# Autonomic Nervous System Changes in Term Infants during Early Skin-to-skin Contact (SSC): Examination of SSC Effectiveness and the Influence of Meconium-stained Amniotic Fluid

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**Aim**: To determine the effects of early skin-to-skin contact (SSC) and the influence of meconium-stained amniotic fluid (MSAF) on infants who satisfied the SSC indications by examining the changes in autonomic nervous system and physiological indices in term infants born with normal vaginal delivery.

Methods: Our study population included cases that satisfied indications for implementing SSC. Study A included 12 infants who underwent SSC (SSC group) and 10 who did not (non-SCC group), and Study B included 9 infants with MSAF (SSC with MSAF group) and 12 without it (SSC with clear AF group). We measured heart rate variability (HRV), heart rate (HR), oxygen saturation (SpO<sub>2</sub>), state of sleep-wakefulness (measured continuously for 2h after birth), respiratory rate (RR), and body temperature (measured every 15 min).

**Results**: In Study A, irrespective of the presence/absence of SSC, sympathetic nervous system (SNS) activities accelerated at 1h after birth, but after 2h, the SNS activity was suppressed in the SSC group. During SSC,  $SpO_2$  progressed within the normal range of >95 %, and the infants were significantly more often in a state of sleep. In Study B, there was no difference in HRV, HR, RR, temperature,  $SpO_2$ , and state of sleep according to the presence/absence of MSAF.

**Conclusions**: SSC is effective in inducing a state of rest because it induces a state of sleep and suppresses SNS hyperactivity at 2h after birth. When SSC indications are satisfied, we demonstrated, for the first time, that MSAF has no adverse effect on infants. *Shinshu Med J 67*: 91—103, 2019

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**Key words**: autonomic nervous system, early skin-to-skin contact, heart rate variability, meconium, oximetry

# I Introduction

Early skin-to-skin contact (SSC) is performed during the sensitive period<sup>1)</sup> when maternal-infant attachment develops immediately after birth. SSC is the infant care<sup>2)</sup> performed by placing a naked term infant in a prone position on the mother's chest after birth and differs from kangaroo care<sup>3)</sup> performed

Respiratory and cardiovascular systems in the neonate rapidly change during the transition from intrauterine to extrauterine life. Respiratory and circulatory functions are immature in neonates, but homeostasis is maintained through interactions between the sympathetic nerve system (SNS) and parasympathetic nerve system (PSNS). SSC provides benefits, such as maintaining respiratory and heart

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on preterm infants in the neonatal intensive care unit. SSC was presented by the WHO<sup>4)</sup> in 1996, and along with "Evidence for the Ten Steps to Successful Breastfeeding" by the WHO<sup>5)</sup>, SCC is recommended worldwide as a means to establish a mother-infant relationship.

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Table 1 Indications for early mother-infant skin-to-skin contact after vaginal deliveries, discontinuation criteria, and implementation methods<sup>2)</sup> in Japan

Indications	Mother: The mother herself is willing to engage in "early mother-infant skin-to-skin contact." Vital signs are stable. The mother is not exhausted. The physician or midwife does not observe any contraindications.	
	Infant: Absence of non-reassuring fetal status. No asphyxia neonatorum (1-min and 5-min Apgar score ≥8). Term newborn. Not a low-birth-weight infant. The physician, midwife, or nurse does not observe any contraindications.	
Discontinuation criteria	Mother: Drowsiness. Physician or midwife judges that it is contraindicated.	
	Infant: Presence of a breathing problem (including apnea and gasping respiration). $SpO_2: <90\%$ . Limp, little vitality. Enters a sleeping state. The physician, midwife, or nurse judges that it is contraindicated.	
Implementation methods	<ul> <li>Explain "early mother-infant skin-to-skin contact" when drafting the birth plan.</li> <li>Start as soon as possible after birth. It is preferable to continue for 30 min or more or until breast-feeding.</li> <li>Set the upper limit to continue at within 2 hours, and conclude at the point when the infant falls asleep or the mother becomes drowsy.</li> </ul>	
	Mother: Confirm that the mother wants "early mother-infant skin-to-skin contact". Elevate the upper body (around 30° is preferable). Wipe away sweat on the chest and abdomen. Hold the nude baby. With the mother and infant chest-to-chest, firmly support the child with both hands.	
	Infant: Dry the infant off. Position the infant's head turned to one side so the infant can breathe easily without the nasal cavity becoming obstructed. Cover the infant with a warmed bath towel. Attach the probe of a pulse oximeter to a lower limb, or the staff member in charge remains in attendance during implementation and does not leave the mother and child alone. The parameters below are monitored, checked, and recorded. Respiratory status: Watch for labored breathing, retractive breathing, tachypnea, groaning, and apnea. Feeling cold, cyanosis. Vital signs (heart rate, respiratory rate, body temperature, etc.). Behavior of mother and infant during implementation. At the conclusion record vital signs and the infant's condition.	

rate stability, maintaining temperature<sup>6)7)</sup>, and promoting breastfeeding<sup>8)</sup> to neonates. Conversely, the need for cardiopulmonary resuscitation during SSC has also been reported<sup>9)</sup>. Therefore, in 2012, the points to bear in mind in regard to the implementation of "Early Mother-Infant Skin-to-Skin Contact"<sup>2)</sup> in Japan outlined indications and discontinuation criteria. However, no studies have examined the autonomic nervous system (ANS) in neonates depending on SSC implemented in accordance with the indications.

Moreover, indications for SSC<sup>2)</sup> list the following exclusion criteria: preterm infants; a low-birth-weight infant; presence of non-reassuring fetal status; asphyxia neonatorum; and contraindications by the physician, midwife, or nurse (**Table 1**). Conversely, the indications do not mention the presence/

absence of meconium-stained amniotic fluid (MSAF). MSAF is not a rare phenomenon; it is observed in an average of 14 % deliveries<sup>10)</sup>, but in severe cases, it is also associated with respiratory condition at birth. However, no studies have examined ANS during SSC according to the presence/absence of MSAF.

Here in Study A, we examined the effects of SSC according to whether the infants underwent SSC; in Study B, we examined the influence of MSAF by changes in the ANS and physiological indices. In this study, heart rate variability (HRV) analysis, widely applied as an evaluation of ANS<sup>11)12)</sup>, was used.

# **I** Methods

# A Subjects

We obtained informed consent from the mothers of the term infants who underwent normal vaginal

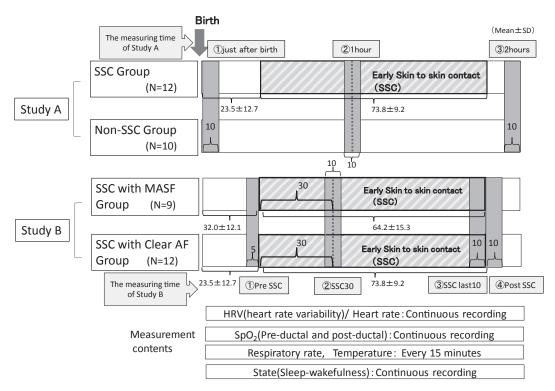


Fig. 1 Study design

deliveries from 2008 to 2011. We included 12 infants with SSC (SSC group) and 10 without SSC (non-SCC group) for Study A and 9 infants with MSAF (SSC with MSAF group) and 12 without MSAF (SSC with clear AF group) for Study B. SSC was promoted in accordance with our hospital implementation criteria, which satisfy the indications published in 2012<sup>2)</sup>. This study was approved by the institutional ethics committee of Shinshu University School of Medicine (approval number 1093).

### **B** Measurement procedures

We designed the protocol for an observational study (**Fig. 1**). We took measurements from term infants born between 8 am and 6 pm, including continuous measurements of HRV, heart rate (HR), oxygen saturation (SpO<sub>2</sub>), and state of sleep-wakefulness for 2h after birth, and measurements of respiratory rate (RR) and body temperature every 15 min. We collected basic infant information, including gestational age, Apgar score, umbilical arterial blood pH, and birth weight, from medical charts.

According to the method of SSC implementation<sup>2)</sup>, it is preferable to commence SSC as early as possible after delivery and perform it for  $\geq 30$  min. In this

study, SSC was performed for 30 min to 2h, and data were collected up to 10 min after SSC completion. For SSC, the naked infant was placed skin-to-skin in the prone position upon the mother's chest in a supine position (maternal upper body angle, approximately 17°). During SSC, the infant's head position turned to one side and was covered with a warmed bath towel, and the mother firmly supported it with both hands. In the non-SSC group, measured data were collected with the infant lying in the supine position in a cot in the delivery room. Observations were performed at room temperature between 24 °C-28 °C and 30 %-40 % humidity.

For Study A, we analyzed the mean of data obtained at three time points, i.e., just after birth (for 10 min after birth), after 1h (for 10 min at 55–65 min after birth), and after 2h (for 10 min at 110–120 min after birth). SSC was commenced at  $23.5 \pm 12.7$  min after birth and was conducted for  $73.8 \pm 9.2$  min. For Study B, we analyzed the mean of data obtained at four time points, i.e., PreSSC (for 5 min prior to commencing SSC), SSC30 (for 10 min at 25–35 min after commencing SSC), SSClast10 (for 10 min prior to completion of SSC), and PostSSC (for 10 min after

Table 2 Infants' Characteristics in Study A

	SSC Group (N = 12)	Non-SSC Group (N = 10)	p-value <sup>†</sup>
Gestational age (weeks)	$39.2 \pm 1.0$	$39.3 \pm 1.4$	N.S.
1-min Apgar score (points)	$8.4 \pm 0.7$	$8.8 \pm 0.4$	N.S.
5-min Apgar score (points)	$9.6 \pm 0.5$	$9.9 \pm 0.3$	N.S.
Umbilical artery blood pH	$7.35 \pm 0.04$	$7.32 \pm 0.07$	N.S.
Neonatal Birth weight (g)	$3,038.2 \pm 315.4$	$3,011.4 \pm 296.9$	N.S.

Data are presented as mean ± SD. † : Student's t-test, N.S.: not significant

completion of SSC). In the SSC with MSAF group, SSC was commenced at  $32.0\pm12.1$  min after birth and was conducted for  $64.2\pm15.3$  min, whereas in the SSC with clear AF group, SSC was commenced at  $23.5\pm12.7$  min after birth and was conducted for  $73.8\pm9.2$  min. No significant differences were found in the times of commencement and durations between the groups.

### C Heart rate variability analysis

HRV serves as an indicator of cardiac autonomic nerve activity by instantaneously measuring variations in the R-R interval per beat 111-131. An Activtracer AC-301A (GMS, Japan) was used for continuously recording the R-R interval, and time series data for 1 min were obtained for power spectral analysis by Memcalc/Tarawa (Suwa Trust, Japan). The analysis was performed with a low frequency (LF) component of 0.04-0.15 Hz and a high frequency (HF) component of 0.15-0.80 Hz<sup>12)</sup> considering the RR of infants. The LF component reflects SNS and PSNS activities, whereas the HF component reflects only PSNS activity. We used the LF/HF as an indicator of SNS activity, and the HF component normalized units (HFnu) calculated by HF/(LF+HF) as an indicator of PSNS activity<sup>13)</sup>.

# D Physiological indices

The HR was measured simultaneously with HRV, and the RR was measured visually for 1 min. Temperature was measured at the infant's forehead using an infrared skin thermometer. SpO<sub>2</sub> was measured using an N-600 pulse oximeter (Nellcor, Covidien). Concerning postnatal circulation <sup>14)</sup>, we measured pre-ductal SpO<sub>2</sub>, reflecting systemic circulation before the ductus arteriosus (sensor attached to the right hand), and post-ductal SpO<sub>2</sub>, after the ductus

arteriosus (right foot), continuously for 2h after birth and used the means of each 1 min for analysis.

### E State of sleep-wakefulness

We analyzed the state of sleep-wakefulness in neonates by the classification of the Brazelton Neonatal Behavioral Assessment Scale<sup>15)</sup>. The scale classifies and evaluates the state of sleep-wakefulness into six stages: state 1 (sleep) to state 6 (crying). Video recordings were used for evaluation, and we calculated the ratio of the state of sleep (states 1–2) for all observation periods.

### F Statistical analysis

Data obtained was statistically analyzed using SPSS21 software. Student's t-test and the  $\chi^2$ -test was used for comparing data between the two groups, and the Bonferroni method was used for multiple comparisons after one factor repeated measures ANOVA. We considered p-values <0.05 to be statistically significant.

### **II** Results

### A Study A: SSC effectiveness

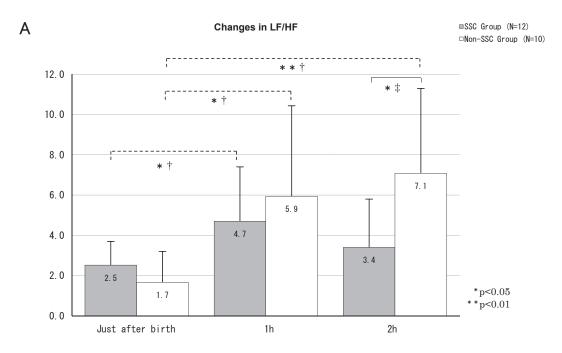
### 1 Infants' background information

**Table 2** shows infants' background information. No differences were found between the two groups in the mean gestational age, Apgar score, umbilical arterial blood pH, and birth weight.

### 2 Changes in heart rate variability

Upon comparing the LF/HF between the two groups, no differences were found just after birth or 1h after birth; however, after 2h, LF/HF was significantly lower in the SSC group than in the non-SSC group (Fig. 2A). Upon examining the shift in LF/HF over the three points in time, the SSC group showed a significant increase in LF/HF only during the

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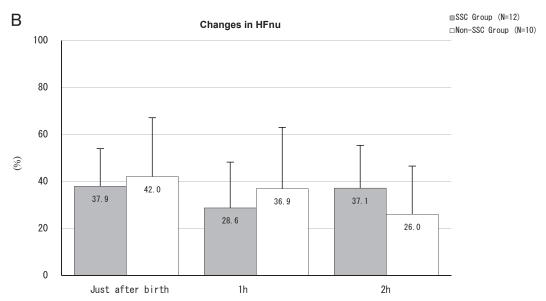


Fig. 2 Changes in heart rate variability for 2h after birth (Study A)
A: Changes in LF/HF B: Changes in HFnu

Data are presented as mean  $\pm$  SD.  $\dagger$  one factor repeated measures ANOVA;  $\ddagger$  Student's t-test Just after birth represents data measured for 10 min after birth, 1h represents data measured for 10 min at 55-65 min after birth, and 2h represents data measured for 10 min at 110-120 minutes after birth.

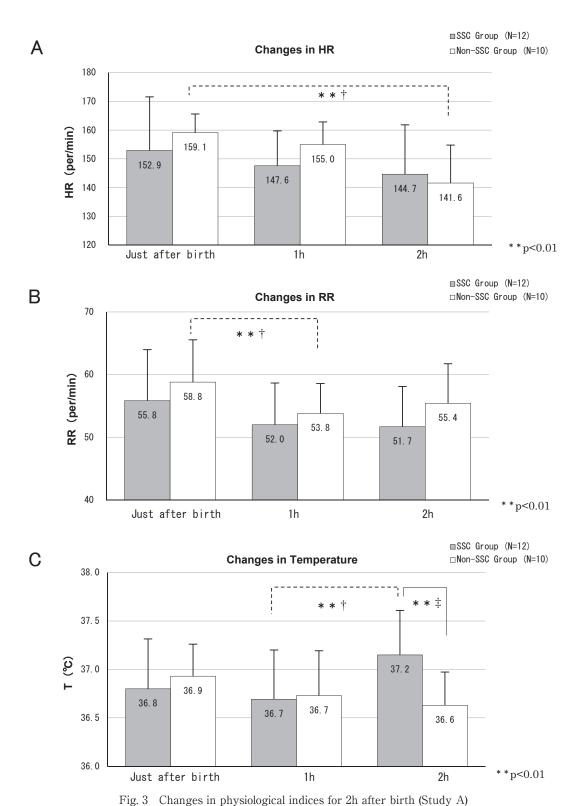
period from just after birth to 1h after birth. On the other hand, the non-SSC group showed significant increases from just after birth to 1h after birth and from just after birth to 2h after birth (**Fig. 2A**).

With regards to HFnu upon comparing the two groups, no significant difference was found at the three time points. Upon examining the shift in HFnu over the three time points, we found no significant changes in either group (Fig. 2B).

# 3 Changes in physiological indices

Upon comparing HR between the two groups, no significant differences were found at the three time points. We found no significant difference in the shift in the HR over the three time points in the SSC group. However, the HR significantly decreased from just after birth to 2h after birth in the non-SSC

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A: Changes in heart rate B: Changes in respiratory rate C: Changes in temperature

Data are presented as mean ± SD. † one factor repeated measures ANOVA; ‡ Student's t-test

Just after birth represents data measured for 10 min after birth, 1h represents data measured for 10 min at 55-65 min after birth, and 2h represents data measured for 10 min at 110-120 minutes after birth.

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group (Fig. 3A).

No significant difference was found in our comparison of RR between the two groups at any of the three time points. Upon examining the shift in RR over the three time points, we found no significant changes in the SSC group, but the RR significantly decreased from just after birth to 1h after birth in the non-SSC group (**Fig. 3B**).

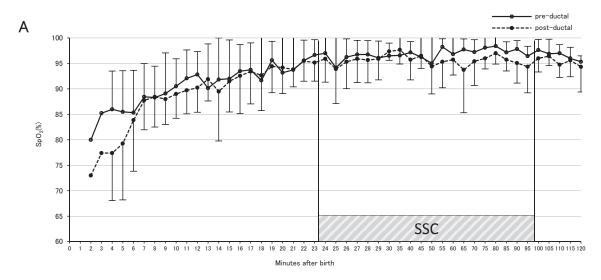
The temperature at 2h after birth was significantly higher in the SSC group than in the non-SSC group. A significant rise was found in the shift in the temperature from 1h to 2h after birth in the SSC group, whereas there was no significant change in the non-SSC group (Fig. 3C).

# 4 Changes in SpO<sub>2</sub>

Pre-ductal SpO<sub>2</sub> reached 90 % after 10 min in the SSC group and after 7 min in the non-SSC group, whereas post-ductal SpO<sub>2</sub> reached 90 % after 11 min in the SSC group and after 12 min in the non-SSC group, with no significant differences between the groups (**Fig. 4A,B**). In addition, SpO<sub>2</sub> shifted almost to >95 % during SSC, and no hypoxia was observed.

### 5 State of sleep-wakefulness

In the SSC group, sleep state accounted for 9.7 % (86 min) during total SSC time (885 min, n=12). In the non-SSC group, we examined the state of sleep during the mean 73.8 min period of SSC after the SSC start time of 23.5 min after birth; sleep state accounted for 1.3 % (9.6 min) during total observa-



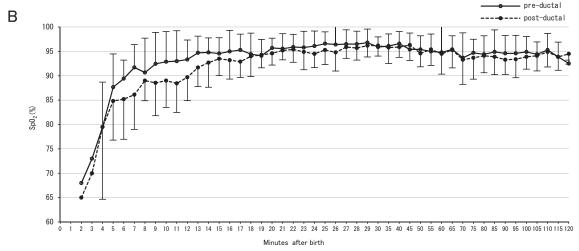


Fig. 4 Neonatal pre- and post-ductal  $SpO_2$  levels during 2h after birth (Study A)  $A:SSC\ Group\ B:non-SSC\ Group$ 

It shows every 1 min for 30 min, and every 5 min for 2h. Data are presented as mean  $\pm$  SD. SSC was commenced at  $23.5 \pm 12.7$  min after birth and was conducted for  $73.8 \pm 9.2$  min.

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Table 3 Infants' Characteristics in Study B

	SSC with MSAF Group (N = 9)	SSC with clear AF Group (N = 12)	p-value <sup>†</sup>
Gestational age (weeks)	$40.2 \pm 0.7$	$39.2 \pm 1.0$	N.S.
1-min Apgar score (points)	$8.7 \pm 0.5$	$8.4 \pm 0.7$	N.S.
5-min Apgar score (points)	$9.2 \pm 0.4$	$9.6 \pm 0.5$	N.S.
Umbilical artery blood pH	$7.37 \pm 0.04$	$7.35 \pm 0.04$	N.S.
Neonatal Birth weight (g)	$3010.9 \pm 342.9$	$3,038.2 \pm 315.4$	N.S.

Data are presented as mean ± SD. † : Student's t-test, N.S.: not significant

tion time (738 min, n = 10), indicating that the SSC group had a significantly higher rate of sleep (p < 0.05).

### B Study B: Influence of MSAF

### 1 Infants' background information

**Table 3** shows infants' background information. No differences were found between the two groups in the mean gestational age, Apgar score, umbilical arterial blood pH, and birth weight.

### 2 Changes in heart rate variability

Upon comparing LF/HF between the two groups, we found no significant differences at all four time points (Fig. 5A). We found no significant differences at all four time points in HFnu between the two groups (Fig. 5B).

# 3 Changes in physiological indices

We found no significant differences at all four time points for the HR (**Fig. 6A**) and RR (**Fig. 6B**) and temperature (**Fig. 6C**) between the two groups.

# 4 Changes in SpO<sub>2</sub>

Pre-ductal SpO<sub>2</sub> reached 90 % after 8 min in the SSC with MSAF group and after 10 min in the SSC with clear AF group, whereas post-ductal SpO<sub>2</sub> reached 90 % after 10 min in the SSC with MSAF group and after 11 min in the SSC with clear AF group, with no significant differences between the groups (Fig. 7A, B). In addition, in both groups, SpO<sub>2</sub> shifted almost to >95 % during SSC, and no hypoxia was observed.

### 5 State of sleep-wakefulness

In the SSC with MSAF group, sleep state accounted for 13.3 % (77 min) during the SSC period (578 min), and in the SSC with clear AF group, sleep state accounted for 9.7 % (86 min) during the SSC

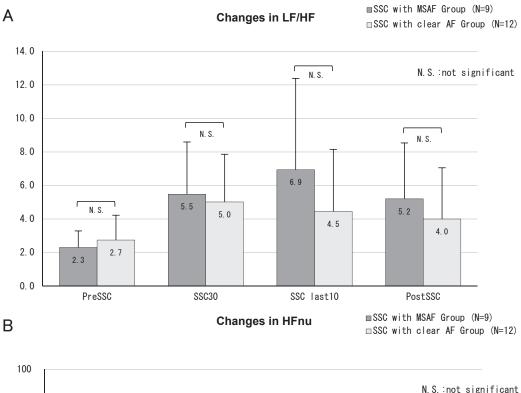
period (885 min), with no significant differences between the groups.

### **IV** Discussion

### A Study A: SSC effectiveness

Comparison of LF/HF between term infants born via normal vaginal delivery in the SSC and non-SSC groups revealed no significant differences, both just after birth and at 1h after birth. At 2h after birth, LF/HF in the SSC group was significantly lower than that in the non-SSC group. In both groups, LF/ HF at 1h after birth was significantly higher than the respective LF/HF just after birth; however, it decreased somewhat in the SSC group from 1-2 h after birth, whereas the non-SSC group increased slightly during this period. Our results suggest that the SNS activity is accelerated at 1h after birth regardless of whether SSC is performed, whereas performing SSC at 2h after birth suppressed SNS activity. This may be attributable to the fact that the SSC group infants were significantly more often in a sleep state. Results pertaining to the non-SSC group are similar to those reported from a previous study<sup>16)</sup>. It is generally thought that the ANS predominantly acts via the PSNS throughout the fetal/ neonatal period<sup>17)</sup>; however, in infants just after birth, the SNS predominantly acts to maintain homeostasis during adaptation to the changing environments.

Conversely, no significant differences with respect to HFnu were observed between the SSC and non-SSC groups as well as in either of the groups over time. Our results do not completely contradict the LF/HF expected based on the relationship of



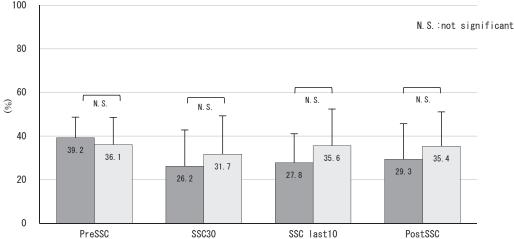


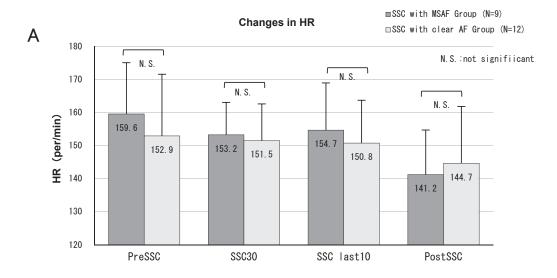
Fig. 5 Changes in heart rate variability for PreSSC, SSC30, SSClast10, and PostSSC (Study B) A: Changes in LF/HF B: Changes in HFnu Data are presented as mean  $\pm$  SD.

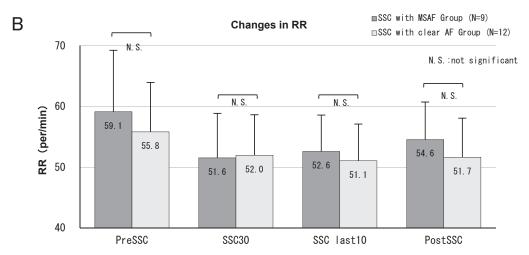
PreSSC represents data measured for 5 min prior to commencing SSC, SSC30 represents data measured for 10 min at 25–30 min after commencing SSC, SSClast10 represents data measured for 10 min prior to completion of SSC, PostSSC represents data measured for 10 min after completion of SSC.

opposing control of SNS and PSNS activity. We may not have detected a clear opposing relationship because the period just after birth requires adaptation to extrauterine life and the ANS is unstable until approximately 3 days after birth<sup>18)</sup>. However, we were unable to clarify whether this result was due to ANS instability just after birth or some other mechanism. Therefore, this appears to be a limitation of this study that needs to be investigated in future

studies.

Among the physiological indices, no difference was observed in the HR and RR according to the presence/absence of SSC; however, at 2h after birth, temperature was significantly higher in the SSC group than in the non-SSC group. Moreover, in the SSC group, the temperature significantly increased from 1h to 2h after birth. This result is comparable with previous results<sup>6)7)</sup>; thus, the temperature-





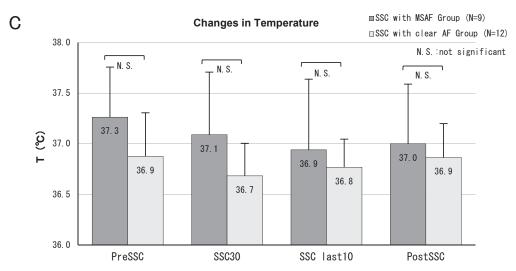
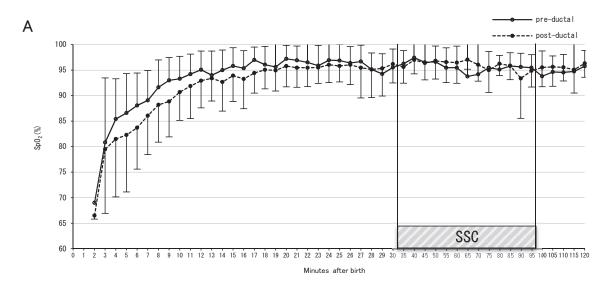


Fig. 6 Changes in physiological indices for PreSSC, SSC30, SSClast10, and PostSSC (Study B)
A: Changes in heart rate B: Changes in respiratory rate C: Changes in temperature

Data are presented as mean ± SD.

PreSSC represents data measured for 5 min prior to commencing SSC, SSC30 represents data measured for 10 min at 25–30 min after commencing SSC, SSClast10 represents data measured for 10 min prior to completion of SSC, PostSSC represents data measured for 10 min after completion of SSC.

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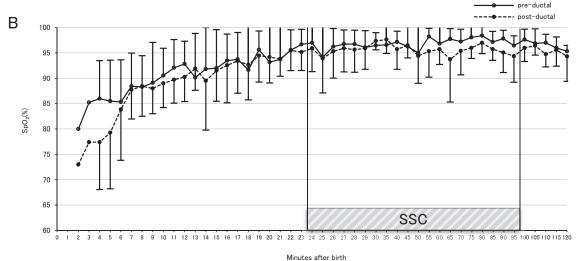


Fig. 7 Neonatal pre- and post-ductal SpO $_2$  levels during 2h after birth (Study B) A:SSC with MSAF Group B:SSC with clear AF Group It shows every 1 min for 30 min, and every 5 min for 2h. Data are presented as mean  $\pm$  SD. SSC was commenced at  $32.0 \pm 12.1(A)$ ;  $23.5 \pm 12.7(B)$  min after birth and was conducted for  $64.2 \pm 15.3$  (A);  $73.8 \pm 9.2(B)$  min

raising effect of SSC was confirmed in this study. The accurate temperature of infants is approximately 37  $^{\circ}$ C  $^{19)}$ , and SSC may help in maintaining this temperature.

In the SSC group, SSC was commenced after  $SpO_2$  reached at least 90 %; however, except for one moment during SSC,  $SpO_2$  shifted to within the normal range of > 95 %. Although early postpartum mother-infant separation has been reported to interfere with arterial blood oxygenation<sup>20)</sup>, we found no such indication in our study. Moreover, preductal  $SpO_2$  reached 90 % after 10 min in the SSC group and after 7 min in the non-SSC group, with

no significant difference between the groups. This result is comparable with that of a report<sup>21)</sup> indicating that in infants who do not require resuscitation, 10 min is required to reach 90 % oxygenation (meeting the target time for  $SpO_2$  to reach >90 % in neonatal cardiopulmonary resuscitation). Moreover, the time taken for  $SpO_2$  to reach 90 % was slower for post-ductal than for pre-ductal, irrespective of the presence/absence of SSC, and postpartum observation while monitoring pre-ductal  $SpO_2$  is important.

SSC increases the state of sleep in infants, suppresses SNS activity 2h after birth, and increases temperature. The results indicated that SSC is effective.

tive in inducing a state of rest at birth and in preventing hypothermia.

### B Study B: Influence of MSAF

Complications in the event of severe MSAF include non-reassuring fetal status, meconium aspiration syndrome, and persistent pulmonary hypertension of the newborn (PPHN)<sup>22)</sup>. A report indicated that when abnormalities are observed on fetal heart rate monitoring and MSAF occur simultaneously, the number of infants with acidosis and who require resuscitation increases<sup>23)</sup>. In our study, we examined changes in ANS and physiological indices according to the presence/absence of MSAF in term infants who received SSC following vaginal delivery. The mean time to SSC commencement was not significantly different according to the presence/absence of MSAF, and therefore, even in the presence of MSAF, if the indications are satisfied, SSC can be commenced at the same time as in the absence of MSAF.

With regards to the ANS, for both LF/HF and HFnu, no significant differences were found at any of the time points according to the presence/absence of MSAF. Therefore, when the indications for SSC are satisfied, changes in ANS are comparable in the presence/absence of MSAF.

We observed no significant differences according to the presence/absence of MSAF in the physiological indices (HR, RR, and temperature), and we found no significant differences in the shifts in these indices from prior to commencing SSC to after having completed SSC. Accordingly, our results indicated that if SSC is commenced after careful observation until the infant satisfies the indications, the physio-

logical indices are stable during SSC, irrespective of the presence /absence of MSAF.

Next, upon examining SpO<sub>2</sub>, no significant difference was found in the time taken for the pre-ductal SpO<sub>2</sub> to reach 90 % according to the presence/absence of MSAF. Moreover, SpO<sub>2</sub> during SSC shifted to almost >95 % in most neonates irrespective of the presence/absence of MSAF, suggesting that postpartum oxygenation does not differ according to the presence/absence of MSAF. Moreover, no significant difference was found in Apgar scores or umbilical arterial blood pH according to the presence/absence of MSAF.

Therefore, when performing SSC, it is still important to monitor the neonate with an attached pulse oximeter and medical staff. In addition, for early detection of PPHN, monitoring of the SpO<sub>2</sub> of the preand post-ductal is essential.

SSC increases the success rate of breastfeeding and promotes parent-infant bonding. Monitoring and careful observation are essential for safely implementing SSC with the recognition of postpartum instability in an infant, in accordance with the "Points to Bear in Mind in Regards to the Implementation of Early Mother-Infant Skin-to-Skin Contact."

### Acknowledgements

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### **Conflict of Interest**

There are no conflicts of interest to declare.

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