

**Sympathetic Nervous System Activity and Pain-Related Response Indexed by Electrodermal Activity during the Earliest Postnatal Life in Healthy Term Neonates**

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**Short title: Electrodermal Activity during the Earliest Postnatal Life**

## Summary

Sympathetic nervous system (SNS) undergoes a prolonged period of fetal and neonatal development and maturation during which is vulnerable to a variety of influences (e.g. painful experiences). Thus, we aimed to evaluate SNS activity at rest and in response to stressful stimulus (pain) within the earliest postnatal life in healthy term neonates using electrodermal activity (EDA) measures. In twenty eutrophic healthy term neonates EDA was recorded within the first two hours after birth (measurement 1 – M1) and 72 hours after birth (measurement 2 – M2) at rest and in response to pain (M1 – intramuscular K vitamin administration; M2 – heel stick). Evaluated parameters were skin conductance level (SCL), non – specific skin conductance responses (NS.SCRs), skin SCL 10 s before pain stimulus (SCL\_10 before pain), skin conductance response (SCR) peak after pain stimulus, SCL 10 s after pain stimulus (SCL\_10 after pain), SCR magnitude, latency, SCR rise/decline time, SCR half recovery time. SCL was significantly decreased at rest during M2 compared to M1 ( $p=0.010$ ). SCL\_10 before pain, SCR peak after pain, and SCL\_10 after pain stimulus were significantly decreased in M2 compared to M1 ( $p=0.014$ ,  $p=0.020$ ,  $p=0.011$ , respectively). SCL was significantly decreased and NS.SCRs were significantly higher in the recovery period after the pain stimulus during M2 compared to M1 ( $p=0.015$ ,  $p=0.032$ , respectively). Our results indicate EDA parameters sensitive to detect sympathetic changes during the earliest postnatal life reflecting its potential in early diagnosis of the autonomic maturation – linked pathological states in neonates.

**Keywords:** Autonomic nervous system, Sympathetic nervous system, Electrodermal activity, Neonates, Pain assessment

## **Introduction**

The autonomic nervous system (ANS) consisting of two branches, sympathetic (SNS) and parasympathetic nervous system (PNS) is extremely important for the proper functioning of the human body and is essential in adequate fetal to neonatal transition, which represents the primary challenge of postnatal life. More specifically, at birth, neural systems including ANS centres in brainstem supported by neuroendocrine changes (predominantly catecholamine levels increase) provides more sustained support to SNS to enable brisk adaptation to postnatal life. Importantly, ANS undergoes a prolonged period of fetal and neonatal development and maturation. In general, the development and maturation of ANS is significant within the second half of pregnancy and requires at least 37 gestational weeks to reach their accurate functioning. While SNS develops steadily throughout the gestation, PNS shows accelerated development between 25 – 32 weeks and also between 36 – 38 weeks of the gestation [1,2]. ANS continues to mature after birth with PNS modulation taking predominance in term neonates [3,4]. The normal increase in PNS activity has been repeatedly evidenced by the evaluation of heart rate variability (HRV), especially by the increased HRV in high – frequency band of spectral analysis in infants born at term [5–7]. However, little is known about the earliest postnatal changes of SNS activity. In this context, electrodermal activity (EDA) also known as skin conductance (SC) represents a useful parameter reflecting the changes in the sympathetic branch of ANS [8]. The measurement of EDA has begun over 100 years ago and refers to all active and passive electrical phenomena in the skin. In other words, SC measures the changes of the skin electrical conductivity due to the activity of eccrine sweat glands which is under the pure control of sympathetic cholinergic nervous system [9]. Moreover, in psychophysiological research EDA is commonly used to index the general states of arousal [10]. In this context, EDA measurement can be used to assess the arousal – linked SNS activity changes during early postnatal life in neonates. To the best of our knowledge, there are no studies concerning solely the issue of SNS maturation within the first postnatal days including immediate postnatal adaptation during 2 hours after birth. Thus, we have focused on evaluation of SNS activity indexed by EDA in healthy term neonates during this earliest postnatal period.

Further, neonates, especially those admitted to intensive care units, commonly undergo many repeated pain experiences (e.g. medically – necessary, often invasive procedures) [11,12]. It is well-known that repeated pain stimulation during the critical period of early life can influence the development of central nervous system in the way of neurocognitive, behavioural, social or emotional impairments [13]. Importantly, pain – related changes are preventable with

appropriate analgesia [14]. Therefore, an appropriate pain management (pain assessment as well as treatment) remains crucial in the prevention of short – and long – term consequences of early life pain. However, to date, no gold standard has been identified for accurate pain assessment in neonates [15]. In this context, physiological measurements such as EDA evaluation can represent potential objective reliable tool for pain discrimination as the pain experience evokes emotional sweating leading to SC increase most evident on palms or soles [16,17]. However, despite relatively good availability of EDA devices, there is still little information regarding pain stimulus – evoked EDA responses in term neonates in the critical earliest postnatal development. Thus, we also aimed to evaluate the postnatal pain-evoked sympathetic changes using EDA evaluation in healthy term neonates.

## **Methods**

### *Participants*

The study included 20 healthy term neonates born spontaneously (10 males, average gestational age:  $39.3 \pm 1.4$  weeks, average birth weight:  $3488.42 \pm 519.95$  g). The inclusion criteria were 38 – 42 weeks of gestational age at birth and physiological immediate postnatal adaptation without need for ventilation support in the delivery room (Apgar score  $\geq 8$  in the first minute and  $\geq 9$  in the fifth minute after birth). The exclusion criteria included all conditions that are proven or very likely associated with abnormal autonomic function: pathological gravidity (preterm birth, preeclampsia, intrauterine growth restriction, etc.), maternal systemic disease (including gestational and other types of diabetes), smoking during pregnancy, congenital anomaly of the neonate, signs of perinatal infection, hypoglycaemia and hyperbilirubinemia requiring phototherapy during hospitalization.

This study presents preliminary data of our prospective observational study „Assessment of Autonomic Nervous Regulation During Postnatal Period in Neonates“, registered in Clinical Trials database (NCT03830424). The study was performed from January 2018 to December 2020 at the Department of Neonatology in University Hospital in Martin and was approved by the Ethical Committee of Jessenius Faculty of Medicine in Martin, Comenius University in Bratislava (EK69/2018). All procedures performed in our study were in accordance with the 2000 Helsinki declaration of the world Medical Association. Subjects were included after meeting the inclusion criteria and with approval of their parents after signing the informed consent. All parents were carefully informed about study protocol.

### *Protocol*

The examination was performed at the Department of Neonatology in University Hospital Martin under standard conditions with the minimization of internal and external stimuli, i.e. in quiet room, warm environment (26°C), air humidity around 50%, during the day between 7 am – 12 am. Two silver – silver chloride bipolar electrodes with sampling rate 256 Hz placed on the sole of the neonate were used for the continuous EDA recording (FlexComp Infinity Biofeedback, Thought Technology Ltd., Canada) according to the recommendation of EDA biosignal measurements [18]. Neonates were all bathed with water and baby liquid washing product approximately 30 min after birth to avoid the possible influence of vernix caseosa after birth on the EDA signal. Thus, the skin surface under the EDA electrodes was clean and dry during the examination. The examination protocol consisted of two EDA measurements: measurement 1 (M1) performed within two hours after birth and measurement 2 (M2) after 72 hours of life at rest and in response to the pain stimulus which was applied as the part of routine neonatal care – intramuscular administration of K vitamin (M1) and heel stick (M2). No analgesia was administered to the subjects during the procedures. The time interval for EDA measurement was 6 minutes at rest and 6 minutes after application of pain stimulus. Recordings were initiated after the stabilization of the subjects (approximately 10 minutes – adaptation interval) during sleep or quiet alertness in the supine position in an incubator (M1) or neonatal cot (M2) under the same temperature conditions.

### *Data analysis*

EDA raw records were carefully checked, and rare artefacts were manually removed for exact data analysis. The tonic EDA component was extracted by the 10<sup>th</sup> order low-pass finite impulse response filter [19]. Furthermore, the skin conductance level (SCL, microSiemens ( $\mu\text{S}$ )) – an index of tonic level of skin electrical conductivity, was evaluated as the average amplitude of the tonic EDA from 5 minutes of artefact – free recordings. Consequently, the non – specific skin conductance responses (NS.SCRs) were evaluated as a rate of spontaneous skin conductance response (SCR) waves without external stimuli reflecting momentary arousal with fixed threshold (0.05  $\mu\text{S}$ ) [20]. SCL and NS.SCRs were computed at rest and during recovery period after the pain stimulus. Further, the SC properties in response to the pain stimulus were evaluated using the following indices: SCL<sub>10 before</sub> ( $\mu\text{S}$ ) – SCL value 10 s before the application of the pain stimulus, latency (s) – temporal distance between the pain stimulus and the initiation of the response (typical range is between 1 and 5 s), SCR peak after pain ( $\mu\text{S}$ ) –

maximal or minimal value after the pain stimulus, SCR magnitude ( $\mu\text{S}$ ) – absolute difference between the peak of the stimulus response and the onset of SCR rise or decline (minimum threshold magnitude is  $\pm 0.0001 \mu\text{S}$ ), SCR rise/decline time (s) – temporal distance between the initiation and the peak value, SCR half recovery time (s) – temporal distance between SCR peak after pain and the reduction of SCR to half of the amplitude, and SCL\_10 after – SCL value 10 s after the application of the pain stimulus.

### *Statistical analysis*

Statistical analysis was performed in jamovi 1.6.9 (Sydney, Australia). The data normality (Gaussian/non – Gaussian distributions) was evaluated by Shapiro – Wilk normality test. All analysed data were not normally distributed. Consequently, the Wilcoxon rank test was used for EDA – SCL, NS.SCRs, and EDA parameters related to pain stimulus – comparisons between M1 and M2. Non-parametric ANOVA (Friedman test) with Durbin – Conover post – hoc test was used for comparisons of SCL at rest, SCL\_10 before pain, SCR peak after pain, SCL\_10 after pain, and SCL in the recovery during M1 and M2 measurement. EDA parameters were expressed as median and interquartile range. A value of  $p < 0.05$  (two – tailed) was considered statistically significant.

## **Results**

### *Comparison of SCL and NS.SCRs at rest*

SCL was significantly decreased during M2 compared to M1 ( $p=0.010$ ). No significant changes were found between resting measurements of the index NS.SCRs ( $p=0.850$ ). The significant result is summarized in Figure 1.

### *Comparison of EDA parameters during the pain stimulus application*

SCL\_10 before pain, SCR peak after pain, and SCL\_10 after pain were significantly decreased during M2 compared to M1 ( $p=0.014$ ,  $p=0.020$ ,  $p=0.011$ , respectively). The other parameters were without significant changes between M1 and M2. The significant results are summarized in Figure 2.

### *Comparison of SCL and NS.SCRs in the recovery period after the pain stimulus*

SCL was significantly decreased in the recovery period after the pain stimulus during M2 compared to M1 ( $p=0.015$ ). Index NS.SCRs was significantly higher in the recovery period during M2 compared to M1 ( $p=0.032$ ). The significant results are summarized in Figure 3.

### *Comparison of the selected EDA parameters during M1 and M2 measurements*

No significant changes were found between rest SCL, SCL\_10 before pain, SCR peak after pain, SCL\_10 after pain, and recovery SCL in neonates during M1 neither M2 measurement.

### **Discussion**

The major findings of this study regarding EDA evaluation during early postnatal life in term neonates are following: 1) SCL was higher within the first two hours after birth associated with significant SCL decreasing after 72 hours of life reflecting a post – delivery stress-related sympathetic withdrawal after postnatal adaptation, 2) in response to pain, SCL\_10 before pain, SCR peak after pain, and SCL\_10 after pain were significantly higher during the first compared to the second measurement. However, it is important to note that this significant difference can be strongly influenced by the SCL baseline level (2 hours compared to 72 hours after birth). In this context, no significant differences were found between SC properties in response to the pain stimulus during either first and second measure indicating these pain-related procedures as not sufficiently aroused conditions for neonates to evoke immediate stimulus-related sympathetic alterations, however 3) NS.SCRs were significantly higher in the recovery period after the pain stimulus during the second compared to the first measurement reflecting increase of stress response with the number of painful procedures. Based on our results we suggest that EDA measurement may represents a sensitive tool to determine the developmental/maturational changes in SNS during the earliest postnatal life.

Birth, i.e. transition from fetal to neonatal physiology, represents the first major life challenge associated with dynamic physiological changes, in which ANS plays a crucial role [21,22]. ANS develops in the fetal period during the development of the central nervous system and continues to mature after birth [22,23]. More specifically, the sympathetic branch of ANS matures steadily throughout the gestation and the parasympathetic division of ANS shows accelerated maturation between 25 – 32 weeks and also between 36 – 38 weeks of the gestation [2,24,25]. Before birth, the SNS outflow from central ANS structures (i.e. structures involved in the central autonomic network (CAN) [26] such as hypothalamus and forebrain) supported by neuroendocrine signalling (e.g. catecholamines) increases to enable the brisk and efficient postnatal adaptation [22]. At birth, the connectivity between higher cortical networks as well as between cortical centres and brainstem structures involved in ANS control increases [27] allowing appropriate SNS functioning. After birth, ANS constantly continues to develop with a predominant increase in the parasympathetic activity during the following two years [3,4]

important for the proper regulation of increasing social interactions the infant encounters [23]. Contrary, the precise understanding of SNS development in the earliest postnatal life is limited [28]. In this context, EDA evaluation represents a non-invasive marker indexing sympathetic cholinergic activity that measures the changes in the activity of eccrine sweat glands resulting in skin conductance changes [20,29]. Moreover, EDA can reflect the nervous system readiness as well as emotional/arousal state in neonates [30]. Thus, EDA evaluation in terms of spontaneous skin conductance changes associated with arousal may represent an optimal tool to investigate SNS development/maturation. From this perspective, peripheral SNS is developed at 18 week of gestation, still continues to mature after birth during first weeks of life [30]. Similarly, the palmar and plantar sweat glands are developed in the second trimester, however, there are inconsistent findings regarding their full maturation and normal functioning. While Harpin and Rutter [31] reported functional emotional sweating occurrence in term neonates, Joergensen et al. [32] suggested that eccrine sweat glands are fully matured and functional even after two postnatal weeks. Our study of the highest SCL during two hours compared to 72 hours after birth confirms the functionality of eccrine sweat glands in term neonates immediately after birth. These findings can provide novel insights into the SNS regulatory mechanisms immediately after birth and during the earliest postnatal life.

Further, neonates are commonly exposed to harmful stimuli from birth, through routine heel lancing for screening tests to repeated invasive procedures in intensive care units [11]. It is widely reported that often recurring stressful and painful stimuli in neonates can result in detrimental short – term (e.g. disruption of the postnatal adaptation or of the development of parent-infant bonding) as well as long – term (e.g. altered sensitivity to pain, worsen psychological outcomes) effects on health [14,33]. As many injury – related changes can be preventable with the proper anaesthetics administration [14], the understanding of the nociceptive system development to pain stimuli is crucial in the neonatal pain management. From the developmental perspective, the somatosensory system undergoes postnatal maturation at peripheral and central levels immediately after birth. Although most areas involved in pain processing are developed around birth, and thus the term neonate's brain should be able to process noxious information [34], when exactly the neonate brain is capable to integrate nociceptive information into a painful experience is still unknown. Nociception is transformed into pain in the complex cortical network including the frontal, parietal, somatosensory, insular, and cingulate cortices [35]. Transformation of the nociception into conscious pain perception has some similarities in infants and adults. While noxious mechanical stimuli evoke similar hemodynamic response in the somatosensory, anterior cingulate cortex, and insular cortex in



both adults and children, the painful stimulus increases hemodynamic response in amygdala and orbitofrontal cortex (regions thought to be involved in sensory stimuli discrimination and identification) only in adults [36] reflecting thus an immature ability to process specific somatosensory input in neonates. On the other hand, additional brain regions such as auditory cortex, caudate, and hippocampus respond to noxious stimuli only in neonates. Importantly, these brain areas can be responsible for encoding and retention of the pain stimulus memories in infant brain [14,36–38]. In addition, excitatory and inhibitory receptive fields in the spinal cord are less “aligned” in neonates compared to adults. Diffuse and large receptive fields are likely associated with poorer discrimination between noxious and non – noxious stimuli as well as poorer spatial localization [39]. Descending inhibition is functionally immature during the first postnatal weeks [40]. More specifically, while in adults the serotonergic projections as a major source of descending control facilitating or inhibiting nociception depend on the state of pain [41], in neonates, the serotonergic descending system facilitates nociceptive and tactile processing. During the first postnatal weeks, there is a maturation – related switch from facilitation to predominant inhibition of nociception [42]. Altogether, as somatosensory neural circuits undergo early postnatal maturation, are highly susceptible to injuries during this critical life period. Thus, the appropriate pain management is very important to prevent the short – term and long – term consequences of early life pain on the nociceptive system development/functioning. Successful pain prevention and treatment should be preceded by a reliable pain assessment. However, as neonates are unable to quantify their pain by self-report, the exact pain assessment in neonates is still challenging for clinicians as well as researchers due to the lack of “gold standard”. The majority of the developed tools in the pain assessment encompass behavioural (e.g. facial expression, body movements, cry qualities) and physiological (e.g. heart rate, blood pressure) responses or combine both into a composite measure. In this context, EDA measurement based on the SNS response to stress could represent a valuable tool to assess the pain as the pain occurrence induces stress – related sympathetic excitation and emotional sweating at palmar or plantar areas resulting in increasing of skin conductance monitored by EDA devices. In addition, EDA measurement is non – invasive, safe, easy to handle method only little influenced by other factors (e.g. circulatory changes) and EDA scores can be also easily understood by clinicians. However, relatively little research examining pain stimulus-related EDA changes exist in the period of the earliest postnatal development [43,44]. Our study revealed no significant changes in the selected pain stimulus – related EDA parameters during M1 neither M2 measurement reflecting EDA evaluation as not appropriate objective tool for pain assessment. However, EDA parameter NS.SCRs (i.e. useful measure of

arousal) was significantly higher in the recovery period after the secondly applied pain stimulus during the second measurement 72 hours after birth compared to firstly applied pain stimulus during the first measurement 2 hours after birth indicating increased stress response after repeated pain stimulus. This finding is in accordance with the study by Storm [45] that revealed the increase of stress response with the number of heel lancing. These findings can suggest that neonates remember pain. However, further research in this area is needed to precisely elucidate this suggestion.

## **Conclusion**

Our study revealed the highest sympathetic activity indexed by EDA early after birth (two postnatal hours) with its significant decrease after 72 hours of postnatal life indicating the post – delivery stress withdrawal linked to appropriate postnatal adaptation in healthy term neonates. EDA evaluation can represent a sensitive tool to detect sympathetic changes during the earliest postnatal life reflecting its potential in early diagnosis of the autonomic maturation – linked pathological states in neonates.

## **Study limitations**

The sleep stage in our subjects during measurements was not specified. We have focused on EDA evaluation in neonates in this study reflecting only the activity of sympathetic branch of ANS. In this context, simultaneous HRV evaluation (mainly in high frequency band) reflecting activity of the parasympathetic branch of ANS could bring more information about complex ANS changes during the earliest postnatal life. Further, vaginal delivery evokes a dramatic surge in vasopressin which has been shown to exert analgesic effects, thus it can influence the neonatal pain responsivity during the first hours after birth. Moreover, regarding the most precise pain assessment in neonates, the multidimensional assessment (e.g. simultaneous monitoring of several physiological parameters in association with behavioural assessment) of responses during and after pain stimuli is nowadays highly recommended. Thus, future research should take this into consideration.

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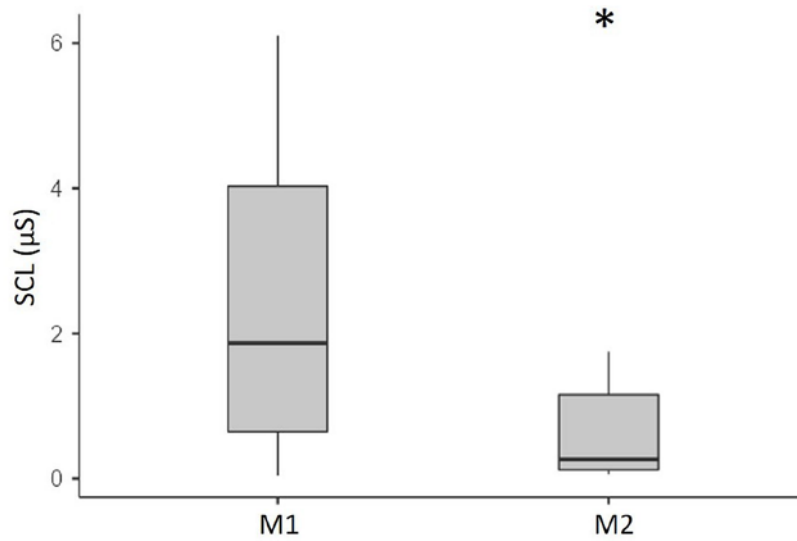


Figure 1. Boxplots of skin conductance level (SCL,  $\mu\text{S}$ ) during measurement within first two hours after birth (M1) and measurement after 72 hours after birth (M2) at rest in newborns. \* represents statistically significant difference ( $p < 0.05$ ) between M1 and M2.

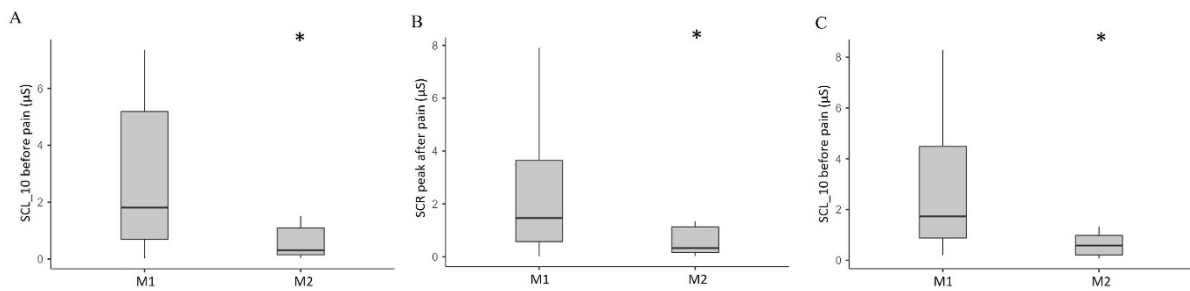


Figure 2. Boxplots of EDA parameters of pain stimulus during measurement within first two hours after birth (M1) and measurement after 72 hours after birth (M2) in newborns. A) SCL<sub>10</sub> before pain – skin conductance level 10 s before the application of the pain stimulus; B) SCR peak after pain – skin conductance response peak after the pain stimulus; C) SCL<sub>10</sub> after pain – skin conductance level 10 s after the application of the pain stimulus. \* represents statistically significant differences ( $p < 0.05$ ) between M1 and M2.

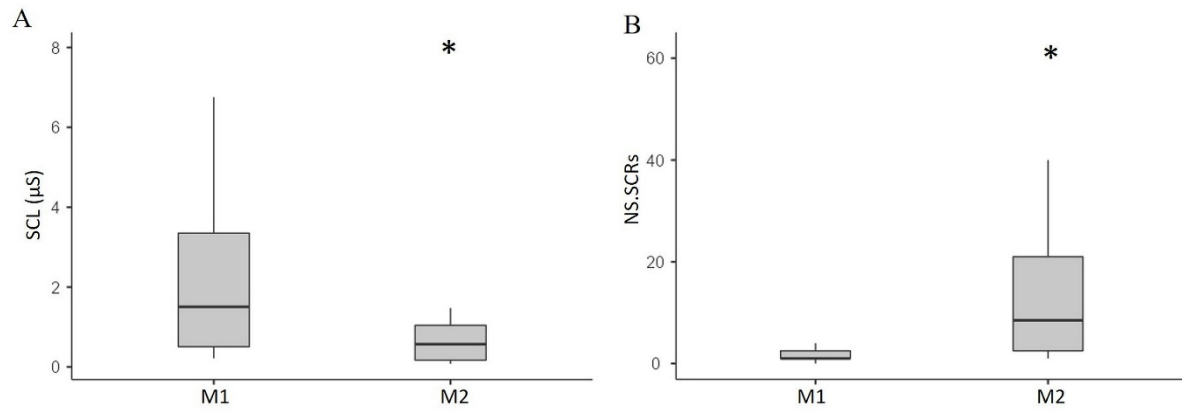


Figure 3. Boxplots of A) skin conductance level (SCL,  $\mu\text{S}$ ), and B) non – specific skin conductance responses (NS.SCRs) during measurement within first two hours after birth (M1) and measurement after 72 hours after birth (M2) during recovery in newborns. \* represents statistically significant differences ( $p < 0.05$ ) between M1 and M2.