells and fresh-frozen plasma if available. (B)
   3) Transport the patient to an appropriate institution. (C)
   4) Administer anti-DIC drugs and platelet concentrate to women with an obstetrical DIC score of ≥8. (C)
4 Uncross-matched group-specific blood, compatible red cell concentrate transfusion with a different ABO, compatible fresh-frozen plasma transfusion with a different ABO, and compatible platelet concentrate transfusion with a different ABO can be administered to women who are suffering from 'obstetrical critical hemorrhage' or imminent cardiac arrest as a result of hemorrhage in the absence of cross-matched group-specified blood. (B)

CQ317: How should 'amnioinfusion' be considered?
Answer

1 Consider the effects of amniotic fluid infusion (amnioinfusion) as follows: (C)
   I. Intrapartum
      1) Amnioinfusion may favorably affect the FHR pattern by decreasing the compression of the umbilical cord.
      2) Amnioinfusion has not been demonstrated to have a prophylactic effect on the development of meconium aspiration syndrome.
   II. Antepartum
      1) Amnioinfusion may be effective for improving the accuracy of antenatal diagnosis using ultrasonography in women with oligohydramnios.
      2) Whether amnioinfusion is beneficial to fetuses with long-term oligohydramnios remains to be studied.
2 Be cautious of amniotic fluid embolism, pulmonary edema and hypertonic uterus during the procedure. (B)

Chapter E. Parturition

CQ401: What medicines and apparatuses should be available in or near the delivery room?
Answer

1. Equip the apparatuses, instruments, and drugs shown in Tables 1 and 2.

CQ402: How should women with a breech presentation be treated?
Answer

1 Confirm that a woman meets all of the following three conditions necessary for the safe performance of external cephalic version. (C)
   1) Emergency cesarean section is available.
   2) No previous cesarean delivery.
   3) Fetus is mature.
2 Choose elective cesarean delivery for a patient with a knee or foot presentation, an estimated fetal weight of <2500 g, GW of <37, or presumed cephalopelvic disproportion. (C)
3 Be able to consider a vaginal delivery in a women without the characteristics described in Answer 2 after fulfilling both of the following two requirements: (C)
   1) Availability of well-trained and full-time medical staff with experience performing breech deliveries.
   2) Women are informed of the risks and benefits of both vaginal and cesarean deliveries.
4 Obtain written informed consent before performing a vaginal breech delivery. (A)

CQ403: How should women who wish to undergo a trial of labor after cesarean delivery (TOLAC) be treated?
Answer

1 Obtain written informed consent for TOLAC in which the risks associated with a TOLAC are described. (A)
2 Confirm that a woman meets all of the following five conditions necessary for a safe TOLAC: (C)
   1) No presumed cephalopelvic disproportion.
   2) Availability of emergency cesarean delivery and emergency treatment for uterine rupture.
   3) Only one previous cesarean delivery.
   4) Previous uterine incision was a low transverse incision with an uneventful postpartum course.
   5) No history of uterine rupture or *trans*-myometrial surgery.
3 Do not use prostaglandin for the induction and/or augmentation of labor. (A)
4 Monitor FHR patterns using cardiotocography during TOLAC. (A)
5 Pay attention to vital signs and abdominal pain in the mother after completing vaginal delivery. (B)

CQ404: How should women with prolonged labor as a result of weak labor pains be treated?
Answer

1 Recommend the oral intake of water or administer i.v. fluids. (B)
2 Adhere strictly to the 'Guidelines for the use of uterotrophic drugs in Japan 2011' and perform all of the following steps when using uterotrophic drugs. (A)
   1) Obtain informed consent for the use of uterotrophic drugs.
   2) Do not use multiple uterotrophic drugs simultaneously.
   3) Apply a cardiotocogram prior to the administration of the uterotrophic drugs.
   4) Use an infusion pump for the i.v. administration of the uterotrophic drug and increase the dose at an interval of ≥30 min.
   5) Assess uterine activities, maternal blood pressure, and pulse rate every hour, in principle.
   6) Record the FHR pattern continuously using a cardiotocogram, in principle.
   7) Transient discontinuation of FHR monitoring with the cardiotocogram is feasible at the physician's discretion.
   8) A well-trained nurse, midwife, or doctor should watch the FHR pattern.
   9) Consider withholding the uterotrophic drug if an abnormal FHR pattern appears.
   10) Remember that no exceptional use is allowed for the initiation dose, the dose increment, or the maximum dose.
3 Remember that cord prolapse may occur after an amniotomy or the spontaneous rupture of the fetal membranes. Perform an amniotomy after confirming 'the fix of the fetal head, station plane of –2 cm'. (B)
4 Record the FHR pattern continuously with a cardiotocogram in febrile women with a body temperature of ≥38.0°C. (B)
5 Be careful of postpartum hemorrhage as a result of uterine atony. (B)
6 Do not inject prostaglandin F2α into the uterine muscle of postpartum women, in principle. (A)

CQ405: How should the induction of labor at term not medically indicated be dealt with?
Answer

1 Be able to induce labor on the demand of a woman or after informed consent with respect to the benefits and risks associated with the induction of labor. (B)
2 Adhere strictly to the Answers in 'CQ412' regarding the induction of labor. (A)
3 Adhere strictly to the 'Guidelines for the use of uterotrophic drugs in Japan 2011' regarding the use of uterotrophic drugs. (A)

CQ406: What criteria are necessary for a safe operative delivery?
Answer

1 Only a well-trained physician or a physician supervised by a well-trained physician should perform operative deliveries, such as vacuum and forceps deliveries. (B)
2 Monitor the FHR patterns continuously during operative deliveries. (C)
3 Use operative deliveries only in women who meet at least one of the following conditions. (B)
   1) Prolonged labor or arrested labor.
   2) A shortened second stage of parturition is desired because of unfavorable maternal conditions.
   3) A non-reassuring fetal status.
4 Use operative deliveries only in women who meet all four of the following conditions.
   1) Gestational age of ≥35 weeks. (C)
   2) No presumed cephalopelvic disproportion. (A)
   3) Completely dilated uterine cervix with ruptured fetal membranes. (B)

4) After engagement of the fetal head (station plane of 0 cm). (B)

5 Pull during uterine contractions, in principle. (B)

6 Do not use vacuum delivery for more than 20 min. Consider forceps or an emergency cesarean delivery if necessary (20-min vacuum trial rule). (C)

7 Do not use vacuum delivery after more than five vacuum trials, even in a situation meeting the 20-min vacuum trial rule (5-time vacuum trial rule). (C)

8 Use outlet-, low-, or lower mid-forceps delivery only in women with both a fetal rotation of <45 degrees and an occiput anterior, in principle. Only a well-trained physician or a physician supervised by a well-trained physician should perform a forceps delivery in situations other than that described above. (B)

CQ407: How should women with meconium staining be treated?

Answer

1 Pay attention to meconium staining in women with ruptured fetal membranes. (B)

2 Apply cardiotocogram to women with meconium staining for at least 20 min to confirm fetal well-being. (B)

3 No specific treatment is required in women with normal FHR patterns. (B)

4 Be cautious of respiratory problems, such as meconium aspiration syndrome, in the neonate. (B)

CQ408: How should a fetus with possible hypoxemia be resuscitated?

Answer

1 Remember that there are no reliable means of improving oxygenation in a fetus. (B)

2 Consider withholding the uterotrophic drug, if such a drug is being used. (A)

3 The following methods may favorably affect the fetal condition: (C)

1) Change in the maternal position from supine to lateral.

2) Oxygen inhalation with a dose of 10–15 L/min.

3) Administration of a tocolytic drug, such as ritodrine (300 mL/h of a bottle containing 50 mg/500 mL), while the mother is in a lateral position.

4) Rapid infusion of lactate Ringer solution (500 mL/20 min).

5) Infusion of warmed normal saline into the uterus (see CQ317).

4 Perform an immediate delivery if fetal compromise as a result of hypoxemia is strongly suspected (see CQ411). (A)

CQ409: How should women at ≥41 GW be treated?

1 Confirm the estimated date of confinement using fetal measurements obtained during the early stage of pregnancy. (A)

2 Assess fetal well-being once or twice a week. (B)

3 Induce labor or conduct watchful waiting (expectant management) depending on the cervical maturation between $41^{-0/7}$ and $41^{-6/7}$ GW. (B)

4 Consider induction of labor in women at ≥42 GW. (B)

5 Adhere strictly to the 'Guidelines for the use of uterotrophic drugs in Japan 2011' when using uterotrophic drugs. (A)

CQ410: How should parturient women be treated?

Answer

1 A physician, mid-wife, or well-trained nurse can manage parturient women. (A)

2 Use a 3-cm/min flow velocity for the paper that traces the FHR pattern when using a cardiotocogram. (B)

3 Obtain a cardiotocogram for at least 20 min upon admission or during the first stage of labor to confirm fetal well-being. (B)

4 Once fetal well-being has been confirmed as described in Answer 3, fetal well-being may be monitored using intermittent FHR auscultation (once every 15–90 min) until the next application of a cardiotocogram within 6 h in women without characteristics described in Answer 5. Continuous FHR monitoring throughout the first stage of labor is feasible. (B)

5 Monitor the FHR pattern continuously in women with the following characteristics. Transient discontinuation of FHR monitoring is feasible at the physician's discretion.

1) During the use of a uterotrophic drug, such as oxytocin. (A)

2) During the second stage of labor, in febrile women (≥38.0°C), during the use of a metreurynter containing ≥41 mL, and during painless labor with anesthetics. (B)

3) Women requiring 'increased monitoring' according to Tables I, II, and III in CQ411. (B)

4) High-risk pregnancies with any of the following characteristics: (B)

- Maternal factors, including diabetes mellitus, pregnancy-induced hypertension, previous stillborn or infant with cerebral palsy as a result of intrapartum fetal hypoxemia at ≥30 GW, or a previous surgical incision into the uterine cavity.
- Fetal factors, including a non-vertex presentation, an estimated fetal bodyweight of <2000 g, fetal growth restriction, and multiple pregnancies.
- Placental factors, such as a low-lying placenta.

5) Other women suspected of having poorly controlled maternal complications. (C)

6 Apply a cardiotocogram for at least 20 min in women with any of the following situations:
1) Rupture of the fetal membranes. (B)
2) Meconium staining or bloody amniotic fluid. (B)
3) When bradycardia or tachycardia is noted during intermittent FHR auscultation. (A)
4) When the rapid progress of labor is noted or a change in the fetal position is anticipated after urination or defecation. (C)

7 Review the FHR patterns that are being continuously monitored by a cardiotocogram at the following intervals: (C)
1) For those who are not at a high risk or those who have an FHR pattern of level 1 or 2: every 30 and 15 min during the first and second stages of labor, respectively.
2) For those at high risk or those who have an FHR pattern of level 3: every 15 and 5 min during the first and second stages of labor, respectively.
3) For those who show an FHR pattern of level 4 or 5: watch continuously.

CQ411: How should various FHR patterns be interpreted and how should women with a non-reassuring fetal status be treated?

Answer

1 Consider that the well-being of a fetus can be assured if the FHR pattern has a normal baseline, a normal baseline variability, the presence of acceleration, and the absence of deceleration. (A)

2 Consider that the well-being of a fetus may be impaired if any of the following FHR patterns are present: (B)
1) Recurrent late decelerations with absent baseline variability.
2) Recurrent variable decelerations with absent baseline variability.
3) Prolonged deceleration with absent baseline variability.
4) Severe bradycardia with decreased or absent baseline variability.

3 Diagnose a fetus as having a non-reassuring fetal status if an FHR pattern level of 3–5 (mild, moderate, and severe variant patterns, as shown in Table I) is present as classified using a combination of three factors: the baseline variability, the baseline, and the presence of various decelerations. (C)

4 Choose one of five treatments (no intervention, increased monitoring, conservative measures for resuscitation of the fetus, preparation for prompt delivery, and prompt delivery) in cases with an FHR pattern of level 1–5, referring to Table III and taking gestational age, the background of the women, and the capacity of the facility into account. (C)

5 Repeatedly assess the feasibility of a successful vaginal delivery taking progression and the stage of labor into account in those who continue to show a level 3 or 4 FHR pattern. (C)

6 Perform an emergency cesarean section soon after abandoning vaginal delivery in the situation described in Answer 5. (C)

CQ412: How should labor be induced?

Answer

1 Adhere strictly to the 'Guidelines for the use of uterotrophic drugs in Japan 2011' regarding the use of uterotrophic drugs, such as i.v. oxytocin, i.v. prostaglandin $F_2\alpha$, and oral prostaglandin PGE_2 tablets. (A)

2 Do not administer multiple uterotrophic drugs simultaneously. (A)

3 Ripen the uterine cervix first if the cervix is unfavorable for the induction of labor. (B)

4 Remember the following three precautions regarding the use of hygroscopic mechanical dilators, such as laminaria rods or a transcervical balloon catheter of ≤40 mL, for the ripening of the cervix.
1) Obtain informed consent as to the indications, methods and possible adverse events associated with these procedures. (B)
2) Perform the procedure in hospitalized women. (B)
3) Pay attention to the possibility of infection regardless of the status of the fetal membranes. Assess the maternal body temperature and the results of laboratory tests, and consider the administration of antibiotics in women with ruptured fetal membranes. (B)

5 In addition, remember the following four cautions regarding the use of a transcervical balloon catheter of ≤40 mL for the ripening of the cervix.
   4) Obtain informed consent regarding the possible risk of umbilical cord prolapse associated with the use of transcervical balloon catheters. (B)
   5) Confirm the absence of the umbilical cord near the presenting part of the fetus prior to the start of the procedure. (B)
   6) Promptly apply a cardiotocogram after the commencement of labor. (B)
   7) Promptly confirm the absence of prolapse or the descent of the cord at the time of the rupture of the fetal membranes or the balloon prolapse. (B)
6 In addition, remember the following three cautions regarding the use of a transcervical balloon catheter of >40 mL for the ripening of the cervix.
   8) Perform continuous FHR monitoring with a cardiotocogram. (B)
   9) Use a transcervical balloon catheter ≤150 mL for vertex presentation. (B)
   10) Ensure the availability of an emergency caesarean section. (C)
7 Do not administer uterotrophic drugs while using laminaria rods and/or sodium prasterone sulfate hydrate. (B)
8 When combining the use of a transcervical balloon catheter and a uterotrophic drug, monitor the FHR for at least 1 h after the application of the transcervical balloon catheter, then initiate the uterotrophic drug. (B)
9 When using an i.v. uterotrophic drug after the use of oral PGE_2, initiate i.v. oxytocin or i.v. prostaglandin $F_2\alpha$ at an 'initiation dose' at least 1 h after the last oral PGE_2 administration. Pay close attention to excessive increases in uterine activity. (B)

CQ413: How should a woman with labor pains or some relevant problem be treated in the absence of data necessary for the management of pregnant women because of a lack of antenatal visits?

Answer

1 Consider her pregnancy to be high risk. (B)
2 Assess the GW. (B)
3 Perform tests recommended during routine antenatal care. (B)
4 Try to identify the patient and to confirm that the address/phone number of her family contact is accurate. (B)
5 Listen to her background in a supportive manner. Consult a city officer to seek public support for her as soon as possible if no support is available from her family. (C)
6 Try to create better surroundings for the newborn by keeping in contact with the woman after discharge from the hospital through visits by regional public health nurses. (C)

CQ414: How may safety be assured in a 'midwife-managed care system'?

Answer

1 A 'midwife-managed care system' is defined as that in which midwives care for pregnant and parturient women in the absence of an attending physician by adhering to institutional rules made by responsible physicians and midwives, with the assurance of a prompt switch from midwife care to physician care in the institution. (B)
2 Midwife care for pregnant and parturient women is low-risk when performed according to institutional rules made by referring to Tables 1, 2, 3, and 4 in the 'midwife-managed care system'. (C)

Chapter F. Incidental complications

CQ501: How should one respond when asked about the outcome of pregnancy complicated with uterine fibroma?

Answer

1 Respond as follows:
   1) The pregnancy outcome is fairly good. However, there may be increased risks of complications, such as premature labor, an abnormal position of the fetus, placenta previa, placental abruption, poly- or oligohydramnios, pregnancy-induced hypertension, or premature membrane rupture. (B)
   2) Approximately 20% of women experience transient pain (for 1–2 weeks) originating from the fibroma. (B)
   3) There may be increased risks of dystocia, increased bleeding, labor arrest, and cesarean delivery. (B)
   4) The risks and benefits of a myomectomy during pregnancy and a cesarean section are uncertain at present. (C)
   5) There may be an increased risk of a hysterectomy postpartum because of the degeneration/infection of the fibroma/uterus. (C)

CQ502: How should women with an abnormal uterine cervical cytology result during early pregnancy be treated?

Answer

1 Perform colposcopy and biopsy, in principle, for patients with abnormal cytology (LSIL, HSIL, and others in the Bethesda system, and classes III, IV, and V in the former system). (B)
2 Treat women with histologically confirmed cervical intraepithelial neoplasia in the absence of cell cytology suggestive of invasive cancer conservatively without conization. (B)
3 Perform conization in cases with any of the following characteristics: (A)
   1) Histologically confirmed microinvasive squamous cell carcinoma.
   2) Histologically confirmed intraepithelial neoplasia but cell cytology indicative of invasive cancer.
   3) Histologically confirmed adenocarcinoma *in situ*.
4 Conservative treatment without termination of the pregnancy is feasible in cases with squamous cell carcinoma stage Ia1 without lymphovascular space invasion or adenocarcinoma *in situ* in the conization specimen. (B)
5 Repeat the cytology examination during pregnancy in women with conservative treatment. (A)
6 Vaginal delivery is feasible in women with conservative treatment. (A)
7 Reevaluate the disease status using cytology, colposcopy, and biopsy 4–8 weeks postpartum. (B)

CQ503: How should pregnant women with postconization uterus be treated?

Answer

1 Note that women are at an increased risk of preterm labor after undergoing cervical conization. (A)
2 Be cautious of signs of preterm labor, such as the shortening of the uterine cervix and increased uterine activity. (B)
3 Consider therapeutic cervical cerclage in women with a shortened uterine cervix. (C)

CQ504: How should women with an ovarian cyst detected during early pregnancy be treated?

Answer

1 Use ultrasonography to visualize the ovarian cyst and assess the possibility of malignancy; monitor the size of the ovarian cyst to rule out the possibility of a common 'corpus luteum cyst', which may resolve spontaneously. (A)
2 Treat women with ultrasonography findings suggestive of an ovarian cyst appearing as a tumor-like lesion, such as a corpus luteum cyst or an endometriotic cyst, conservatively. (B)
3 Treat women with an ovarian cyst with the characteristic ultrasonographic features of a benign lesion as follows: (C)
   1) An ovarian cyst with a largest diameter of <6 cm or a unilocular ovarian cyst with a largest diameter of <10 cm: conservative treatment without surgical intervention.
   2) An ovarian cyst with a largest diameter of ≥10 cm or a multilocular ovarian cyst with a largest diameter of ≥6 cm: surgical removal of the cyst, preferably after 12 GW.
4 Remove the tumor surgically, irrespective of the size and gestational age if a borderline or malignant tumor is suspected. (B)
5 Remove the cyst surgically, irrespective of the nature of the cyst and the gestational age in cases with severe abdominal pain as a result of ovarian torsion, rupture, or bleeding. (A)

CQ505: How should women with decayed teeth and/or periodontal diseases be treated?

Answers

1 Recommend a visit to a dentist when asked about decayed teeth and/or periodontal diseases, as pregnancy may have an unfavorable effect on the progression of these diseases. (B)
2 Remember that favorable effects of treatment on the risks of preterm birth and FGR have not been demonstrated, although periodontal diseases are reportedly associated with preterm birth and FGR. (C)

Chapter G. Infection

CQ601: How should pregnant women with bacterial vaginosis (BV) be treated?

Answer

1 Treat women with symptomatic BV using antibiotics. (B)
2 Detect patients with BV by screening all women with known risk factors of preterm delivery, such as previous preterm delivery, and treat patients with BV using antibiotics as soon as possible. (C)

CQ602: How should pregnant women with urogenital *Chlamydia trachomatis* infections be treated?

Answer

1 Screen all women for the detection of *C. trachomatis* infection using a swab specimen sampled from the endocervix for the prevention of neonatal *C. trachomatis* infection. (B)

2 Detect *C. trachomatis* in specimens using nucleic acid amplification tests, nucleic acid hybridization tests, direct immunofluorescence, enzyme immunoassay (EIA), or culture method. (B)
3 Treat with a single dose of oral azithromycin (1.0 g) or oral clarithromycin (200 mg × 2/ day, 7 days). (B)

CQ603: How should women with genitourinary group B streptococcal (GBS) infection be treated?
Answer

1 Screen all women at 33–37 GW for the detection of GBS infection using a culture method for specimens from the vagina, perineum, and rectum. (B)
2 Administer i.v. penicillin/ampicillin to women with any of the following characteristics to prevent early-onset GBS diseases of the infant during labor or after premature membrane rupture: (B)
   1) GBS infection in a previous infant (even in the absence of GBS in the current pregnancy).
   2) All women with a positive GBS result except for women undergoing an elective cesarean section.
   3) Women with an unknown GBS status.
3 Remember that a 3-day antibiotic administration period is sufficient to eradicate GBS in women with premature membrane rupture. (C)

CQ604: How should pregnant women with an antibody against toxoplasma be treated?
Answer

1 Estimate the timing of the toxoplasma infection using specific IgG and IgM antibodies against toxoplasma. (B)
2 Remember that a positive IgM antibody does not necessarily indicate a recent infection occurring within several months, since the phenomenon of 'persistent IgM' in which positive IgM antibodies sometimes persist for a long time (≥3 months) has been known to occur. (B)
3 Administer oral acetylspiramycin to women infected after the establishment of the current pregnancy. (B)
4 Consider the administration of pyrimethamine and sulphadiazine between 16 and 27 GW in women with a suspected fetal infection. (C)

CQ605: How should women with rubella infection during pregnancy be treated?
Answer

1 Screen all women to detect patients with rubella infection during an early stage of pregnancy using HI (titer of antibody against rubella). (A)
2 Obtain the following information from women during an early stage of pregnancy. (B)
   1) Contact with rubella patients during the past 3 months.
   2) Skin rash during the past 3 months.
   3) Febrile disease during the past 3 months.
   4) Swelling of cervical lymph nodes during the past 3 months.
   5) Occupation during the past 3 months.
3 Take diagnostic measures in women with any of the following findings: (B)
   1) Symptoms suggestive of rubella infection, such as skin rash, febrile condition, and swollen lymph nodes.
   2) Contact with rubella patients.
   3) Titer of antibody (HI) ≥ ×256 during early pregnancy.
4 Remember that diagnostic measures should include both repeated measures of the HI titer in paired sera samples and measures of specific IgM antibody. (B)
5 Administer vaccine to postpartum women with an HI antibody titer ≤ ×16. (C)

CQ606: How should women with HBs-antigen be treated?
Answer

1 Determine the status of HBe-antigen and liver function and tell women about the risk of HBV vertical transmission. (A)
2 Recommend a visit to an appropriate physician. (C)
3 Take measures, including the administrations of HB immunoglobulin and vaccination against HBV to prevent HBV vertical transmission, in cooperation with pediatricians. (A)
4 Tell women that breast-feeding does not increase the risk of HBV vertical transmission, providing that preventive measures are being taken. (B)

CQ607: How should women with HCV-antibody be treated?
Answer

1 Quantify HCV-RNA in the blood and examine liver function. (A)
2 Tell women with undetectable HCV-RNA that HCV vertical transmission does not occur. (B)
3 Tell women with detectable HCV-RNA that there may be a risk of HCV vertical transmission. Recommend visit to appropriate physicians. (B)
4 Tell women with detectable HCV-RNA that breast-feeding does not increase the risk of HCV vertical transmission. (C)

5 Provide information on the Japanese vertical transmission rates according to titers of HCV-RNA and delivery modes in women with a higher HCV-RNA. Help women to choose a delivery mode according to these data. (C)

CQ608: How should pregnant women with genital herpes be treated?

Answer

1 During the first trimester, apply acyclovir ointment to the lesions and advise the patient to refrain from sexual activities. (B)
2 During the second or third trimester, administer systemic anti-viral drugs to women with a primary infection or first-episode disease. (B)
3 Recommend an elective cesarean section in women with any of the following statuses.
   1) Presence of genital lesions at supposed time when labor will occur. (A)
   2) Labor pains occurring within 4 weeks of the manifestation of the primary infection. (C)
   3) Labor pains occurring within 1 week of the manifestation of a recurrence or non-primary first-episode disease. (C)
4 Pay attention to the neonate with respect to symptoms derived from vertical transmission. (B)

CQ609: How should women with cytomegalovirus (CMV) infection be treated?

Answer

1 Remember that the clinical usefulness of screening for CMV infection has not been established. (C)
2 Consider that fetuses with growth restriction, enlarged cerebral ventricle, microcephalus, a high-echoic periventricular area, ascites, and/or hepatosplenomegaly may be infected with CMV. (C)
3 Interpret the results of maternal CMV-antibody as follows. (B)
   1) Diagnose as primary infection during pregnancy when seroconversion (change from a negative CMV-IgG during early pregnancy to a positive CMV-IgG during pregnancy) is observed.
   2) The effects of CMV on the fetuses are milder in women who acquired CMV before pregnancy (a positive CMV-IgG during early pregnancy), compared with those of a primary infection during pregnancy, although adverse effects on the fetuses may occur.
   3) Consider recent infection in women with a positive CMV-IgM, but be cautious of persistent CMV-IgM (the phenomenon of long-lasting IgM positivity).
4 Tell women with CMV infection that no helpful fetal therapy has been established. (B)
5 Remember that infected fetuses are likely to have an abnormal FHR pattern during labor. (C)
6 Diagnose as congenital infection when CMV-IgM is detected in the cord blood and/or CMV is detected in the urine of neonates within 2 weeks after birth. (B)
7 Refer infants with congenital infection to an appropriate physician for the long-term follow-up of auditory function. (B)

CQ610: How should women with HIV infection be treated?

Answer

1 Screen all women for the detection of patients with HIV infection early during pregnancy using tests for HIV screening. (B)
2 In cases with a positive screening test result, do the following: (A)
   1) Inform the woman that 19 out of 20 women with a positive screening test result are not actually infected.
   2) Perform a Western blotting test and a nucleic acid amplification test simultaneously to confirm the screening result.
3 Consult with a regional designated hospital regarding patients with HIV/AIDS. (C)
4 Perform all of the following measures to prevent vertical transmission. (B)
   1) Treat infected women with antiretroviral drugs during pregnancy.
   2) Elective cesarean delivery.
   3) Formula milk feeding.
   4) Prophylactic administration of antiretroviral drugs to the neonate.

CQ611: How should pregnant women with varicella infection be treated?

Answer

1 Tell women as follows when asked about varicella infection during pregnancy:
   1) Women with neither a history of varicella infection nor vaccination against varicella should refrain from contact with patients with varicella. (A)
   2) Congenital varicella syndrome is reportedly seen in 0.55%, 1.4%, and 0.0% of neonates born to mothers infected with varicella during the

first-, second-, and third-trimesters of their pregnancies, respectively. (B)
   3) No infants with congenital varicella syndrome and/or malformation as a result of varicella infection have been born to mothers in whom an erroneous vaccination was administered during the 3 months prior to the establishment of pregnancy or during pregnancy. (B)
2 Do not administer varicella vaccine to pregnant women. (A)
3 Administer prophylactic i.v. gammaglobulin (2.5 g to 5.0 g) to women who have been in close contact with a patient infected with varicella during the previous 2 weeks and who may be susceptible to varicella infection because of a possible lack of antibody. (C)
4 Administer acyclovir to pregnant women with varicella infection to prevent serious complications. (C)
5 Treat the mother and neonate as follows when the mother manifests a varicella infection during the 5 days prior to delivery or 2 days postpartum:
   1) Administer acyclovir to the mother. (B)
   2) Administer i.v. gammaglobulin to the neonate. (B)
   3) Administer acyclovir to neonates with symptoms of varicella infection. (B)
6 Isolate in-hospital pregnant women with varicella infection in a private room away from other women to prevent in-hospital horizontal transmission. (C)

CQ612: How should women with a positive screening test result for HTLV-1 infection be treated?
Answer

1 Note that a considerable number of women show a false-positive result on screening tests for HTLV-1 infection (particle agglutination or enzyme-linked immunosorbent assay). (A)
2 Diagnosis as an HTLV-1 carrier only after a confirmation test (Western blot analysis) shows a positive result. (A)
3 Inform women of their diagnosis as an HTLV-1 carrier very carefully, with consideration of ethical problems. (A)
4 Inform the patient's family of her diagnosis as an HTLV-1 carrier only after receiving the patient's permission. (B)
5 Instruct the patient in the following methods as alternatives to breast-feeding for the prevention of HTLV-1 vertical transmission: (B)
   1) Formula milk feeding.
   2) Frozen-thawed breast milk.
   3) Short-term breast-feeding within the first 3 months after birth.

CQ613: How should women with syphilis be treated?
Answer

1 Screen all women for the detection of patients with syphilis using two methods (a non-specific test and a specific test for *T. pallidum*). (A)
   1) Non-specific tests include serological tests for syphilis (STS), such as an agglutination test, VDRL test, or RPR card test.
   2) Specific tests include FTA-ABS and TPHA.
2 Promptly administer antibiotics, such as penicillin, to women with active syphilis. (A)
3 Assess the fetus during the latter half of the pregnancy with respect to signs of infection, such as hepatomegaly, ascites, hydrops, and a thickened placenta, in infected women. (C)
4 Assess the effect of treatment at 28–32 GW and perinatally using the STS titer. (C)
5 Remember that physicians must notify the regional public health center within 7 days of diagnosing a new patient with syphilis according to the Infectious Diseases Control Law, in which syphilis is classified as a fifth-class infectious disease. (A)
6 Examine the neonate born to an infected mother with respect to congenital syphilis, according to Table 2. (A)

CQ614: How should women with parvovirus B19 (PB19) infection be treated?
Answer

1 Remember the following two points: (B)
   1) Co-living with a patient with PB19 infection is a risk factor.
   2) Flu-like symptoms associated with erythema and arthralgia are signs of a PB19 infection.
2 Determine the anti-PB19 IgM titer if a PB19 infection is suspected. (B)
3 Assess fetal anemia and hydrops in cases with maternal PB 19 infection, since approximately 10% of such fetuses develop anemia, hydrops and/or die. (C)
4 Consider PB19 infection as a differential diagnosis for fetal hydrops. (B)
5 Recommend that infected women wash their hands and wear a flu mask to prevent in-hospital horizontal transmission. (C)
6 Remember that the following facts are known about PB infection during pregnancy: (C)

1) Ninety percent of hydrops fetalis cases develop within 8 weeks (median, 3 weeks) after maternal infection with PB19.
2) Fetal mortality is higher for maternal infection at <20 GW than for maternal infection at >20 weeks.
3) Spontaneous remission occurs in one-third of hydrops fetalis cases.
4) A blood transfusion to the fetus may be effective for improving the outcome.
5) The outcome of surviving fetuses with PB19 infection is similar to that of non-infected fetuses.

Chapter H. Twin pregnancies

CQ701: How should chorionicity and amnionicity be determined for a twin pregnancy?

Answer

1 Determine chorionicity until the end of 10 GW. (A)
2 Count the numbers of chorions and amnions using ultrasonography to determine the chorionicity and amnionicity. (A)
   1) Diagnose as monochorionic and dichorionic twins in cases with one and two gestational sacs (GS), respectively, as the number of GS equals that of the chorion.
   2) Diagnose as dichorionic in cases with a relatively thick dividing membrane (inter-twin septum).
   3) Diagnose as diamniotic in cases of monochorionic twins with a thin dividing membrane.
   4) Presume monochorionic monoamniotic twins and repeat the ultrasonography examination in cases with an unrecognizable dividing membrane.
3 Determine the chorionicity and amnionicity referring to the presence or absence of a twin peak sign, the number of placentas, and the fetal sex in cases with undetermined chorionicity at ≥14 GW. (B)

CQ702: How should women with monochorionic twin pregnancies be treated?

Answer

1 Refer women to secondary or tertiary institutions or treat the women in cooperation with those institutions. (B)
2 Provide information on the risks associated with monochorionic twins. Be cautious of the occurrence of twin-to-twin transfusion syndrome and twin reversed arterial perfusion sequence. (B)
3 Determine amnionicity (mono- or di-) before 14 GW. (B)
4 Examine using ultrasonography at least once every 2 weeks in cases with monochorionic diamniotic twin pregnancy, paying attention to discordances in volumes of the amniotic fluid and/or fetal development. (C)
5 Provide information on the risk of sudden fetal death as a result of cord entanglement in women with monoamniotic twins. (C)

CQ703: How may twin-to-twin transfusion syndrome (TTTS) and a twin reversed arterial perfusion (TRAP) sequence be detected in monochorionic twin pregnancies?

Answer

1 Presume TTTS and examine extensively in a case with a tendency toward polyhydramnios in one twin and oligohydramnios in the co-twin. (B)
2 Presume a TRAP sequence and examine extensively when a dead twin is growing. (B)

CQ704: How should women with twin pregnancies and a single fetal death be treated?

Answer

1 Manage expectantly, but pay attention to maternal DIC in dichorionic twin pregnancies. (B)
2 Manage expectantly, but pay attention to anemia and the well-being of the surviving twin in monochorionic twin pregnancies. (C)
3 Inform women with a monochorionic placenta that the surviving twin is at an extraordinarily high risk of developing permanent disabilities or perinatal mortality, even with the best of treatment. (C)

CQ705: What general cautions are needed when managing women with twin pregnancies?

Answer

1 Pay attention to clinical signs of preterm labor in the latter half of the pregnancy. (A)
2 Provide adequate tests for the detection of pregnancy-induced hypertension, HELLP syndrome and venous thromboembolism during the latter half of the pregnancy. (C)
3 Pay closer attention to fetal well-being at ≥37 GW in twin pregnancies than in singleton pregnancies. (B)
4 Refer to the following when choosing a delivery mode: (C)
   1) Both twins are cephalic presentation: vaginal delivery.
   2) First twin is cephalic and the second twin is non-cephalic: similar cautions to those for the vaginal delivery of a singleton with a breech presentation

are required during the vaginal delivery trial (see CQ402).
3) First twin is non-cephalic presentation: elective cesarean section.

5 Monitor the FHR patterns of both fetuses continuously and simultaneously during labor. (B)
6 Confirm the fetal position and FHR pattern of the second twin immediately after the vaginal birth of the first twin. (B)
7 Pay attention to postpartum hemorrhage and venous thromboembolism. (C)

Chapter I. Newborns

CQ801: How should neonates with birth asphyxia be resuscitated?

Answer

1 Physicians, midwives and nurses are required to make every effort to acquire knowledge and the necessary skills to perform neonatal resuscitation, as one in 100 neonates requires resuscitation just after birth. (A)
2 Assess the following three points just after birth. (A)
1) Not an immature infant.
2) Good breathing/crying.
3) Good muscle tone.
3 Care for neonates routinely as shown in Table 1 in cases meeting all three conditions described in Answer 2. (B)
4 Perform the 'primary resuscitative procedures' shown in Table 2 if any abnormality is present among the three conditions shown in Answer 2. (B)
5 Take further resuscitative measures in cases in which 'primary resuscitative procedures' have failed, referring to Figure 1 posted on the wall of the delivery room. (C)
6 Determine the Apgar scores at 1 and 5 min and record them. (B)
7 Analyze the umbilical arterial blood gas and record the findings. (C)
8 Be cautious of maintaining an adequate neonatal body temperature. (B)
9 Consult neonatologists or experienced physicians if there is any concern regarding the neonate's condition. (B)

CQ802: How should newborns be cared for within 10 days after birth?

Answer

1 Apply eye drops or ointment containing antibiotics to the eyes of newborns to prevent conjunctivitis. (B)
2 Attempt to find any congenital anomalies. (B)
3 Confirm the absence of extremity paralysis and cephalohematoma. (A)
4 Regularly assess body temperature, bodyweight, respiration, feeding conditions, activities, and skin color (jaundice and cyanosis). (B)
5 Remember that clinical impressions, such as a lack of vigor, bad skin color, or tachypnea, are often clues for the diagnosis of specific abnormalities in newborns. (B)
6 Suspect a possible infection, hypoglycemia, congenital heart disease, gastrointestinal disease, hemolytic disease, or congenital metabolic disease if a neonate exhibits any of the abnormalities described in Answer 4. (B)
7 Administer vitamin K to neonates in an adequate manner (refer to the Discussion). (B)
8 Provide mass-screening tests for the detection of congenital metabolic diseases and an auditory test to neonates after obtaining the parents' informed consent. (C)
9 Remember that discharge from the hospital within 3 days after birth is associated with an increased risk of re-hospitalization for jaundice and/or dehydration. (C)
10 Keep in contact with regional public health nurses or city officers who may be concerned with neonatal health, if necessary. (C)
11 Support mothers to establish breast-feeding. (C)
12 Pay attention to the safe preparation and preservation of formula milk, referring to Table 2. (B)

CQ803: What information concerning respiratory syncytial virus (RSV) is helpful for preterm infants (<36 GW) leaving the hospital?

Answer

1 Preterm infants (<36 GW) with RSV infection are likely to develop severe complications. (C)
2 The prophylactic administration of certain drugs to preterm infants during the RSV season prevents or reduces severe complications caused by RSV. (C)
3 Provide information on clinics at which drugs for RSV prophylaxis are available. (C)

CQ804: How should the cause of intrauterine fetal death (IUFD) at ≥22 GW be determined and how should women with IUFD be treated?

Answer

1 Estimate the time of IUFD in an integrated manner. (A)
2 Determine the cause of death using the following tests:
Fetal factors
1) Macroscopic inspection of the stillborn infant, placenta and the umbilical cord. (A)
2) Histopathological examination of the placenta and umbilical cord. (C)
3) Autopsy of the stillborn infant. (C)
4) X-ray examination of the whole body of the stillborn infant, or equivalent examinations. (C)
5) Chromosomal analysis. (C)
Maternal factors
6) Tests such as the indirect Coombs test for atypical antibodies against erythrocytes in cases with an undetermined antibody status. (B)
7) Tests for antiphospholipid antibody, including lupus anticoagulant, anti-cardiolipin antibody, and anti-cardiolipin β2GP1 antibody. (C)
8) Tests for syphilis in undetermined cases. (B)
9) Tests for parvovirus B19 or other TORCH infections. (C)
10) Tests for glucose tolerance and thyroid function. (C)
11) Tests for coagulation-fibrinolysis system. (C)
12) Test for feto-maternal transfusion. (C)
3 Provide information regarding the risk of recurrence in siblings of IUFD cases with structural malformations and/or chromosomal aberrations at the request of the mother/family. (B)
4 Support the mother and family psychologically and emotionally with counseling and other forms of assistance. (B)

Chapter J. Others

CQ901: How should one respond when asked about the effects of car seatbelts during pregnancy?
Answer

1 Explain as follows.
Damage from car accidents is reduced if seatbelts are applied in an appropriate manner. The chest belt should pass between the breasts, and the waist belt should pass below the pubic bone; neither belt should cross the protruding abdomen. (A)

CQ902: How should female patients involved in multiple casualty incidents be treated?
Answer

1 Consider the possibility of a casualty being pregnant while performing triage and while treating and transporting female casualties. (B)
2 Try to identify pregnant women using ultrasonography or a similar apparatus to detect the fetal cardiac activity if female casualties are not able to respond to questions during secondary triage. (C)
3 Tag pregnant patients who are suffering from a rupture of fetal membranes, genital bleeding, pain in the abdomen, and/or fetal death with a red card. (B)

CQ903: How should one behave after experiencing an incidental maternal mortality?
Answer

1 Notify the 'accident investigation committee' of each hospital. (A)
2 Contact the central and prefectural offices of the Japan Association of Obstetricians & Gynecologists (JAOG) and report the incident in detail. (A)
3 Make every effort to obtain consent to perform an autopsy. (A)

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RESEARCH ARTICLE

Complete restoration of phenylalanine oxidation in phenylketonuria mouse by a self-complementary adeno-associated virus vector

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Abstract

Background Classical phenylketonuria (PKU) arises from a deficiency of phenylalanine hydroxylase (PAH) that catalyses phenylalanine oxidation in the liver. Lack of PAH activity causes massive hyperphenylalaninemia and consequently severe brain damage. Preclinical studies showed that conventional adeno-associated virus (AAV) vectors could correct hyperphenylalaninemia in a mouse model of PKU, although limitations such as very large dose requirement and relative inefficiency in female animals were recognized.

Method An AAV8-pseudotyped vector was constructed with a self-complementary AAV (scAAV) genome for efficient liver transduction and expression. Following vector injection to PKU mice, blood Phe was periodically measured by an enzymatic fluorometric assay. *In vivo* Phe oxidation was evaluated by a non-invasive breath test using [1-^{13}C]Phe. Vector copy number in the host tissues was determined by quantitative polymerase chain reaction.

Results A single injection of 1×10^{11}–1×10^{12} particles of the scAAV8 vector resulted in a reduction of blood Phe to normal or near-normal levels for more than 1 year in both genders. The treated animals showed normal level of *in vivo* Phe oxidation. The presence of >1 copy of vector DNA per diploid genome in the liver was associated with normal blood Phe in the AAV-treated PKU mice.

Conclusions Complete phenotypic correction of PKU mice was achieved by the scAAV8 vector for the longest duration reported to date. The vector overcame the female-specific disadvantage in AAV-mediated liver transduction; thus, it offers a promising platform of long-lasting gene therapy for PKU. Copyright © 2011 John Wiley & Sons, Ltd.

Keywords adeno-associated virus; gene therapy; phenylketonuria; phenylalanine oxidation

Introduction

Phenylketonuria (PKU; OMIM 261 600) is an autosomal recessive disorder caused by a deficiency of phenylalanine hydroxylase (PAH; EC 1.14.16.1) in the liver [1]. The enzyme is responsible for the major part of phenylalanine (Phe) clearance by converting Phe to tyrosine (Tyr) with the aid of tetrahydrobiopterin (BH_4) and molecular oxygen. Consequently, PAH deficiency leads to a massive accumulation of Phe under a normal

diet, which is toxic to the developing brain. Untreated patients are afflicted with severe mental retardation, seizure and growth failure, as well as hypopigmentation of the hair and skin. The current management for PKU mandates an early diagnosis by newborn screening followed by a Phe-restricted diet to prevent irreversible brain damage in infancy and childhood. Such a diet is also recommended for adult PKU patients to avoid problems associated with hyperphenylalaninemia, such as psychomotor dysfunction and teratogenic effects on fetuses carried by the female patients (so-called 'maternal PKU syndrome'). However, the unpalatable and expensive diet loads heavy burdens on the patients and their families. Therefore, an alternative approach to PKU, preferably achieving a life-long cure, is desired.

We and other investigators have explored the development of somatic gene therapy for PKU, which offers a novel therapeutic paradigm [2,3]. The most straightforward approach is to deliver the functional PAH gene to the liver, where the enzyme is normally expressed. In most preclinical studies, a mouse model of PKU (*Pah*^{enu2} strain developed in BTBR background) has been used because it shows a similar phenotype to human PKU [4,5]. Thus far, recombinant adeno-associated virus (AAV) vectors have shown the most promising results in correcting hyperphenylalaninemia in *Pah*^{enu2} mice [6–10]. Through these studies, however, two major problems were recognized. One was the relatively large dose requirement of AAV vectors to correct murine PKU phenotype compared to other disease models such as haemophilia B. The other problem was gender-dependent effectiveness of AAV vectors, particularly when they were targeted to the liver. That is, female *Pah*^{enu2} animals required greater AAV doses to reduce blood Phe, and the therapeutic effect was not long-lasting. This phenomenon was investigated in some detail with another animal model [11]. To enhance gene delivery and overcome these problems, attention has turned to alternative AAV serotypes with distinct tissue tropism (e.g. AAV8 for liver transduction) [12–14]. Pseudotyping with AAV8 capsid showed higher efficiency in treating *Pah*^{enu2} mice, together with *cis*-acting elements to enhance liver-specific transcription and mRNA transport [9,10].

In the quest for a further improvement in liver transduction, we realized that another drawback in AAV transduction should be circumvented. Because the single-stranded (ss) DNA genomes of conventional AAV vectors are transcriptionally inactive, they must become double-stranded (ds) to be expressed. The ss- to dsDNA conversion requires host cell-mediated DNA synthesis or annealing of complementary genomes from separate virions, which appears to be the rate-limiting step in AAV transduction [15,16]. This process can be bypassed with a self-complementary (sc) AAV vector because its genome DNA spontaneously self-anneals to form stable dsDNA in the host cell [17–19]. Therefore, we investigated the efficacy of a self-complementary AAV (scAAV) vector for the treatment of *Pah*^{enu2} mice.

Materials and methods

Vector construction

A serotype 8-pseudotyped ssAAV vector for PKU (ssAAV8/CAG-mPAH) was constructed with ssAAV/CAG-mPAH plasmid (Figure 1, top) used in our previous study [7,20]. A scAAV8 vector was constructed with scAAV/LP1-hFIX plasmid carrying the human factor IX (hFIX) gene (kindly provided by Dr John T. Gray, St Jude Research Hospital, Memphis, TN, USA) [21]. For vector-derived PAH expression, the hFIX sequence (*Eco*R I-*Xho* I) in scAAV/LP1-hFIX was replaced with the murine PAH (mPAH) cDNA (*Eco*R I-*Sal* I) from ssAAV/CAG-mPAH (Figure 1, bottom). AAV8-pseudotyoed vector stocks were propagated by an adenovirus-free, three-plasmid transfection method [22]. Briefly, a 10-tray Cell Factory container (CF10; Nalge Nunc International, Rochester, NY, USA) of semiconfluent 293 cells were cotransfected with 650 μg of AAV/CAG-mPAH or scAAV/LP1-mPAH plasmid, 650 μg of AAV2 rep-AAV8 cap helper plasmid (pRep-Cap8 from Dr J. M. Wilson [12]) and 650 μg of adenoviral helper plasmid (pAdeno [23]) by standard calcium phosphate method. Cells were incubated with active gassing for 3 days and harvested [24]. AAV vectors were purified from the crude cell extract by serial ultracentrifugation with CsCl. Vector genome (vg) titers were determined by dot blot hybridization with the mPAH cDNA probe [7]. For scAAV8/LP1-mPAH, titration was also carried out by quantitative polymerase chain reaction (qPCR), where scAAV8/LP1-mPAH and the vector plasmid standard were amplified with a primer set (5′-ACAGTGAATCCGGACTCTAAGG-3′ and 5′-CTGCTCAGGACTCCGTTCTC-3′) using a real-time PCR instrument (7900HT; Applied Biosystems, Foster City, CA, USA). A 136-bp region between LP1 promoter and the mPAH cDNA was amplified and confirmed by agarose gel electrophoresis. The qPCR-based titer was calibrated with the result of other titration methods described previously [25,26].

Animals and gene delivery

Colonies of a PKU model mouse, BTBR-*Pah*^{enu2} and its wild-type (WT) strain BTBR (obtained from Jackson Laboratories, Bar Harbor, ME, USA) were maintained in the animal facility of Jichi Medical University. All animals were fed standard mouse chow (CE-2 from Clea Japan, Tokyo, Japan) *ad libitum* providing approximately 25% of energy as protein. Genotyping for the presence of the *Pah*^{enu2} mutation was performed by PCR analysis of tail biopsy DNA. In brief, exon 7 (136 bp) of the mPAH gene was amplified with a primer set (5′-CTTGTACTGGTTTCCGCCTC-3′ and 5′-GG TTCAGGTGTGTACATGGG-3′). The amplified DNA from WT allele is cleaved by *Mbo*II into 70-bp and 66-bp fragments, whereas the counterpart from *Pah*^{enu2} allele is uncleavable as a result of the c.835T → C (F263S) mutation [5]. The scAAV vector (1×10^{11} to 3×10^{12}

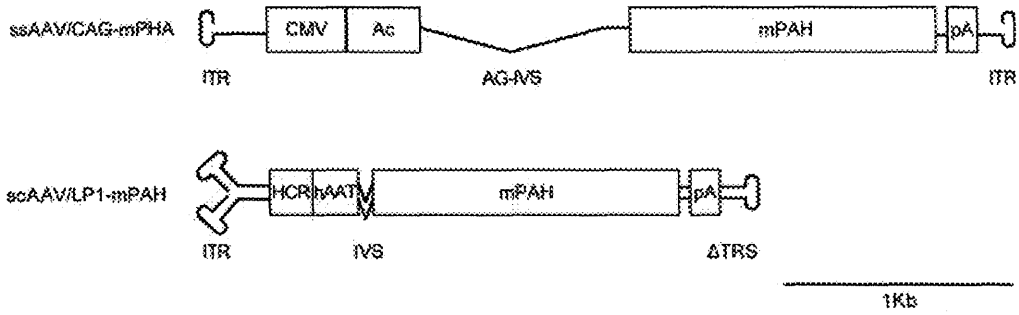

Figure 1. Structure of AAV vectors for PKU gene therapy. Top: ssAAV/CAG-mPAH vector. CAG promoter consists of the human cytomegalovirus immediate-early enhancer (CMV), the chicken β-actin promoter (Ac) and a chicken β-actin/rabbit β-globin composite intron (AG-IVS). CAG promoter, the murine phenylalanine hydroxylase cDNA (mPAH) and the SV40 late polyadenylation signal (pA) are flanked by the AAV inverted terminal repeats (ITR). Bottom: scAAV/LP1-mPAH vector. LP1 promoter consists of the human apolipoprotein E/C-I hepatic control region (HCR) and the human α1-antitrypsin promoter (hAAT). In addition, the vector contains a modified SV40 small t-antigen intron (IVS), mPAH and pA. The entire expression cassette is flanked by the intact AAV ITR and the terminal resolution site-deleted ITR (ΔTRS)

particles) was dissolved in 0.5 ml of saline and injected into the peritoneal cavity of *Pah*^{enu2} mice. All animal experiments were carried out in accordance with the institutional guidelines under protocols approved by the Institutional Animal Care and Use Committee at Jichi Medical University.

Blood Phe assay

Blood Phe was measured by an enzymatic fluorometric assay using an Enzaplate PKU-R kit (GE Healthcare, Tokyo, Japan) [7]. Mice were tail phlebotomized and 30–40 μl of blood was spotted onto a paper filter (No. 545 for newborn mass screening; Advantec Toyo, Tokyo, Japan). A disc (3 mm in diameter) was punched out from the dried blood spot and placed in a round-bottom, 96-well microtiter plate. Phe was eluted from the disc and incubated with resazurin and Phe dehydrogenase, an NAD-dependent enzyme. The enzyme reaction produces NADH, which in turn converts resazurin to resorufin with the aid of diaphorase. The resultant resorufin was measured on a Fluoroskan Ascent plate reader (Labsystems, Helsinki, Finland) with a 544 nm/590 nm filter set.

Evaluation of *in vivo* Phe oxidation

In vivo Phe oxidation activity was evaluated by a method of Kure *et al.* [27] with slight modification. [1-^{13}C]L-Phe (^{13}C-Phe; from Cambridge Isotope Laboratories, Andover, MA, USA) and L-Phe (from Wako Pure Chemicals, Osaka, Japan) were dissolved in saline at 200 mg/ml and 20 mg/ml, respectively, and sterilized through 0.22-μm syringe filters (Millex-GV, Millipore, Yonezawa, Japan) immediately before intraperitoneal (i.p.) injection. Mice were preloaded with 20 mg/kg of L-Phe 30 min prior to ^{13}C-Phe challenge (2 mg/kg). Each mouse was kept in a lid-sealable plastic box for 15 min before and 45 min after ^{13}C-Phe challenge, and a total volume of 120 ml of air was transferred to a sampling bag for [^{13}C]urea breath test (Otsuka Pharmaceuticals, Tokyo, Japan) with a glass syringe. The ratio of $^{13}CO_2$ and $^{12}CO_2$ was measured by gas chromatography-mass spectrometry (GC-MS; by SRL, Tokyo, Japan), and the difference was designated as $\Delta^{13}CO_2$.

Determination of vector biodistribution

Tissue genomic DNA was extracted by a standard method using proteinase K (Boehringer Manheim, Manheim, Germany) and phenol:chloroform (Nippon Gene, Toyama, Japan). To quantify genomic DNA copy number, a unique region of the murine β-actin gene was amplified by qPCR with a primer set (5′-GGTCCTGG ATCACTCAGAACGGACACCA-3′ and 5′-AGCCTCAATAC GCACGCGCAGCTAAC-3′) along with a plasmid control. Vector copy number in tissue DNA was estimated by qPCR in the same manner as in vector titration.

Statistical analysis

Statistical analysis was performed using StatView, version 5.0, for Macintosh (SAS Institute, Cary, NC, USA). A paired *t*-test and unpaired *t*-test (Student's *t*-test or Welch's *t*-test) were used for comparison between the two groups. $p < 0.05$ was considered statistically significant for all analyses.

Results

Construction of AAV8 vectors for PKU

For comparison with scAAV8, a reference ssAAV8 vector was constructed with ssAAV/CAG-mPAH plasmid (Figure 1, top) [7]. Although the CAG promoter allowed very high hepatic expression in our previous studies

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with ssAAV2 and ssAAV5 [7,28], the size of CAG-mPAH expression cassette (3.5 kb) exceeded the limit of packaging capacity of a scAAV vector (up to 2.2 kb). To meet the packaging requirements of scAAV, Nathwani *et al.* [21] developed a compact hFIX expression cassette (LP1-hFIX; 2.1 kb) and assembled it in the modified AAV2 backbone with an intact 5′ terminal resolution site (*trs*) and a deleted 3′ *trs* (scAAV/LP1-hFIX). The LP1 hybrid enhancer/promoter consists of the core liver-specific elements from the human apolipoprotein E/C-I gene hepatic control region (HCR) and the human α1-antitrypsin promoter (hAAT). The expression cassette also contains a modified SV40 small t-antigen intron and the SV40 late polyadenylation signal. Because the coding sequences of mPAH (1362 bp) and hFIX (1386 bp) have almost identical lengths, we constructed scAAV/LP1-mPAH vector by replacing the hFIX cDNA in scAAV/LP1-hFIX with the mPAH cDNA from ssAAV/CAG-mPAH (Figure 1, bottom). PAH expression from the resultant plasmid (scAAV/LP1-mPAH) was confirmed by transfection of Huh7 cells and immunoblotting (data not shown). The ssAAV/CAG-mPAH and scAAV/LP1-mPAH genomes were packaged into AAV serotype 8 capsid and titrated by dot blot hybridization. The determined titers of viral stocks were approximately 1×10^{13} vg/ml for ssAAV8/CAG-mPAH and 6×10^{13} vg/ml for scAAV8/LP1-mPAH, respectively. scAAV8/LP1-mPAH was also titrated by qPCR along with a plasmid control and calibrated with the result obtained from dot blot hybridization [25].

Efficacy of ssAAV8 in PKU mice

We previously reported that 1×10^{13} vg of ssAAV5/CAG-mPAH partially corrected hyperphenylalaninemia in male *Pah*^{enu2} mice when administered through the portal vein (PV) [7]. Meanwhile, ssAAV8 vectors delivered reporter genes to adult mouse liver with comparable efficiency following either i.p. or intravenous injection, with an apparent gender-specific barrier [29,30]. Therefore, we administered a log-smaller dose (1×10^{12} vg) of ssAAV8/CAG-mPAH vector to the peritoneal cavity of male PKU mice. This procedure resulted in an almost complete correction of hyperphenylalaninemia for 24 weeks (Figure 2a), confirming a very efficient liver transduction by AAV8. This observation prompted us to carry out a similar comparative study with female *Pah*^{enu2}. When female PKU mice were given 1×10^{13} vg of ssAAV8/CAG-mPAH through PV, blood Phe was rapidly decreased to normal levels (<2 mg/dl), and the initial impact on blood Phe was identical to that of PV-injected 1×10^{14} vg of ssAAV5/CAG-mPAH (Figure 2b). The therapeutic effect was transient, however, and hyperphenylalaninemia gradually resumed to the pretreatment level by 24 weeks post-injection. The same dose (1×10^{13} vg) of i.p.-injected ssAAV8/CAG-mPAH exhibited minimal effect on blood Phe during the observation period (Figure 2b). Taken together, the liver transduction efficiency of the ssAAV8 vector was

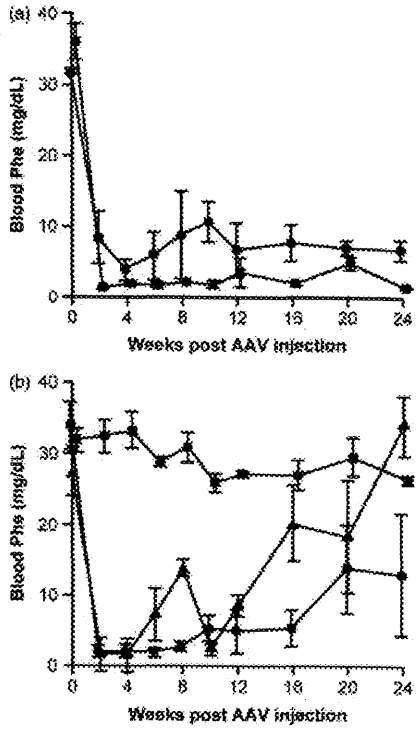

Figure 2. Efficacy of ssAAV5 and ssAAV8 vectors in *Pah*^{enu2} mice. (a) Male *Pah*^{enu2} mice were given either 1×10^{13} vg of ssAAV5/CAG-mPAH through the portal vein (PV) (circles, $n = 4$; data adopted from Mochizuki *et al.* [7]), or 1×10^{12} vg of ssAAV8/CAG-mPAH by i.p. (squares, $n = 3$). Blood Phe (mg/dl) levels are shown as the mean ± SD. (b) Female *Pah*^{enu2} mice were given either 1×10^{14} vg of ssAAV5/CAG-mPAH via the PV (circles, $n = 5$; data adopted from Mochizuki *et al.* [7]), 1×10^{13} vg of ssAAV8/CAG-mPAH via the PV (triangles, $n = 4$), or 1×10^{13} vg of ssAAV8/CAG-mPAH by i.p. injection (squares, $n = 3$). Blood Phe (mg/dl) levels are shown as the mean ± SD

greater than that of ssAAV5 by tenfold, although the gender-specific barrier was not overcome. Regarding the administration route of AAV8, PV injection was superior to i.p. injection, although the latter procedure was useful in male mice.

Short-term efficacy of scAAV8 in Phe metabolism of female PKU mice

Based on the above result with the ssAAV8 vector, we realized that further improvement was required to cure female *Pah*^{enu2} mice. Therefore, we addressed whether the self-complementary AAV genome would boost liver transduction. We gave 1×10^{11} or 1×10^{12} vg of scAAV8/LP1-mPAH to the peritoneal cavity of adult female *Pah*^{enu2} (8 weeks of age). These doses were two to three logs smaller than those of ssAAV5 and ssAAV8 vectors showing a transient therapeutic effect on female *Pah*^{enu2} (Figure 2b). When fed standard chow, the blood Phe concentration in WT BTBR mice was below 1.7 mg/dl (100 μM), whereas that in untreated *Pah*^{enu2} mice was above 20 mg/dl (1200 μM) (Table 1).

Table 1. Blood Phe, *in vivo* Phe oxidation and liver vector DNA in female *Pah*^{enu2} mice after 8 weeks of scAAV8/LP1-mPAH injection

Genotype/dose (vg)	Phe (mg/dl)	$\Delta^{13}CO_2$ (‰)	Vector DNA (c/dg)
WT/none ($n = 7$)	0.7 ± 0.1	38.9 ± 14.8	ND
Pah^{enu2}/none ($n = 8$)	32.5 ± 6.2	1.1 ± 0.8	ND
Pah^{enu2}/1×10^{11} ($n = 4$)	1.8 ± 0.5	32.8 ± 13.4	1.5 ± 0.3
Pah^{enu2}/1×10^{12} ($n = 4$)	1.0 ± 0.3	39.5 ± 13.0	27.3 ± 16.0

ND, not determined.

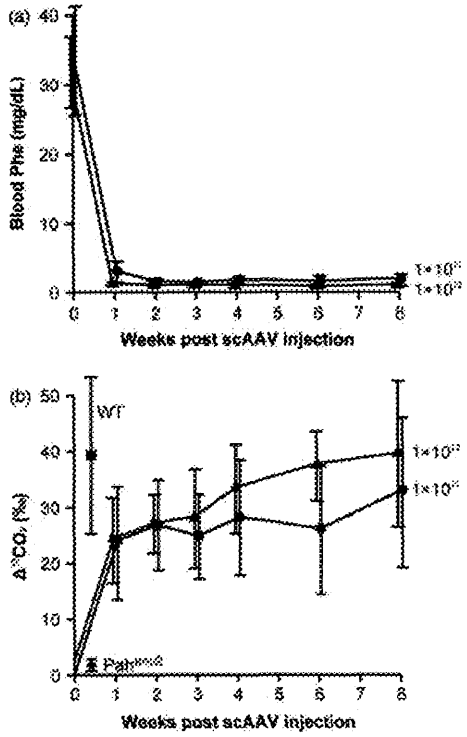

Figure 3. Short-term efficacy of scAAV8/LP1-mPAH in female *Pah*^{enu2} mice after i.p. injection. (a) Weekly blood Phe (mg/dl) levels are depicted as the mean ± SD. (b) *In vivo* Phe oxidation ($\Delta^{13}CO_2$‰) levels are represented as the mean ± SD of each dosage group. Circles, BTBR-*Pah*^{enu2} given 1×10^{11} vg of scAAV8 ($n = 4$); triangles, BTBR-*Pah*^{enu2} given 1×10^{12} vg ($n = 4$); square, wild-type BTBR (WT, $n = 7$); diamond, untreated BTBR-*Pah*^{enu2} (Pah^{enu2}, $n = 8$)

Following a single i.p. injection of scAAV8/LP1-mPAH, a dramatic decrease of blood Phe was observed in female *Pah*^{enu2} (Figure 3a). In mice receiving 1×10^{11} vg of vector, blood Phe was decreased from 33.3 ± 7.8 mg/dl to 3.0 ± 1.5 mg/dl ($n = 4$; $p < 0.01$) in 1 week and kept within the normal range 2–8 weeks post-injection (Table 1). In mice receiving 1×10^{12} vg of vector, hyperphenylalaninemia was corrected even more rapidly and uniformly; blood Phe was normalized in 1 week (from 31.7 ± 5.1 to 1.2 ± 0.3 mg/dl, $n = 4$; $p < 0.01$) through 8 weeks after treatment (Table 1). Between these two dosage groups, the blood Phe concentration was not significantly different, except for the data obtained 2 weeks post-i.p. ($p < 0.05$). With normalization of blood Phe, hypopigmentation in the treated mice was gradually ameliorated. Patchy black hair emerged by week 2, and the animals recovered the WT coat color by week 8.

In parallel with blood Phe analysis, we evaluated the *in vivo* oxidation capacity for Phe by a non-invasive breath test. In this assay, administered ^{13}C-Phe is converted to [1-^{13}C]Tyr by PAH, which is then broken down to yield homogentisic acid and $^{13}CO_2$ by two enzymatic reactions. Eventually, $^{13}CO_2$ is liberated into breath, and the $^{13}CO_2$ concentration (as a ratio to $^{12}CO_2$) is measured by infrared spectrophotometry or GC-MS. Because our initial experiments showed that GC-MS offered lower background, this method was used in the subsequent investigation. The amount of $^{13}CO_2$ production ($\Delta^{13}CO_2$) is determined by the difference between the $^{13}CO_2$ concentration of the breath samples collected before and after ^{13}C-Phe infusion. Because a significant fraction of input ^{13}C-Phe is metabolized through the above pathway where PAH catalyses the rate-limiting step, we can evaluate PAH activity by measuring $\Delta^{13}CO_2$ [27]. WT mice showed positive $\Delta^{13}CO_2$ values without an apparent gender difference (males: 45.5 ± 8.8‰, $n = 7$; females: 38.9 ± 14.8‰, $n = 7$). By contrast, untreated *Pah*^{enu2} mice produced very little, if any, $\Delta^{13}CO_2$ in the breath test (males: 1.0 ± 0.6‰, $n = 3$; females: 1.1 ± 0.8‰, $n = 8$), resulting from the absence of PAH activity (Table 1). The impaired *in vivo* Phe oxidation was rapidly corrected following scAAV8/LP1-mPAH injection, in a reciprocal fashion to blood Phe reduction (Figure 3b). In the female *Pah*^{enu2} mice receiving 1×10^{11} vg of vector, $\Delta^{13}CO_2$ was improved from 0.9 ± 0.8‰ to 23.6 ± 10.2‰ in 1 week ($n = 4$; $P < 0.05$) and maintained in a near-normal to normal range thereafter. In mice receiving 1×10^{12} vg of vector, $\Delta^{13}CO_2$ was increased from 1.4 ± 0.9‰ to 24.2 ± 7.5‰ at week 1 ($n = 4$; $p < 0.01$) with an upward tendency until week 8 (Table 1).

After 8 weeks post-i.p., the animals were euthanized for tissue DNA analysis for vector biodistribution. The vector content in the liver was 1.5 ± 0.3 and 27.3 ± 16.0 copies/diploid genome (c/dg) in the low- (1×10^{11} vg, $n = 4$) and high-dosage (1×10^{12} vg, $n = 4$) groups, respectively ($p < 0.05$; Table 1). Although the vector DNA was barely detected (<0.01 c/dg) in the spleen and gonads from mice given 1×10^{12} vg of vector, a trace amount of vector (0.3–0.5 c/dg) was present in these viscera from the mice receiving 1×10^{12} vg. The DNA analysis recapitulated a very strong liver tropism of scAAV8 vectors as reported previously [21].