the fetal circadian clock observed in these studies was synchronized in almost antiphase to the maternal rhythm (6, 7). Information on the postnatal synchronization of the circadian clock to the day-night rhythm is sparse. Studies that investigated the adrenal circadian rhythm of the newborn infant demonstrated the presence of both diurnal and non-24-h changes in salivary and urinary cortisol (8–10). However, the phases of the observed rhythms were not synchronized with clock time, and the acrophase was random among subjects. The adult-type cortisol rhythm, with its acrophase in the early morning, becomes evident only about 2 months after birth (11). It remains unknown whether the circadian rhythm of maternal origin is temporally lost or if it is modified to be finally entrained to the day-night rhythm during the first few months of life. Detailed information on how a mature circadian rhythm is developed may help our understanding of how to induce a successful transition to extrauterine life and early establishment of a day-night sleep cycle in infants.

To investigate how the fetal adrenal clock alters its rhythm within a short period after birth, we monitored the changes in salivary cortisol in medium-risk newborn infants throughout a 24-h period. We hypothesized that the adrenal clock of the newborn infant, which has been synchronized in antiphase with the maternal rhythm, would be transiently reentrained to the time of birth due to a range of hormonal events occurring at or shortly after birth.

## Materials and Methods

This study was conducted at the neonatal intensive care unit of Kurume University Hospital (Kurume, Fukuoka, Japan) with the approval of the local ethics committee. Written informed consent was obtained from a parent of each infant.

### Study population

Between October 2009 and April 2010, we allocated 20 d for 24-h saliva sample collection. On each of these days, we recruited up to three near-term to term newborn infants who were older than 1 d and physiologically stable. Eventually, saliva samples were collected from 27 infants (33.7–41.8 wk gestational age and 2–11 d postnatal age in range), who were hospitalized because of low birth weight (20 infants), maternal hyper- or hypothyroidism (two infants), or maternal gestational diabetes mellitus (five infants). Infants who underwent phototherapy during or within 24 h before the study and infants who required resuscitation and/or intensive life support were not included because of the potential bias of stress on cortisol values.

### Sample collection

Sample collection was started at 0900 h to obtain eight saliva samples per infant with 3-h intervals. In our unit, during the first few days of life, term and near-term infants are regularly fed every 3 h starting at 0100 h. *Ad libitum* feeding is started after the oral intake exceeds 140 ml/kg/d, usually after d 4. For infants who were regularly fed, samples were collected 2 h after feeding. For infants who were *ad libitum* fed, the timing of feeding was adjusted so that samples were collected at least 90 min after feeding. The timing for the sample collection was adjusted by up to ± 20 min from the scheduled time to obtain samples during sleep or calmly awake states.

For the sample collection, an absorbent swab stick (Sorbette; Salimetrics LLC, State College, PA) was gently inserted into the infant's mouth for approximately 1 min, allowing the swab end to absorb sufficient saliva. The sample was immediately centrifuged at 3000 rpm, and kept at 4 C until the eighth sample for the subject was collected; at this temperature range, salivary cortisol concentrations remain stable for up to 3 months (12). To better assess the pattern of diurnal cortisol changes, subjects with two or more invalid data points (mainly due to insufficient sample volume <50 μl) were excluded from the analysis.

### Cortisol assay

Saliva samples were frozen at −80 C until assayed. The concentrations of salivary cortisol were determined by enzyme immunoassay (high sensitivity salivary cortisol ELISA kit; Salimetrics). The limit of detection of this assay in our laboratory was 0.19 nmol/liter, and the intra- and interassay coefficients of variation were 5.43 and 6.41%, respectively.

### Clinical data collection

Background data were collected for both inter- and intrasubject variables, including antenatal steroid administration, multiple birth, intrauterine growth restriction, delivery mode (vaginal delivery, elective cesarean section, or emergency cesarean section), age (gestational, corrected, or postnatal), incubator type (open or closed), feeding mode (oral or tube), venipuncture or heel lance shortly before the sample collection, and the sleep status and the mode of lighting at the time of sample collection. In our unit, we provide cycled lighting aiming at 100–200 lux during the day (0700 to earlier than 1900 h) and 10–30 lux during the night (1900 to earlier than 700 h). Quilt covers are used over the closed incubators.

### Data analysis

The influence of the infant's age on cortisol values (maximum, minimum, mean, and SD) were assessed using the Pearson's correlation coefficient. The potential influence of other variables on cortisol values was evaluated using the ANOVA. To assess the global trends in diurnal cortisol changes, samples obtained at 0900 and 1200, 1500 and 1800, 2100 and 2400, and 0300 and 0600 h were averaged and were compared using the repeated-measures ANOVA and the Fisher's least significant difference test; the latter was chosen considering the exploratory nature of the analysis, in which strict control for type I error is unnecessary (13). Also, to assess the influence of the clock time of birth (birth time), values were averaged for four time periods of 0 to less than 6, 6 to less than 12, 12 to less than 18, and 18 to less than 24 h after the birth time.

Based on the finding from these exploratory analyses, a new hypothesis was formed that the acrophase of cortisol occurs with some delay after the birth time. To test the hypothesis, we first objectively estimated the acrophase in hours to the first decimal place for the range of 0900 to less than 0900 h on the second day of the study by fitting a quartic polynomial to the eight-point

time-series data (14); this polynomial was chosen to optimize curve fitting to the time-series data with up to two peaks (Supplemental Fig. 1, published on The Endocrine Society's Journals Online web site at http://jcem.endojournals.org). Because of uncertainty around the presence of the diurnal cortisol cycle in the newborn infant, periodic functions were not applied; for the analysis only, the sample collection time and the acrophase in cortisol were transformed into an interval scale of 0900 to earlier than 3300 h by adding 24 h to the clock time on the second day of the study. The dependence of the acrophase on the birth time was assessed using a linear regression model without adjusting value ranges for the intercept (Supplemental Fig. 2). The presence of the linear correlation was assessed on the whole study population and on two subgroups of relatively younger infants (<d 5, n = 13) and older infants (≥d 5, n = 10) to evaluate the influence of the postnatal age on the pattern of diurnal cortisol changes. *P* values were adjusted for subgroup analyses using the Bonferroni correction.

## Results

### Data profile

Eight saliva samples were successfully collected from all 27 infants (224 samples in total); however, nine samples were of insufficient volume for the assay. Consequently, three infants had only six valid data sets; another infant started phototherapy halfway through the study; to minimize the bias, these four infants were excluded from further analyses. Physiological and clinical backgrounds of the study population are presented in Table 1 and Supplemental Fig. 3; 74% of subjects were born during the period 0900 to earlier than 2100h (Supplemental Fig. 4).

**TABLE 1.** Background clinical variables

| Variables | |
|---|---|
| Intersubject variables | |
| Clinical background | |
| Female/ male | 7/16 |
| Multiple births | 14 |
| Birth weight (g) | 2344 (441) |
| Intrauterine growth restriction | 5 |
| Antenatal steroid | 4 |
| Vaginal delivery | 8 |
| Elective/emergency cesarean section | 8/7 |
| Age at birth and sample collection | |
| Gestational age (wk) | 36.6 (1.7) |
| Postnatal age (d) | 5.3 (3.4) |
| Postconceptional age (wk) | 37.1 (2.1) |
| Treatment and environment | |
| Continuous venous infusion | 7 |
| Tube feeding | 4 |
| Closed incubator | 9 |
| Intrasubject variables[a] | |
| Sleep state (asleep) | 133 |
| Blood sampling before saliva collection | 14 |

Values are shown as the mean (SD) or the number of incidences.

[a] Values are based on 182 salivary samples from 23 subjects.

### Infant's age, body weight, and salivary cortisol

Maximum cortisol values per individual were dependent on the gestational age ($P < 0.005$) and postconceptional age ($P < 0.01$). Minimum cortisol values were only dependent on the postnatal age ($P < 0.05$). Mean cortisol values were associated with the gestational age ($P < 0.05$). SDs of cortisol values per individual were dependent on the gestational age and postconceptional age (both $P < 0.005$) (Table 2). The influence of the delivery mode to the cortisol level and its individual SD was not evident (Supplemental Fig. 5).

### Independent variables of salivary cortisol

Infants who were orally fed had higher cortisol values than their peers ($P < 0.05$). The awake state was noted only two (zero to three) times per subject [median (range)] the observation of the awake state did not vary between sample collection times except that no infant was noted to be awake at 1200 h. The awake state was associated with higher cortisol levels than the sleep state ($P < 0.01$) (Table 3). No other clinical factors were identified as independent variables of salivary cortisol.

### Clock time, birth time, and diurnal changes in salivary cortisol

Cortisol levels during the period from 1500 to earlier than 21:00 were higher than those of 0900 to earlier than 1500 and 0300 to earlier than 0900 h (both $P < 0.05$) for the whole study population; these trends were not significant for subgroups of younger and older infants (Fig. 1, A–C). Cortisol levels were significantly higher during 0 to less than 6, 6 to less than 12, and 12 to less than 18 h after the birth time than during 18 to less than 24 h after the birth time for both the whole study population ($P < 0.005$, 0.05, and 0.05, respectively) and the subgroup of younger infants ($P < 0.005$, 0.005, and 0.05, respectively); these trends were not observed in the subgroup of older infants (Fig. 1, D–F).

Curve fitting to individual time-series data was satisfactory with mean (SD) $r^2$ values of 0.63 (0.17) (Supplemental Figs. 1 and 6). The acrophase of cortisol was positively correlated with the birth time in the whole study population ($P < 0.01$) and the younger infant subgroup ($P < 0.005$); the linear relationship was not observed in the subgroup of older infants (Fig. 2; also see Supplemental Figs. 7 and 8 for findings in other subgroups of the delivery mode and individual variability in cortisol).

## Discussion

We confirmed the presence of a 24-h adrenal circadian cycle in newborn infants. Increase in salivary cortisol was

**TABLE 2.** Dependence of individual cortisol values on age

| | Cortisol (nM) | | Correlation coefficients | | |
|---|---|---|---|---|---|
| | Mean | (SD) | Gestational age | Postnatal age | Postconceptional age |
| Individual maximum | 21.11 | (8.45) | 0.61[a] | −0.18 | 0.56[a] |
| Individual minimum | 3.32 | (1.32) | 0.10 | −0.46[b] | −0.02 |
| Individual mean | 9.08 | (2.96) | 0.47[b] | −0.41 | 0.36 |
| Individual SD | 6.47 | (3.22) | 0.65[a] | −0.06 | 0.64[a] |

[a] $P < 0.01$, Pearson correlation coefficient.

[b] $P < 0.05$, Pearson correlation coefficient.

observed around 1500 to less than 2100 and 0 to less than 6 h after the birth time. During the first 5 d of life, the acrophase of the cortisol rhythm was predominantly defined by the birth time, the influence of which disappeared thereafter. Our current findings suggest that, as previously demonstrated in the fetal species (15), the adrenal circadian rhythm of the newborn infant is first entrained by maternal stimuli *in utero* and is then transiently reentrained by strong but short-lasting stimuli at birth, both of which appear to induce circadian cycles with different phases from the day-night cycle. Such complex regulation of the adrenal clock, although potentially relevant for protecting the infant from life-threatening events during the transitional period, may disturb the swift synchronization of the infant's circadian rhythm to the day-night cycle. Our current findings build on results from previous studies that addressed the developmental process of the fetal circadian rhythm and may present a vital piece of the puzzle by providing information on the presently unclear function of the adrenal circadian clock during the early neonatal period.

**TABLE 3.** Dependence of cortisol values on clinical variables

| Variables | Mean (SD) (nM) |
|---|---|
| Intersubject variables | |
| Gender | |
| Male | 9.68 (0.69) |
| Female | 7.71 (1.21) |
| Intrauterine growth restriction | |
| No | 8.96 (3.21) |
| Yes | 9.51 (2.04) |
| Antenatal steroid | |
| No | 9.44 (2.74) |
| Yes | 7.38 (3.84) |
| Cesarean delivery | |
| No | 10.69 (3.40) |
| Yes | 8.38 (2.55) |
| Multiple births | |
| No | 9.88 (3.70) |
| Yes | 8.57 (2.39) |
| Feeding mode | |
| Oral | 9.66 (0.63)[a] |
| Tube | 6.33 (2.71) |
| Incubator type | |
| Open | 9.47 (3.07) |
| Closed | 8.48 (2.85) |
| Continuous venous infusion | |
| No | 8.50 (3.06) |
| Yes | 10.40 (2.41) |
| Intrasubject variables[b] | |
| Sleep status | |
| Sleep | 8.13 (6.04)[c] |
| Awake | 12.00 (9.99) |
| Blood sampling before saliva collection | |
| No | 8.99 (7.42) |
| Yes | 9.68 (6.19) |
| Lighting mode | |
| Night | 8.39 (7.39) |
| Day | 9.38 (6.85) |

[a] $P < 0.05$ from the ANOVA.

[b] Values are based on 182 salivary samplesfrom 23 subjects.

[c] $P < 0.01$ from the ANOVA.

## Monitoring the adrenal circadian rhythm using salivary cortisol

For adults, salivary cortisol has been proposed as a noninvasive surrogate for plasma cortisol (16). Salivary cortisol levels reflect those in the free plasma fraction without being affected by the salivary flow rate. Similar to serum cortisol, salivary cortisol in the adult has a well-documented diurnal pattern with peak levels in the early morning, typically decreasing over the day, and a nadir at night (17). This technique has successfully been applied in the newborn infant (18, 19). In our subjects, sufficient saliva was obtained in 96% of the cases. A tight linear relationship between plasma and salivary cortisol levels has been demonstrated even in very low-birth-weight infants (20), supporting the usefulness of this technique in the newborn infant.

## Adrenal circadian rhythm of the fetus and newborn infant

In adult mammals, the suprachiasmatic nucleus (SCN) of the hypothalamus serves as a master clock that regulates the biological rhythm of peripheral clocks and systemic organs (21). Until fetal monitoring techniques became available, it was long accepted that infants were born without a circadian rhythm. However, studies that used fetal physiological monitoring techniques demonstrated 24-h rhythms of the fetal heart rate and respiratory and

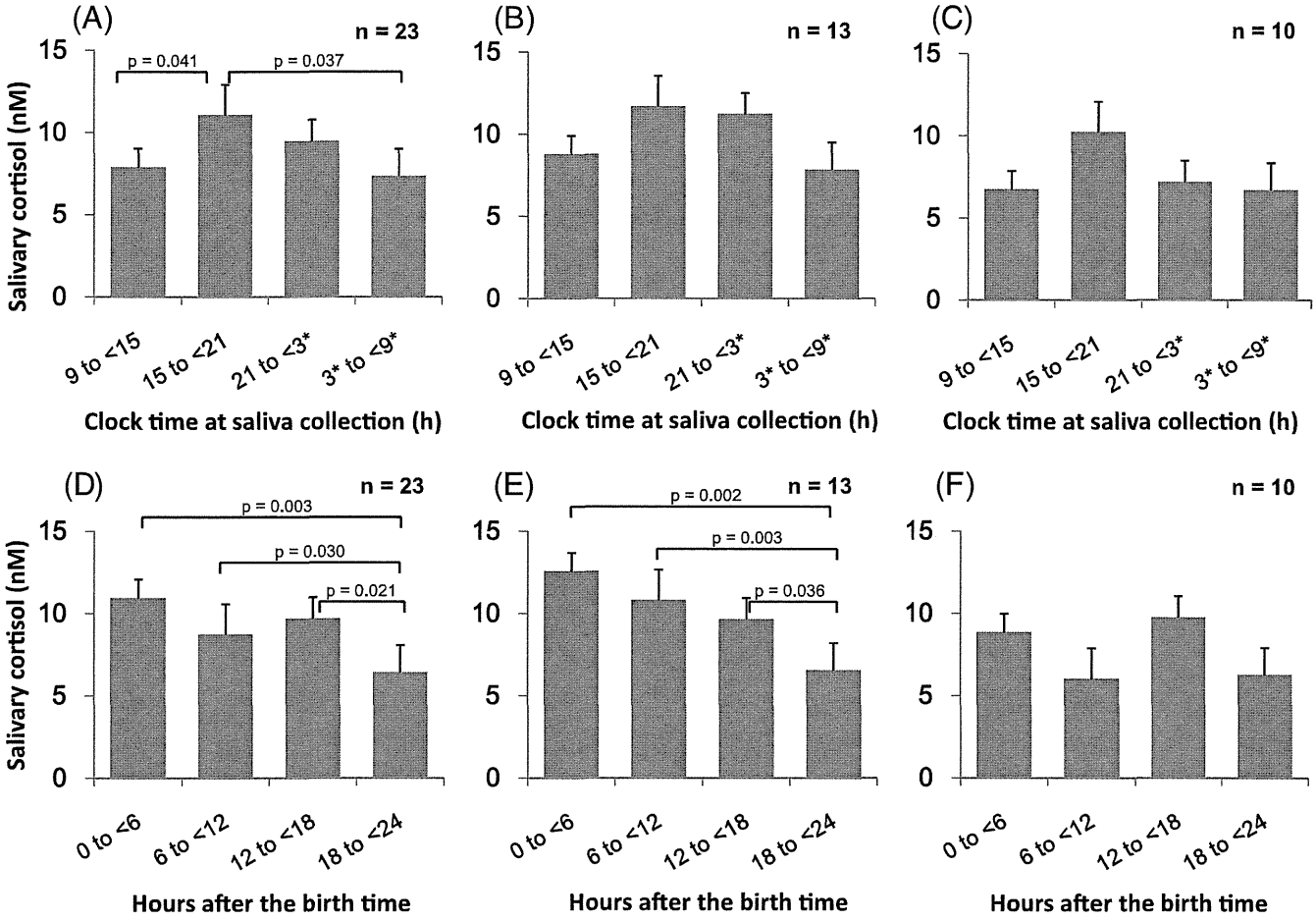


**FIG. 1.** Dependence of diurnal changes in cortisol on clock time and birth time. Diurnal cortisol changes over four averaged bands of (A–C) the clock time and (D–F) the time after the birth time, for the entire study population (A and D), younger subjects earlier than d 5 (B and E), and older subjects d 5 or later (C and F). Values are shown as mean and SE. *P* values are from repeated-measures ANOVA with the Fisher's least significant difference test. Label on the x-axis indicates the sample collection time on the clock time (A–C) and the time interval after the birth time (D–F). Higher salivary cortisol values were observed during the periods of 1500 to earlier than 2100 and 0 to earlier than 6 h after the birth time. *, Clock time on the second day of the study.

body movements (15). Recent studies that monitored maternal and fetal hormones and clock genes revealed that the fetal SCN is not involved in the modulation of circadian rhythm, whereas the fetal adrenal gland plays the central role in the entrainment of the fetal circadian clock (6, 15, 21, 22). A study in cord blood of infants born by scheduled elective cesarean section demonstrated the presence of a diurnal cortisol rhythm with its acrophase in the afternoon (7). A study in the rat confirmed that the fetal adrenal clock was entrained almost in antiphase to the maternal rhythm (6).

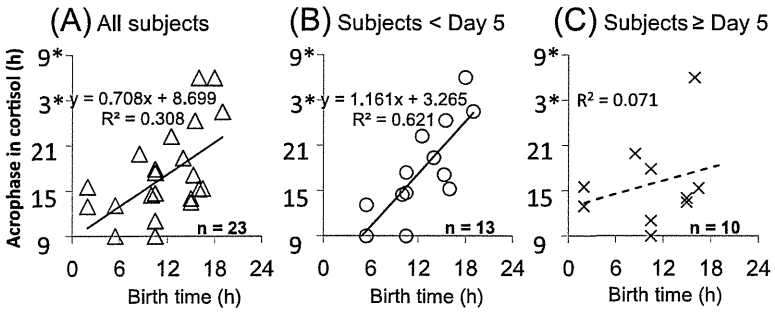


**FIG. 2.** Association between the birth time and the acrophase of cortisol rhythm. Scatter plot demonstrating the association between the birth time and the acrophase in cortisol. The acrophase was positively correlated with the birth time in the whole study population ($P < 0.01$) (A) and the younger infant subgroup ($P < 0.005$) (B) but not in the older infant subgroup (C). *Solid lines* are for regression with $P < 0.05$, and *broken lines* are for reference from the Pearson's correlation coefficient and Bonferroni correction. *, Clock time on the second day of the study.

Although the function of the peripheral circadian clock in the adrenal gland is well established *in utero*, diurnal cortisol changes in the newborn infant are unsynchronized with the external clock time shortly after birth, presumably because of the loss of maternal hormonal stimuli and the immaturity of the SCN (8–10). This condition is likely to persist for several months until the adrenal clock is finally entrained to the day-night cycle (23, 24). Our study also suggested the presence of a diurnal cortisol rhythm in the newborn infant, which

showed a modest trend toward increased cortisol in the late afternoon, suggesting the synchrony with the external clock. This observation contrasted with previous studies in the newborn infant (8–10) but was consistent with observations in the fetus (6, 7). Given that other circadian and ultradian rhythms are likely to be entrained in association with birth and subsequent extrinsic stimuli (*e.g.* cold stress, light-dark cycle, feeding, and other cares) (25–27), it may not be surprising that previous studies failed to identify a cortisol rhythm of maternal or fetal origin shortly after birth (8–10). The exact mechanism and the relevance of the antiphase synchronization of the fetal circadian clock are unknown. However, this time difference may in part explain the prolonged period required for the entrainment of the circadian clock to the day-night rhythm.

### Influence of the birth time on the adrenal circadian clock

Thus far, the regulating factor for the adrenal circadian clock in the newborn infant has not been identified. In our current study, the influence of the birth time on the diurnal changes in cortisol was revealed when the acrophase of cortisol rhythm was studied relative to the birth time. For infants younger than 5 d, the acrophase was observed approximately 3 h after the birth time, whereas such an association was not observed for the older infants. Given the series of hormonal events observed around delivery, which include the stimulation of hypothalamus, pituitary, and their downstream systems (25, 26), birth itself may provide a sufficient stimulus to entrain the infant's circadian clock to the time of birth. However, the influence of the birth-related stimuli to the adrenal circadian clock was observed only during the first 5 d of life. Such transient synchronization of the adrenal circadian clock with the birth time may be relevant only for the safe transition of the fetus to the extrauterine life. However, given that natural onset of labor most frequently occurs in the early morning hours (28), entrainment of the adrenal clock to the morning may boost early synchronization of the circadian clock to the day-night rhythm rather than introducing an additional, confusing signal to the fetal circadian clock. Future studies need to address the mechanism of and the relevance of reentrainment of the adrenal clock at birth.

### Determination factors of noncircadian changes in cortisol

Consistent with the observations in adults (29), salivary cortisol levels were dependent on the sleep or wake states in our current population. The rhythm of cortisol secretion in the newborn infant may include both diurnal and ultradian rhythms (30). In our subjects, oral feeding was also associated with higher cortisol levels as opposed to tube feeding. Spangler (24) reported that the type and the timing of feeding affect salivary cortisol levels in the newborn infant. However, Shulman *et al.* (31) reported that urinary cortisol levels were not associated with the mode of feeding in preterm infants. The difference between the feeding modes observed in our current study may merely be caused by the difference in the corrected age of the infants, which was another prominent independent variable of salivary cortisol. Stressful environments within an intensive care unit, represented by continuous bright lighting and painful procedures, may have negative effects on the development of the newborn infant (32, 33). In our current study, clinical variables such as blood sampling, lighting mode, and type of incubator were not recognized as independent variables of salivary cortisol, which may be in part because of the commitment of our unit to developmental care, including low-intensity cycled lighting and quilt covers.

### Limitations

Our study was not powered to give a solid overview of the diurnal hormonal cycle in the newborn infant mainly because of the limited population size and study duration. Because of the lack of direct evidence to support the presence of diurnal cortisol cycles, we were unable to use routine statistical analysis such as periodic functions. Application of a linear regression model to the birth time (000 to $<$ 2400 h) and acrophase (0900 to $<$ 3300 h) means the mandatory use of a coordinate plane with the birth time axis intersecting the acrophase axis at 0900 h, suggesting that the model is optimal only when the intercept for the acrophase is close to 0900 h (Supplemental Fig. 2). However, we decided not to correct the intersection of the axis because the positive intercepts were expected for the acrophase from the exploratory analysis, and we aimed to keep the statistical model as simple as possible. Consequently, linear relationships were demonstrated in the whole study subjects and the subgroup of younger subjects without introducing complex corrections; calculated intercepts for these populations were 8.7 and 3.3 h respectively (Fig. 2). Only a few subjects in our population were born during 0000 to earlier than 0600 h, which might also help minimize the number of subject influenced by the fixed intercept. Future studies are required to confirm our findings using data collected over at least several days.

We observed that the sleep-wake state at the time of saliva collection affected the cortisol level; however, we were unable to monitor the sleep-wake rhythm of the infant over the whole study period; noninvasive continuous monitors such as actigraphy may enable the direct com-

parison of circadian cycles identified using serial salivary cortisol and limb motions (34). Our current study cohort consisted of infants who were hospitalized at a neonatal intensive care unit but are not under intensive life support. However, their clinical backgrounds and environmental factors were still different from their peers; the impact of high-risk birth may appear even long after weaning off intensive cares (35); caution is required when generalizing the finding into normal newborn infants. Measurement of melatonin may provide additional information about the function of the master circadian clock. This information was not available for the current study because of the greater minimum sample volume (150 $\mu$l) required for the assay.

### Clinical implications and conclusions

A 24-h adrenal circadian rhythm was present in the newborn infant; salivary cortisol increased around 1500 to earlier than 2100 and from 0 to earlier than 6 h after the birth time. These presumably reflect the fetal circadian rhythm and the superimposed rhythm entrained at birth, respectively. Given that the fetal circadian rhythm is entrained in antiphase to the maternal cycle (6, 7) and that the birth-induced reentrainment may occur at any time of the day, the newborn infant is unlikely to acquire an optimal circadian rhythm for extrauterine life. Further studies are required to determine how the circadian rhythm is acquired. In addition, studies are needed to elucidate the relevance of the following: 1) the antiphase entrainment of the fetal circadian rhythm to the maternal rhythm, 2) the transient entrainment of the infant's adrenal clock to the birth time, and 3) the subsequent disappearance of the overt adrenal rhythm. This information may help predict the time when a mature sleep-wake cycle is established and promote early acquisition of a mature sleep-wake cycle.

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# Fetoscopic Laser Photocoagulation for the Treatment of Twin-Twin Transfusion Syndrome in Monochorionic Twin Pregnancies

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**Abstract:** Fetoscopic laser surgery for severe twin-twin transfusion syndrome (TTTS) has become the optimal treatment choice since the release of the Eurofetus randomized clinical trial. These techniques have been adopted throughout the globe, and many institutions have instituted or will soon institute fetoscopic laser surgery procedures; however, laser surgery has a steep learning curve because of the following: challenging placental location, complex and unexpected communicating anastomoses, residual anastomoses after surgery, or discolored amniotic fluid. We have been performing laser surgery since 2002 in Japan; to date, we have compiled a series of 170 cases. Our data indicates a 78% of overall survival with 5% neonatal morbidity, 63% of survival of both twins, and 93% survival of at least one twin. The recurrent TTTS rate was 1% and the residual vessel rate was 3%.

To improve the learning curve of laser surgery, the employment of various techniques is recommended to achieve a successful surgical outcome: (1) Mapping: before laser ablation, a very thorough mapping of vascular anastomoses should be done, and should be repeated after ablation; (2) Sequential order: obliteration of arterio-venous anastomoses from donor to recipient should be done first to avoid donor hypotension and/or anemia; (3) Trocar (cannula) assisted technique: Trocar assisted technique: Using gentle indent the trocar to the placenta by withdrawing the scope shortly, then anastomoses could be ablated easily; (4) Line method: to avoid residual anastomoses, the laser should draw a virtual line at the hemodynamic equator; The operator must be careful not to miss small anastomoses.

These techniques can help achieve a successful outcome for fetoscopic laser surgery and improve the outcome for cases of severe TTTS.

**Keywords:** Twin-twin transfusion syndrome, fetoscopy, laser, amnioreduction, and ultrasonography.

## INTRODUCTION

Fetoscopic laser surgery for severe twin-twin transfusion syndrome (TTTS) has been conducted since the early 1990s in US and Europe. After the conclusion of Eurofetus randomized clinical trial [1], fetoscopic laser surgery has become the standard and optimal treatment for the condition. Recently, these techniques have been implemented throughout the globe; many institutions have instituted or will soon institute the performance of fetoscopic laser surgery. As with many new procedures, fetoscopic laser surgery has a steep learning curve for a variety of reasons (i.e., challenging placental location, complex and unexpected communicating anastomoses, dividing membrane lifting, residual anastomoses after surgery, or discolored amniotic fluid. In Japan, we have been performing laser surgery since 2002 and five laser centers employ the same protocols. To date, more than 500 TTTS cases, including 170 cases at our institution, were performed by laser surgery. In this article, we introduce and review the new technical tips to improve the achievement of successful outcome for laser surgery and indicate our data of perinatal outcome and complication of fetoscopic laser surgery for severe TTTS.

## PATHOPHYSIOLOGY AND DIAGNOSIS OF TTTS

Because of the vascular anastomoses between the fetuses, monochorionic twin pregnancies have a high-risk profile compared with dichorionic twin pregnancies. TTTS is one of the major complications resulting from vascular communications and their imbalanced blood distribution, involving about 5 - 10 % of monochorionic twin. TTTS can be characterized by an imbalanced blood distribution: a net flow from one fetus (the donor twin) to the other (the recipient twin) through placental communicating vessels. The donor twin is characterized by a hypodynamic status, manifested by hypovolemia, hypotension, oliguria, oligohydramnios, fetal growth restriction, and renal failure. These processes ultimately result in fetal demise. In contrast, the recipient twin is characterized by a hyperdynamic status, hypervolemia, hypertension, polyuria, polyhydramnios, heart failure, and hydrops fetalis; thus, it often also results in a fetal demise. The prognosis for severe early onset TTTS is dismal, with perinatal mortality rates of up to 90 % if untreated.

TTTS is defined prenatally by ultrasonography as: a monochorionic diamniotic twin pregnancy; polyuric polyhydramnios in the recipient twin (maximum vertical pocket > 8 cm, and large distended bladder) with oliguric oligohydram-

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nios in the donor twin (maximum vertical pocket < 2 cm and collapsed or non-visible bladder) simultaneously; and no signs of abnormality due to poly- or oligo-hydramnios. Once the diagnosis of TTTS is made, the severity is classified by Quintero's stage [2] from I to V. Stage III TTTS is sub-classified into two sub-groups defined by whether the donor bladder is visible or non-visible. Sub-classification of Stage III [2, 3] is defined as follows: Stage III classical (Doppler studies are critically abnormal in either twin and the bladder of the donor is not visible); and Stage III atypical (Doppler studies are critically abnormal in either twin and the bladder of the donor is still visible).

## METHODS AND SUBJECTS

### Concept of Fetoscopic Laser Surgery for TTTS

Fetoscopic laser surgery of communicating vessels for severe TTTS consists of a few basic principles: in as much as imbalanced blood distribution due to placental vascular anastomoses are thought to be the main cause of TTTS, laser ablation of communicating vessels can eliminate the cause of TTTS; and all anastomoses (AV (arterio-venous), AA (arterio-arterial), VV (veno-venous anastomoses)) can be visualized and ablated by a fetoscopic procedure.

### Preparation for Fetoscopic Laser Surgery

Essentially, before attempting the procedure, operators should be knowledgeable of the complex pathophysiology of TTTS and other TTTS-related events such as twin anemia polycythemia sequence (TAPS), acute feto-fetal hemorrhage after single fetal demise, selective intrauterine growth restriction (sIUGR) in monochorionic twin, and twin reversed arterial perfusion (TRAP) sequence. Ultrasound assessment should be conducted and the echocardiographic features of TTTS must be evaluated. The donor twin is characterized by a hypovolemic status of the placenta and circulatory insufficiency. Fetal growth restriction and umbilical arterial Doppler abnormalities are common ultrasound features. Doppler examination reveals a decrease in the end-diastolic velocity of the umbilical artery, especially the absence or reverse end-diastolic velocity in Stage III or IV. Decreased peak systolic velocities of the descending aorta are also common. Coarctation of the aorta in the donor or smaller fetus in a monochorionic twin pregnancy has been reported and, based on the hemodynamic theory, decreased blood flow into the donor or smaller twin might increase the risk of a coarctation of the aorta [4]. Most recipient fetuses develop cardiac dysfunction complicated by cardiomegaly, tricuspid and mitral valve regurgitation, ventricular hypertrophy, increased reverse flow in the inferior vena cava, and pulmonary stenosis; they also develop reverse flow of the ductus venosus and pulsatile flow in the umbilical vein [5, 6]. Typically, mild cardiomegaly and increased reverse flow in the inferior vena cava occurs first; moreover, right ventricle compromise occurs earlier than left ventricle compromise. Congestive heart failure and hydrops fetalis in the recipient may originate from chronic volume and pressure overload of the right ventricle. These conditions lead to cardiomegaly and atrio-ventricular valve regurgitation. Occasionally, some cases of a severely affected recipient can develop into acquired pulmonary stenosis/atresia with an intact ventricular septum [5, 7].

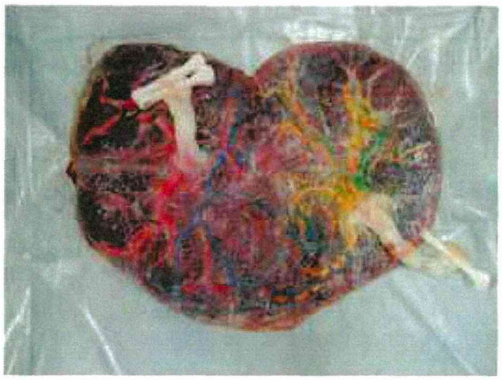

**Fig. (1). Placental Dye Injection Examination of Monochorionic Placenta after Delivery**
Color dye was injected into artery (blue or green) and vein (red or yellow).

Additionally, the operator should be trained to identify and characterize the vascular anastomoses of the monochorionic placenta. Placental dye injection examination [8, 9] of the monochorionic placenta should be an important step before attempting laser surgery (Fig. **1**). All vessels on the placental surface can be precisely differentiated by fetoscopic inspection; arteries principally cross over veins and the color of arteries is dark blue due to deoxygenated blood, whereas veins appear bright red due to oxygenated blood from the placenta. AA and VV anastomoses are directly linked artery-to-artery or vein-to-vein, and have no terminal ends. While AV anastomosis is not anatomical anastomosis itself, the artery (feeding artery) comes from a fetus to cotyledon, and goes to the other fetus as drainage vein. It is called an AV anastomosis. Occasionally, three vessels or four vessels cotyledons are seen, in which three or four types of different vessels are in to same cotyledon. AA anastomoses are theoretically complex and bidirectional transfusion depends on the location of the hemodynamic equator and branch of artery. This mechanism can provide AA anastomoses as functional AV behavior for both directions [10].

### Setting and Performance of Fetoscopic Laser Surgery (Procedural Steps)

Epidural anesthesia or local anesthesia with maternal conscious sedation can be chosen for fetoscopic laser surgery. In our first 36 cases, general anesthesia was chosen; this option was similar to that of other institutions in the early period of fetoscopic laser surgery because immobility of the fetuses especially in the recipient fetus; however, after operator skills improved, epidural or local anesthesia were chosen because they were less invasive for the mother and could decrease maternal complications [11].

After adequate anesthesia was achieved, a 3.8 mm trocar (Richard Wolf, Vernon Hills, IL) was inserted into the recipient amniotic sac with ultrasonographic guidance (Fig. **2**). Appropriate fetoscopes (i.e. Richard Wolf angled-view endoscope, 2.8 mm diameter, 30 cm length; 25 degree (RW-8930.402), 30 degree (RW-8930.422), 70 degree (RW-8660.412), operative 12 degree with working channel for 5 Fr instruments (RW-8746.401); and a 2 mm diameter, 26 cm, 0 degree rigid telescope (K26008AA, Karl Storz, Tuttlingen, Germany) with sheath (K11630KH)) were selected

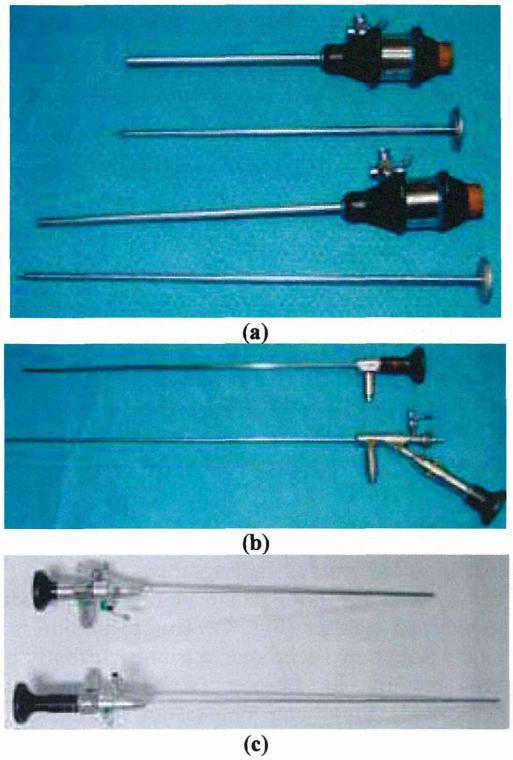

**Fig. (2). Instruments for fetoscopic laser surgery**
(**a**) 3.8-mm Trocar
(**b**) Diagnostic (0, 25, 30, 70 degree) and operative (12 degree with 5 Fr. channel for operating devices) fetoscope by Richard Wolf
(**c**) 3-mm fetoscope with sheeth by Karl Storz

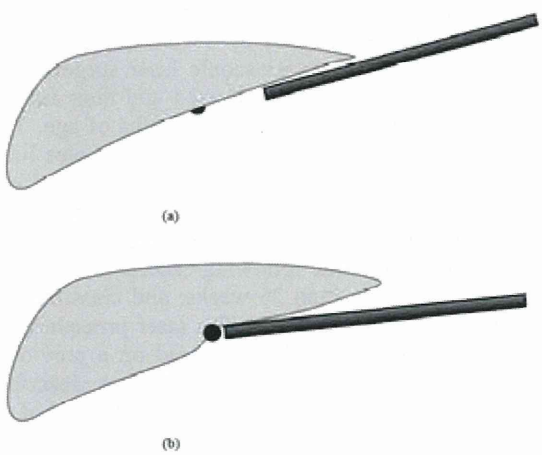

**Fig. (3). Trocar assisted technique**
Gentle indent the rigid trocar to the placenta by withdrawing the scope within the trocar a short distence. Angle of fetoscope and target vessel is tangential. After trocar assisted technique, pushing the trocar close to the target vessel, angle will be adequate to ablate the vessels as perpendicular.

according to the placental and fetal location (Figs. **2, 3**). All communicating vessels were initially mapped and then ablated by neodymiu:yttrium-aluminium-garnet (Nd:YAG) laser (Surgical Laser Technology, Montgomery, PA); this was conducted via the non-contact method with fetoscopic guidance. Laser fibers were inserted into the operating channel of the fetoscope and the laser power was usually set from 15 to 40 watts for Nd:YAG (1,064 nm) laser. Re-examination and re-lasering of anastomoses with mapping was then done; subsequently, the hemodynamic equator was drawn by laser. Finally, amniodrainage was done if indicated.

### Mapping System

During the procedure, placental vessel mapping helps the operator to identify and orient the direction and location the anastomoses. Before laser ablation, a very thorough mapping of vascular anastomoses must be done by the operator and navigator. Each vascular anastomosis was labeled as AV-DR, AV-RD, AA, or VV (for example, AV-DR represented an arterio-venous anastomosis from donor to recipient); the navigator records this information as figures or comments. During the laser ablation, the operator eliminates each anastomosis by referring to the mapping system. After ablation, reevaluation of all placental anastomoses should be done. Additionally, by using the mapping system before ablation, we can choose an appropriate sequence for the ablations.

This system also has the potential to reduce the incidence of residual anastomoses and recurrence of TTTS. A low incidence of residual anastomoses and recurrence of TTTS was reported by Cincotta *et al.* [12], Chmait *et al.* [13] and our series (3); all three studies employed a mapping system.

### Sequential Order

To reduce the incidence of a fetal demise after laser surgery, especially a donor with absent or reversed umbilical arterial flow, Quintero *et al.* and Nakata *et al.* proposed the new technique that all anastomoses should be ablated in a specific order: first, AV-DRs; then, AV-RDs [14, 15]. In particular, the donor twin with an abnormal Doppler of the umbilical artery appears logically to be more vulnerable to an acute hemodynamic change such as hypotension or anemia. If AVRDs are obliterated first, intertwin transfusion from donor to recipient occurs; thus, the donor twin develops increased hypotension and anemia followed by fetal demise. Sequential laser ablation of anastomoses and elimination of the AVDRs prior to the AVRDs could result in improved blood pressure of the donor via an intraoperative intertwin transfusion, rescue as well as stabilization of the hemodynamics of the donor. It is currently controversial whether arterio-arterial and veno-venous anastomoses should ablated first, prior to AV anastomoses, or last; however, an AVDR first policy could reduce fetal demise after laser surgery especially in donors with abnormal Doppler [14, 15]. The US Fetus Consortium is currently undergoing a randomized control trial to compare outcomes between the standard laser approach and the sequential laser approach.

### Trocar (Cannula) Assisted Techniques

Generally, an anterior placenta is the one of the most difficult settings for FLP cases. Because of the tangential angle of target vessels and fetoscope alignment, it becomes quite difficult to confirm the anastomoses and to ablate the vessels by laser. Quintero *et al.* originally proposed the technique of trocar-assisted selective laser photocoagulation [16]. Using the rigid trocar, gently indent the placenta by withdrawing the scope within the trocar a short distance. At this point, the anastomoses can be easily ablated because the

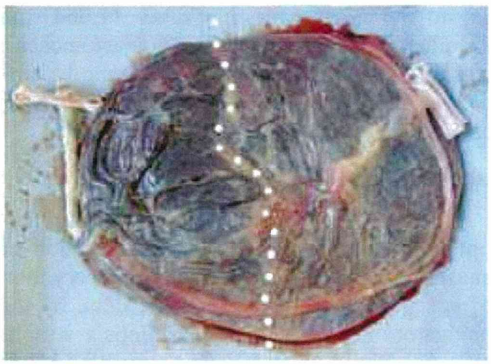

**Fig. (4). Line method.**
Draw the laser line along with the hemodynamic equator (dot line), creating dichorionized placenta.

**Table 1. Baseline and Surgical Characteristics (n=152)**

| Maternal Age (Year) | 30 (15 – 40) |
|---|---|
| Gestational age at surgery (weeks) | 21 (16 – 25) |
| Location of placenta | |
| Anterior | 77 (51%) |
| Posterior | 75 (49%) |
| Quintero stage | |
| Stage I | 18 (12%) |
| Stage II | 27 (18%) |
| Stage III | 84 (55%) |
| atypical | 29 |
| classical | 55 |
| Stage IV | 23 (15%) |
| Complete surgery | 152 (99%) |
| Anesthesia | |
| General | 36 (24%) |
| Epidural | 116 (76%) |
| Operation time (minutes) | 60 (25 – 158) |

Data are shown as median (range) or number (%)

target vessel and fetoscope are perpendicular rather than tangential (Fig. **3**).

This trocar-assisted technique has three potential benefits: (1) It allows perpendicular rather than tangential alignment of the target vessels as described above; (2) Reduction of the blood flow in large communicating vessels, which are difficult to ablate with normal laser energy; the pressure exerted by the trocar reduces the flow and allows for ablation with less laser energy; and (3) We can use this technique to avoid inadvertent injury to the fetus and dividing membrane (The fetus and membrane can move unexpectedly toward the fetoscope and laser; thus, slightly withdrawing the fetoscope within the trocar allows for safe ablation of the target vessels).

Using the trocar has another technical benefit. If we choose direct sheath centesis without the trocar, we can only use one type of fetoscope; however, we can use an appropriate fetoscope (i.e., 0 degree, 30 degree, or 70 degree for an anterior placenta. Furthermore, both Richard Wolf and Karl Storz instruments fit a 3.8 mm cannula.

**Table 2. Pregnancy Outcome and Survival Rates (n=152)**

| Gestational Age at Delivery (Weeks) | 33 (19 – 40) |
|---|---|
| Miscarriage (delivery < 22 weeks) | 6 (3.9%) |
| Recurrent TTTS | 1 (0.7%) |
| TAPS | 2 (1.3%) |
| Residual anastomoses | 4 (2.6%) |
| Over all survival (n=304) | 237/304 (78%) |
| Neurological sequale (n=237) | 13/237 (5.5%) |
| 2 survivors | 96 (63%) |
| 1 survivor | 45 (30%) |
| 0 survivor | 11 (7%) |
| At least 1 survivor | 141 (93%) |

Data are shown as median (range) or number (%)

## Line Drawing Methods

To avoid residual anastomoses, a virtual line should be drawn with the laser at the hemodynamic equator, not the membrane equator (Fig. **4**). This technique is also reported as the Solomon Technique; a trial is currently ongoing to test this method in Europe (www.trialregister.nl, trial ID: NTR1245). Small anastomoses are not missed by the virtual line method. First, selective laser ablation of each vascular end of anastomotic vessels is performed; second, construct a dotted line with the laser; and finally construct as virtual line along with the laser along the hemodynamic equator.

## TTTS PATIENTS

From 2002 to 2010, 152 Japanese women whose pregnancy was complicated by severe TTTS before 26 weeks gestation underwent fetoscopic laser surgery in our institution. All patients were delivered and their infants were followed-up for until at least six months of age. TTTS was diagnosed in monochorionic twin pregnancies based on standard ultrasound criteria: polyhydramnios and oligohydramnios with the deepest vertical amniotic pocket measuring at least 8.0 cm in the recipient and at most 2.0 cm in the donor. All patients met the following criteria for laser surgery: gestational age less than 26 weeks; and classification by Quintero's stage from I to IV. The laser procedure for placental communicating vessels was based on a previously reported method [3] with additional techniques described above if indicated: mapping system; sequential order of AV-DR first policy if possible; using a trocar of appropriate diameter for the fetoscope; employing trocar-assisted techniques; and laser line drawing methodology. Patient baseline and surgical characteristics are presented in Table **1**. Seventy percent of the patients were stage III (55%) and IV (15%) and 50% of the patients had an anterior placenta.

## RESULTS

We completed laser surgery on 99% of the patients. The median surgical time was 60 minutes; however, surgical time was counted from the insertion of the trocar to amniodrainage with the following intervening steps: fetoscopic inspection, mapping, and lasering. Tables **2** and **3** present the perinatal outcomes. The overall survival rate was 78%; 5.5% of the cases had neurological sequelae including periventricular

**Table 3. Perinatal Outcome According to Quintero Stage**

| Stage | I n=18 | II n=27 | III Atypical n=29 | III Classical n=55 | IV n=23 |
|---|---|---|---|---|---|
| 2 survivors | 12 (67%) | 18 (67%) | 13 (45%) | 39 (71%) | 14 (61%) |
| 1 survivor | 5 (28%) | 5 (18%) | 14 (48%) | 12 (22%) | 9 (29%) |
| 0 survivor | 1 (5%) | 4 (15%) | 2 (7%) | 4 (7%) | 0 (0%) |
| At least 1 survivor | 17 (95%) | 23 (95%) | 27 (93%) | 51 (93%) | 23 (100%) |
| Overall survival | 29/36 (81%) | 41/54 (76%) | 40/58 (67%) | 90/110 (82%) | 37/46 (80%) |
| Neurological sequale | 1/29 (3.4%) | 4/41 (9.8%) | 3/40 (7.5%) | 2/110 (1.8%) | 13/237 (5.5%) |

**Table 4. Comparison of Perinatal Outcomes in Published Series**

| | Ville *et al.* 1998 n=132 | Hecher *et al.* 1999 n=73 | Hecher *et al.* 2000 n=200 | Quintero *et al.* 2003 n=95 | Senat *et al.* 2004 n=72 | Huber *et al.* 2006 n=200 | Middledorp *et al.* 2007 n=100 | Cincotta *et al.* 2009 n=100 | Sago *et al.* 2010 n=181 | Chmait *et al.* 2011 n=682 | Present study 2011 n=152 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Median gestational age at delivery (weeks) | - | 33 | 34 | 32 | 33 | 34 | 33 | 31 | 33 | 33 | 33 |
| Perinatal survival (%) | 55 | 61 | - | 64 | 56 | 72 | 70 | 76 | 76 | 79 | 78 |
| Neurological sequale (%) | 4 | 6 | 6 | 4 | 7 | - | - | 3 | 5 | | 5 |
| 2 survivors (%) | 36 | 42 | 50 | 44 | 36 | 60 | 58 | 66 | 62 | 72 | 63 |
| 1 survivor (%) | 38 | 37 | 30 | 38 | 38 | 24 | 23 | 19 | 28 | 18 | 30 |
| 0 survivor (%) | 26 | 21 | 20 | 17 | 26 | 17 | 19 | 15 | 10 | 10 | 7 |
| At least 1 survivor | 74 | 79 | 80 | 82 | 74 | 84 | 81 | 85 | 90 | 90 | 93 |

neurological sequale including periventricular leukomalasia, interaventricular hemorrhage grade III and IV and cerebral palsy

leukomalacia, intraventricular hemorrhage grade 3 and 4, and cerebral palsy. At six months after delivery: in 63% of the cases, both twins survived; in 30% of the cases, one twin survived; and in 93% of the cases at least one twin survived. The Quintero stage did not worsen in any of the survivors; however, stage III atypical, which was defined as abnormal Doppler flow with visible donor bladder, had a decreased survival rate especially in 2 survivors.

## DISCUSSION

Table **4** presents the perinatal outcomes in published series including early series of pioneer operators [1, 17-20] and published data [12, 21-24] from the conclusion of the Eurofetus trial comparison to the present study. Early series reported approximately a 60% overall survival rate, a 5% neurological complication rate; and a 40% survival rate of both twins. Middledorp *et al.* [21], Cincotta *et al.* [12], Huber *et al.* [22], and Chmait *et al.* [23] describe improved perinatal outcomes: > 70% overall survival, 58-69% with two survivors and; and > 80% with at least one survivor. Hecher *et al.* and Huber *et al.* reported the data from their 400 consecutive case series divided into two groups: the first 200 [20] and last 200 [22]. As their experience increased, they reported an increasing overall survival rate, especially for cases of two survivors. In our series, the overall perinatal survival for at least six months was 78%; the neurological complication rate was 5% of neurological complications; the rate for both twins surviving was 63%; and at least one twin survived in 93% of the cases. These data appear favorable 93% of the cases. These data appear favorable and are comparable to that of the latest 200 case series of Huber *et al.* [22] We attribute our favorable results to mapping, trocar assisted techniques, selection of the appropriate fetoscope, sequential order ablation, and the laser line drawing method.

## CONCLUSION

In view of our experience regarding the management of TTTS, comprehensive techniques including preparation of various new devices, selection of instruments, and advanced laser ablation techniques have contributed to the progress of fetoscopic laser surgery for TTTS in monochorionic twins.

## CONFLICT OF INTEREST

None declared.

## ACKNOWLEDGEMENT

None declared.

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# Perinatal outcome of monochorionic diamniotic twin pregnancies managed from early gestation at a single center

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## Abstract

*Aim:* The aim of this study was to evaluate the perinatal outcome of monochorionic diamniotic (MD) twin gestations managed from early gestation onward at a single center.

*Material and Methods:* This was a retrospective single-cohort study, spanning 6 years, of 219 MD twin pregnancies who received prenatal care before 14 weeks of gestation and delivered at our center. The subjects were all under the same perinatal management protocol. The incidence of fetal or neonatal death, major neurological complications at 28 days of age, twin–twin transfusion syndrome (TTTS), twin anemia-polycythemia sequence, and discordant birth was evaluated. Laser surgery was offered for TTTS at less than 26 weeks; however, selective feticide was not performed.

*Results:* Pregnancy termination was selected in two cases. Miscarriage occurred in two (1%) of the cases and preterm delivery occurred in 91 (42%). In regard to perinatal outcome at 28 days of age, 195 (89%) women possessed two live infants and 205 (94%) possessed at least one live infant. The overall survival rate was 92% (403/438). The prevalence of TTTS was 17 cases (8%), seven of whom underwent laser surgery. Four cases of twin anemia-polycythemia sequence were diagnosed postnatally (2%); discordant birth was diagnosed in 24 (12%). Major neonatal neurological abnormalities were noted in six cases (2%).

*Conclusions:* The incidence of perinatal complications in 219 sets of MD twins managed from early gestational age to the neonatal period in one perinatal center was demonstrated. The incidence of TTTS was 8%; the survival rate was 89% at 28 days of age.

**Key words:** discordant twin, monochorionic twin, perinatal outcome, twin anemia-polycythemia sequence, twin–twin transfusion syndrome.

## Introduction

Monochorionic diamniotic (MD) twin gestations have a higher risk of perinatal complications than both dichorionic twins and singleton pregnancies.[1–3] Almost all MD twins have placental vascular anastomoses.[4] These can occasionally play a causal role in specific pathologic conditions, such as twin–twin transfusion

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syndrome (TTTS) and twin anemia-polythemia sequence (TAPS); in addition, the anastomoses can have a harmful effect on the surviving twin following an intrauterine demise of the co-twin due to acute feto-fetal hemorrhage.[5–8] Furthermore, selective intrauterine growth restriction caused by unequal placental sharing and vascular anastomoses can also contribute to the poor prognosis of an MD twin.[9–12] Meticulous perinatal management is indicated because, according to a previous study, approximately 30% of MD twins suffer complications.[13]

The incidence of TTTS is approximately 8–9% of MD twins; this is a major cause of death and neurological sequelae for these fetuses.[2,14,15] Recently, fetoscopic laser coagulation of vascular anastomoses has been reported to improve the perinatal outcome of twins with TTTS.[16,17] However, in Japan, the overall perinatal prognosis of MD twins has not been fully evaluated in the period after the introduction of laser surgery for TTTS. To evaluate the disease prevalence of MD twins, the fetal and neonatal outcomes in cases managed from the first trimester onward in a single center was retrospectively investigated.

## Material and Methods

This was a retrospective study of the perinatal outcome of women with MD twin pregnancies delivered at a single referral center in Japan. All women were consecutively asked to participate in the study. Inclusion criteria were women with an MD twin pregnancy who received prenatal care before 14 weeks of gestation and delivered at our center. The first visit of all subjects occurred during a 6-year period from January 2004 to January 2010. Exclusion criteria were: women with single or double fetal demise before 14 weeks of gestation; twin-reversed arterial perfusion sequence (TRAP); triplets containing a monochorionic twin pair; and cases of major anomalies diagnosed before 14 weeks of gestation. During the study period, monochorionicity and an accurate gestational age were determined by transvaginal ultrasound between 11 and 14 weeks' gestation. Ultrasound examination, including fetal biometry and the estimation of amniotic fluid volume using an amniotic fluid pocket, was performed every week beginning at 16 weeks' gestation. Detailed anatomical examinations at 20 and 30 weeks' gestation were performed. Patients were hospitalized for obstetrical complications, such as threatened premature delivery and pregnancy-induced hypertension (PIH). In the case of threatened premature delivery at approximately 35 weeks' gestation, the patient was hospitalized and a continuous intravenous drip of ritodrine hydrochloride and/or magnesium sulfate was administered. When a physician deemed preterm delivery to be imminent before 34 weeks' gestation, prophylactic antenatal betamethasone was administered. The decision to deliver was based on obstetrical indications. If the course of the pregnancy was uncomplicated, elective delivery was planned at around 38 weeks' gestation. A non-vertex, presenting twin, a non-vertex, second twin with an estimated fetal weight (EFW) less than 1500 grams, or other common contraindications to labor were deemed to be contraindications to vaginal delivery. If vaginal delivery was not contraindicated, the mode of delivery was decided after counseling with the patient. All patients could select elective cesarean delivery independent of fetal position. Selective feticide was not performed under any circumstances because of medical-legal concerns. In all cases, monochorionicity was confirmed by placental examination after birth. Neonatologists were present for resuscitation at all deliveries. All infants were neurologically evaluated by neonatologists 28 days after birth. If an infant was suspected to be neurologically abnormal or was admitted to the neonatal intensive care unit (NICU), brain ultrasound, computed tomography, and/or magnetic resonance imaging were performed.

TTTS was defined as the presence of ultrasound findings of polyhydramnios in one twin and oligohydramnios in the other, as previously defined.[16] Laser surgery for TTTS was offered if it was diagnosed before 26 weeks' gestation. Expectant management, serial amniodrainage, or elective preterm birth was performed for TTTS diagnosed after 26 weeks' gestation. The diagnostic criterion for TAPS was based on postnatal criteria, which were defined by the presence of anemia (hemoglobin of 11 g/dL) in one twin and polycythemia (hemoglobin of 20 g/dL) in the other twin at the birth without signs of TTTS.[14] Major congenital anomalies were defined as lethal anomalies, anomalies requiring postnatal surgical intervention, or anomalies caused by functional impairment. Patients with suspected severe growth restriction were usually hospitalized for monitoring. Birthweight discordancy was calculated according to the following formula: (weight of larger twin – weight of smaller twin) × 100/ weight of larger twin. When the birthweight discordant rate was ≥25%, excluding cases of intrauterine fetal demise (IUFD), we defined them to be severe discordant growth.

Neonatal death was defined as death within 28 days of birth. The definition of overall survival was calculated as the number of live-birth infants divided by the number of all fetuses registered in the study, including termination of pregnancy (TOP) cases. Overall mortality was calculated as the sum of IUFD and neonatal death divided by the all fetuses registered. Neonatal mortality was calculated as neonatal death per total number of live-birth infants.

## Results

A total of 219 women with MD twins participated in this retrospective study; one patient carrying a fetus with a cystic hygroma and one carrying a fetus with fetal acrania were excluded. The maternal clinical characteristics are presented in Table 1. Detailed information regarding delivery and birthweights of live-born infants are summarized in Table 2. In Japan, miscarriage is defined as delivery at less than 22 weeks of gestation and preterm birth is between 22 and 36 weeks of gestation. TOP was elected in two cases, one with TTTS and one with a single IUFD. In regard to complications at <22 weeks of gestation, spontaneous miscarriage occurred in two cases (1%) and IUFD of both fetuses occurred in eight cases (4%). The median gestational age at delivery, excluding the two TOP cases, was 37.3 weeks (range: 15–40). Almost half (42%) resulted in preterm birth. Of 207 cases >22 weeks' gestation, 38% resulted in a vaginal delivery for both twins; the rate of cesarean delivery was 62%, including 3% of patients with an elective cesarean section for the second twin. Low-birthweight infants occurred in 67% of the cases, including 5% with an extremely low-birthweight infant (<1000 grams).

Table 3 presents the incidence of perinatal complications, including IUFD, TTTS, TAPS, congenital anomalies, and discordant growth >25%. In addition, five of nine cases with single IUFD were diagnosed before 22 weeks; IUFD of both twins was diagnosed before 22 weeks in all eight cases. The percentage of TTTS in cases with a single IUFD was 33%; that for IUFD of both twins was 0%. In 17 patients complicated with TTTS, 11 (65%) were diagnosed before 26 weeks; furthermore, nine patients underwent fetoscopic laser photocoaglation. For cases that developed TTTS after 26 weeks, immediate delivery was considered. Of the 17 cases with TTTS, excluding one TOP case, the rate of two surviving twins 28 days after birth was 69%; in 25% of the cases, one twin survived and in one of the 16 cases, neither twin survived. For cases of TTTS, the neonatal survival rate was 29/32 (91%), the neonatal

**Table 1** Maternal characteristics ($n = 219$)

| Maternal age (years) | 30 ± 5 | |
|---|---|---|
| Nulliparous | 130 | (59%) |
| Spontaneous conceptions | 191 | (87%) |
| Ovulation induction | 16 | (7%) |
| IVF/ICSI | 12 | (5%) |

Data presented as mean ± SD or $n$ (%). ICSI, intracytoplasmic sperm injection; IVF, *in vitro* fertilization.

**Table 2** Pregnancy outcomes and birthweight of live-born infants

| Gestational age at delivery | $n = 219$ | |
|---|---|---|
| TOP | 2/219 | (1%) |
| Miscarriage <22 weeks | 2/219 | (1%) |
| Double IUFD <22 weeks | 8/219 | (4%) |
| Delivery at 22–29 ± 6 weeks | 10/219 | (5%) |
| Delivery at 30–33 ± 6 weeks | 25/219 | (11%) |
| Delivery at 34–36 ± 6 weeks | 44/219 | (20%) |
| Delivery at ≥37 weeks | 128/219 | (58%) |
| Delivery mode after 22 weeks | n = 207 | |
| Vaginal delivery | 79/207 | (38%) |
| Cesarean section | 122/207 | (59%) |
| Cesarean section for second twin | 6/207 | (3%) |
| Iatrogenic vs spontaneous birth between 22 and 36 weeks | n = 79 | |
| Spontaneous preterm delivery | 46/79 | (58%) |
| Iatrogenic preterm birth for fetal indication | 27/79 | (34%) |
| Iatrogenic preterm birth for other obstetrical indication | 6/79 | (8%) |
| Birthweight of live births after 22 weeks | n = 403 | |
| <1000 g | 20/403 | (5%) |
| 1000–1500 g | 23/403 | (6%) |
| 1500–2500 g | 227/403 | (56%) |
| >2500 g | 133/403 | (33%) |

IUFD, intrauterine fetal demise; TOP, termination of pregnancy.

**Table 3** Perinatal complications as monochorionic twins

| | | |
|---|---|---|
| IUFD in one fetus | 9/219 | (4%) |
| IUFD in both fetuses | 8/219 | (4%) |
| TTTS | 17/219 | (8%) |
| Both IUGR | 22/219 | (10%) |
| TAPS | 4/219 | (2%) |
| Major congenital anomalies | 15/438 | (3%) |
| Severe discordant growth (>25%) | 24/198 | (12%) |

Above cases may have more than one complication. IUFD, intrauterine fetal demise; IUGR, intrauterine growth restriction; TAPS, twin-anemia polycythemia sequence; TTTS, twin–twin transfusion syndrome.

mortality rate was 3/29 (10%), and the overall mortality rate was 26/32 (81%). Seven of nine cases underwent laser surgery; in seven of these cases, both twins survived and in the other two, one twin survived. However, in one of these cases, both twins expired in the neonatal period. TAPS was confirmed postnatally in four cases (2%); one was a post-laser surgery complication. All of these cases required a transfusion and/or a partial exchange transfusion after birth. The frequency of spontaneous TAPS was 1%; that of post-laser surgery was 6%. Major congenital anomalies were detected in 15 of 438 (3%) fetuses. In two cases, both twins were found to have congenital anomalies; however, in 11 cases, one twin was found to have a congenital anomaly. The anomalies included two fetuses with congenital obliteration, one with hypospadias, one with hypospadias and cleft palate, one with a broncho-esophageal fistula, two with hypertrophic pyloric stenosis, one with a multicystic, dysplastic left kidney, one with hypoplasia of the sylvian fissure, one with bilateral ventriculomegaly, one with a right congenital diaphragmatic hernia, one with atrophic kidneys in the surviving twin and intrauterine demise of the co-twin, and three with cardiac defects (double outlet right ventricle, tricuspid valve dysplasia, and pulmonary stenosis). In regard to birthweight discordance, 24 (12%) twin pairs had >25% weight discordance. In 198 cases, both neonates survived.

Neonatal and maternal perinatal complications are presented in Table 4. Of the 403 live-birth infants, 100 (25%) were admitted to the NICU. Neonatal deaths occurred in four infants (1%). In one case, neonatal death occurred in both twins and in two cases, one infant expired. Three neonatal deaths occurred in TTTS cases; a congenital heart defect (pulmonary stenosis) was found in one case. At 28 days after birth, abnormal neurological symptoms were noted by a neonatologist in five infants (1%). In regard to maternal complications, four women (2%) required a transfusion because of massive hemorrhage, 21 women (10%) developed PIH, and three women (1%) developed abruptio placentae.

In regard to perinatal outcome at birth of the 219 MD twins, in 199 (91%) cases, both twins were live-born and 195 (89%) were alive at 28 days of age; at least one twin survived at birth in 207 cases (95%) and 205 (94%) were alive 28 days after birth (Table 5). The overall survival rate was 404/438 (92%). Neonatal mortality was 6/403 (1%) and overall mortality was 41/438 (9%).

**Table 4** Neonatal and maternal complications

| Neonatal complications in live births after 22 weeks | $n = 403$ | |
|---|---|---|
| NICU admission | 100/403 | (25%) |
| Neonatal death | 6/403 | (1%) |
| Apgar score <7 at 5 min | 14/403 | (3%) |
| Abnormal neurological findings at 28 days after birth | 6/399 | (2%) |
| Maternal complications in MD twins | $n = 219$ | |
| Maternal blood transfusion | 4/219 | (2%) |
| PIH | 21/219 | (10%) |
| Abruptio placentae | 3/219 | (1%) |

MD, monochorionic diamniotic; NICU, neonatal intensive care unit; PIH, pregnancy-induced hypertension.

**Table 5** Survival rates ($n = 219$)

| | | |
|---|---|---|
| At least one twin survived at birth | 207 | (95%) |
| Both twins survived at birth | 199 | (91%) |
| At least one twin survived at 28 days after birth | 205 | (94%) |
| Both twins survived at 28 days after birth | 195 | (89%) |
| Both twins survived without abnormal neurological findings at 28 days after birth | 190 | (87%) |

## Discussion

This paper presents the perinatal outcome of MD twins followed from the first trimester to 28 days after birth under the defined management protocol at one tertiary perinatal care center. In 219 women with MD twin pairs, the rate of both surviving was 91%, the rate of one surviving was 4%; neither twin survived in 5% of the cases. These results are similar to those of a previous prospective study of 202 MD twins by Lewi *et al.*[14] However, both of these studies represent a higher survival rate than a survey of 102 MD twins by Sebire *et al.*[1] Their results were: two survivors, 82%; one survivor, 6%; and no survivor, 12%. This difference might be explained by the subsequent introduction of fetal therapy, such as laser surgery for TTTS. The perinatal outcome could also vary across the study period, because our study was conducted from 2004 to 2010, whereas their study period was from 1992 to 1996; at that time, fetal therapy for pathologic MD twins, including TTTS, was uncommon. Due to ethical issues, selective feticide has not been performed in Japan. Therefore, improvement of the prognosis might be partially due to the introduction of laser surgery as well as advances in overall perinatal management.

The pathogenesis of TTTS is an imbalance of inter-twin blood transfusion via placental vascular

anastomoses, which connect both circulations. In general, the recipient twin exhibits hypervolemia and polyhydramnios, and the donor twin exhibits hypovolemia, oliguria, and oligohydramnios. When untreated, the mortality rate of both twins is approximately 90%.[18] In recent years, laser surgery has improved the perinatal outcome of twins with TTTS compared to serial amniodrainage.[16,17,19,20] TTTS occurred in 8% of the cases in our study, which was similar to the incidence of 8–9% previously reported.[2,14,15] Of 17 TTTS cases, the survival rate at 28 days of age was 85% and all patients possessed at least one live infant. The survival rate for nine patients who underwent laser surgery for TTTS was 89%, which was consistent with the results of a previous Japanese study.[21] Weekly ultrasound evaluation to detect amniotic fluid volume abnormality at an early stage was performed in our institution as an experiment, however, there was insuficient evidence that close monitoring could improve the prognosis among monochorionic twins.

An inter-twin difference in hemoglobin levels has been described as TAPS. TAPS is thought to be a chronic feto-fetal transfusion caused by smaller anastomoses than that of TTTS.[9,22,23] The incidence of spontaneous TAPS in this series was 3/202 (1%) and that of TAPS after laser surgery for TTTS was 1/9 (6%). The incidence of TAPS was similar to that of previous studies.[14,24–26] Although the incidence of TAPS is low, neonates with TAPS often require a blood transfusion for the anemic twin and a partial exchange transfusion for the polycythemic twin.[27] Routine middle cerebral artery peak systolic velocity measurement in all MD twins might be useful for the antenatal diagnosis of TAPS.[28,23]

Major congenital anomalies were found prenatally in 13/219 (6%) twin pairs, which was compatible with the 6% reported in previous studies.[14,15] However, because the cases of intrauterine fetal demise and anomalies detected before 14 weeks, 6 days of gestation were excluded from our study, and the actual incidence of congenital anomalies could be higher than 6%. The incidence of congenital anomalies has been reported to be higher in MD twins than in singletons.[29] Zygotic splitting may particularly affect midline defects.[30] Furthermore, characteristic vascular events from early pregnancy to delivery may possibly lead to brain and cardiac anomalies.[31,32] Detailed anatomical ultrasound examinations are indicated for MD twins.

In live-born twin pairs, severe discordant growth complicated 24 of 198 cases (12%), which was similar to the incidence of other reports.[1] According to a report by Lewi *et al.*,[14] the incidence was 14%; however, this value included cases of IUFD and TTTS. In this study, severe discordant growth was diagnosed after birth and we did not take into account ultrasonographic fetal weight estimations. Recent studies have shown that selective intrauterine growth restriction caused by unequal placental sharing and vascular anastomoses can also contribute to a poor prognosis for both fetuses.[1,14,33] In particular, cases of selective intrauterine growth restriction with abnormal umbilical artery Doppler and early onset discordant growth carry a high mortality rate.[9,10,34–36] Detailed prenatal ultrasound with particular attention to not only TTTS signs but also to fetal growth restriction and abnormal Doppler findings may possibly be useful to diagnose high-risk MD twins.

In conclusion, our study demonstrates the incidence of perinatal complications in 219 monochorionic diamniotic twins managed from the first trimester to the early neonatal period at one referral center. The incidence of TTTS was 8% and that of both neonates surviving was 91%. The evaluation of long-term survival of MD twins is planned for a future study.

## Disclosure

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# 一絨毛膜性二羊膜性胎盤を用いた血管吻合検索方法の検討

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monochorionic twin
placental vascular anastomoses
placental injection

**概要**　一絨毛膜性二羊膜性胎盤では多くの場合に血管吻合が存在し，Twin anemia-polycythemia sequenceや双胎間輸血症候群の原因となる．これらの病態を理解するため，さらには吻合血管レーザー遮断術の治療効果判定のため，血管吻合検索を軸とした胎盤の病理学的検査は重要である．胎盤血管吻合検索は血管内に各種物質を注入して行うが，どの方法が優れているかは定まっていない．今回我々は，色素注入法，樹脂注入法および色素樹脂併用法を施行した一絨毛膜性二羊膜性胎盤を用いて，それぞれの検索方法の長所と短所を比較検討した．その結果，色素注入法は胎盤表面からの微細な血管吻合検索に優れ，樹脂注入法は立体構造の把握に適していた．また，今回新たに行った色素樹脂併用法では微細な血管吻合・深部血管吻合，双方の検索に優れていた．さらに，症例に合わせた適切な血管吻合検索法の選択法を示す．

## はじめに

一絨毛膜性二羊膜性胎盤（monochorionic diamnionic（MD））では多くの場合に血管吻合が存在し，血管吻合を介した胎児間の血液移動はTwin to twin transfusion syndrome（TTTS）やTwin anemia-polycythemia sequence（TAPS）などの発症要因となる．これらの病態を理解するため，さらには吻合血管レーザー遮断術（fetoscopic laser photocoagulation of placental communicating vessels（FLP））の治療効果判定のため，血管吻合検索は重要である．現在，生理食塩水注入法，色素注入法，樹脂注入法など様々な血管吻合検索法が行われている[1)]が，どの方法が最適かは定まっておらず各施設で独自の方法が行われている．

## 目的

今回我々は，当センター病理診断部に提出されたMD胎盤を用いて，色素注入法，樹脂注入法および色素樹脂併用法を施行し，それぞれの検索方法の長所と短所を比較検討した．また，症例に応じた血管吻合検索法を明らかにし，今後の診断法の資とすることを目的とする．

## 方法

2008年12月～2011年10月に当センター病理診断部に提出されたMD双胎胎盤3例を用いて，色素注入法・樹脂注入法・色素樹脂併用法を施行した．

症例1（色素注入法）：Twin amniotic fluid discordance with abnormal dopplerのため妊娠24週でFLP施行（臨床試験）．妊娠35週5日で分娩となったMD双胎．

症例2（樹脂注入法）：TTTS（stage Ⅱ）のため妊娠24週でFLP施行．妊娠35週4日で分娩となったMD双胎．

症例3（色素樹脂併用法）：妊娠36週5日に分娩となったMD双胎．

各検索方法は以下の通りである．

①色素注入法

4Frの経鼻・経口胃チューブ（アトムメディカル）4本，10mlシリンジ（テルモ）4本，タコ糸，臍帯クリップ（アトムメディカル）2個，4色の不溶性色素（THE DAVIDSON MARKING SYSTEM, BRADLEY PRODUCTS, INC, MS, USA）を20%中性緩衝ホルマリンで50%に希釈したものを用意する．各臍帯動脈・静脈に4Frチューブを1本ずつ，計4本 挿入し生理食

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