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Topical Sheep Oil and Pain Intensity and Crying Duration Following Pentavalent Vaccination in Two-Month-Old Infants: A Quasi-Experimental Study

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Abstract

Background: Vaccination is essential for preventing infectious diseases, but injection-related pain is a common painful experience in infancy. This study evaluated the association of topical sheep oil with pain intensity and crying duration during pentavalent vaccination in two-month-old infants.

Methods: In this quasi-experimental study, 72 healthy two-month-old infants were recruited via convenience sampling and nonrandomly assigned to intervention (n = 36) and control (n = 36) groups. Purified sheep oil (2.5 mL) was applied over the vastus lateralis muscle 60 minutes before vaccination. Pain was assessed using the Modified Behavioral Pain Scale before, during, and after vaccination, and crying duration was measured using a digital stopwatch.

Results: Baseline characteristics were comparable between groups (all $P > 0.05$). Pain scores were lower in the sheep oil group during vaccination ($P = 0.024$) and 15 seconds after vaccination ($P < 0.001$), with a significant time × group interaction ($P < 0.001$). Crying duration was 15 (13–17) seconds in the intervention group versus 18.5 (15–20) seconds in the control group ($P = 0.050$).

Conclusion: Topical sheep oil was associated with lower pain intensity and shorter crying duration after pentavalent vaccination. Given the non-randomized design, randomized controlled trials are needed to confirm these findings.

Key Words: Complementary medicine; Infant; Pentavalent vaccine; Procedural pain; Sheep oil; Vaccine Methods.

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1- INTRODUCTION

Vaccination is among the most successful and cost-effective public health interventions, substantially reducing childhood morbidity and mortality from vaccine-preventable diseases worldwide. Despite these remarkable achievements, pain associated with vaccine injections remains one of the earliest and most frequent painful experiences in infancy. Although transient, vaccination pain can trigger immediate physiological and behavioral stress responses and may negatively affect both infants and their caregivers (1).

Accumulating evidence indicates that painful vaccination experiences contribute to parental anxiety, increase fear of future injections (2), and may reduce adherence to recommended immunization schedules. It has been estimated that pain-related fear and distress contribute to vaccine hesitancy in approximately 8–28% of children (3). Furthermore, combination vaccines, particularly the pentavalent vaccine, have been reported to produce greater pain intensity than other childhood vaccines (4).

Pain experienced during early infancy is not merely an unpleasant transient event. Repeated exposure to untreated procedural pain has been associated with alterations in pain sensitivity, stress regulation, neurodevelopment, and behavioral outcomes later in life (5). In preterm infants, repeated painful procedures may cause physiological instability and adversely affect neurodevelopment. Pain and stress may also influence cerebral hemodynamics and have been associated with an increased risk of intraventricular hemorrhage (IVH), a major complication of prematurity linked to increased morbidity, mortality, and adverse neurodevelopmental outcomes. Therefore, effective management of procedural pain is essential in neonatal care (6).

Consequently, effective pain management during routine vaccination has become an essential component of family-centered pediatric care (7).

Current evidence supports the use of both pharmacological and non-pharmacological approaches for reducing vaccination-related pain (8). Pharmacological interventions, including topical local anesthetics such as lidocaine–prilocaine cream, have demonstrated analgesic efficacy but may be associated with limitations (9), including delayed onset of action, local skin reactions, systemic absorption, methemoglobinemia in susceptible infants, and potential drug interactions (10). These agents increase healthcare costs and are not always readily available in primary healthcare settings (11).

In contrast, non-pharmacological interventions are inexpensive, simple to administer, and generally associated with minimal adverse effects. Breastfeeding, oral sucrose, skin-to-skin contact, tactile stimulation, distraction techniques, and optimized injection procedures have all demonstrated varying degrees of effectiveness in reducing procedural pain during childhood immunization (11). Most of these interventions are believed to exert their analgesic effects through sensory modulation mechanisms described by the Gate Control Theory of Pain and through activation of endogenous pain-inhibitory pathways (12).

In recent years, increasing attention has been directed toward complementary and traditional therapies for pain management. Natural oils derived from plants and animals have demonstrated anti-inflammatory, antioxidant, moisturizing, and wound-healing properties that may contribute to pain reduction following tissue injury. Among these traditional remedies, topical sheep oil has long been used in several regions of Iran for the management of burns, musculoskeletal

pain, skin injuries, and delayed wound healing (13). Sheep oil contains a variety of biologically active compounds, including saturated and unsaturated fatty acids, particularly oleic and linoleic acids, together with fat-soluble vitamins such as vitamins A, D, E, and K. Experimental evidence suggests that these bioactive constituents may suppress inflammatory mediators, reduce oxidative stress, enhance tissue repair, and improve local microcirculation, thereby potentially attenuating nociceptive responses following tissue trauma (12).

Despite its traditional use, evidence regarding the analgesic effects of topical sheep oil remains limited. To our knowledge, its potential role in reducing vaccination-related pain in infants has not been clinically evaluated. Therefore, this study aimed to assess the association of topical sheep oil with pain intensity and crying duration during pentavalent vaccination in two-month-old infants.

2- MATERIALS AND METHODS

This quasi-experimental study was conducted between September 2024 and September 2025 at a Comprehensive Health Service Center in Rafsanjan, southeastern Iran. The study investigated the association between topical sheep oil application one hour before vaccination and procedural pain intensity and post-vaccination crying duration in healthy two-month-old infants receiving routine pentavalent vaccination.

Healthy two-month-old infants who attended the health center for their scheduled first dose of pentavalent vaccination were recruited using convenience sampling. Written informed consent was obtained from the parents or legal guardians before enrollment.

Infants were eligible if they were two months of age and scheduled to receive their first dose of the pentavalent vaccine; had no history of chronic pain or serious medical conditions; had no skin lesions, dermatitis, or infection at the intended application or injection site; had not received any pharmacological or non-pharmacological pain-relieving intervention before vaccination; and had normal birth weight and physical growth appropriate for age.

Infants were excluded if parental consent was withdrawn; a severe adverse reaction occurred during or immediately after vaccination, including seizure, syncope, anaphylaxis, or an extensive local reaction; crying persisted for more than five minutes after vaccination; or the intervention protocol was not completed as planned.

Participants were allocated to the intervention and control groups using a non-randomized sequential recruitment procedure. Eligible infants were first recruited into the control group using convenience sampling. After 36 eligible infants had been recruited into the control group, recruitment of eligible infants into the intervention group was initiated and continued until 36 infants had been enrolled. Thus, a total of 72 infants participated in the study, with 36 infants in each group.

Because group assignment was based on the order and timing of recruitment rather than random sequence generation, allocation concealment was not used, and group assignment was predictable based on the recruitment period. Parents and healthcare personnel involved in administering the intervention could not be blinded because of the visible nature of the topical application.

However, outcome assessment was performed under blinded conditions. All vaccination procedures were video-recorded, and pain scoring was independently performed by observers who were blinded to group allocation. The outcome assessors did not participate in

the administration of the intervention or vaccination procedure.

The sample size was determined based on previous research investigating pain during infant vaccination, assuming a two-sided significance level of 0.05 and a statistical power of 80%. A total of 72 infants were included, with 36 participants in each group (14).

Parents of infants assigned to the intervention group received 2.5 mL of purified sheep oil in a sterile 2.5-mL syringe, together with standardized written and verbal instructions regarding its application.

Approximately one hour before vaccination, the entire 2.5 mL of sheep oil was applied as a thin layer to a standardized 3 × 3 cm area over the vastus lateralis muscle of the thigh. To standardize the location and size of the application area, mothers were provided with a cardboard template containing a 3 × 3 cm opening in the center. The application site and procedure were explained and demonstrated to the mothers before the intervention.

The mother applied the oil within the standardized area using the provided template. Following application, the treated area was covered with a transparent plastic dressing until the infant arrived at the health center for vaccination. Upon arrival, the dressing was removed, and the skin was disinfected according to routine clinical practice before vaccine administration.

The sheep oil used in the study was prepared using a traditional purification process involving butter extraction followed by gentle heating to remove impurities. Laboratory analysis confirmed an approximate purity of 99%. Microbiological testing of the oil was also performed by a laboratory before its use in the study. The oil was stored in a sterile container and subsequently transferred into sterile 2.5-mL syringes for administration.

Because the intervention involved topical application of an animal-derived product to infants, local skin reactions and potential hypersensitivity were monitored. Following application of the sheep oil, each infant was observed for evidence of local sensitivity or allergic reaction.

Mothers were instructed to monitor the application site for redness, rash, swelling, urticaria, or other signs of sensitivity. They were instructed to wash the area with cold water and seek medical attention if any signs of an allergic or hypersensitivity reaction occurred.

Immediately before vaccination, the intended injection site was examined for redness, urticaria, or other clinically apparent local reactions. Vaccination was not performed if such a reaction was present. No infant presented with redness, urticaria, or other clinically apparent local reaction at the injection site before vaccination.

Following vaccination, infants were monitored for possible adverse reactions, and mothers were instructed to report any subsequent skin reactions or other adverse events. No hypersensitivity-related adverse events associated with topical sheep oil were reported during the study. Parental acceptability of the intervention was assessed formally through mothers' willingness to apply the oil according to the standardized procedure and their ability to complete the intervention as instructed. Infants in the control group received routine vaccination care without any topical intervention. No placebo was used.

The pentavalent vaccine (0.5 mL) was administered intramuscularly into the vastus lateralis muscle using a 23-gauge auto-disable syringe by the same experienced vaccinator throughout the study. The vaccination procedure, injection

technique, vaccinator, and environmental conditions were kept as consistent as possible between the two groups.

2-1. Behavioral Pain Assessment

The primary outcomes were behavioral pain intensity and post-vaccination crying duration. Pain intensity was assessed using the Modified Behavioral Pain Scale (MBPS) (14). The MBPS evaluates three behavioral domains: facial expression (0–3), body movement (0–3), and crying response (0–4), yielding a total score ranging from 0 to 10, with higher scores indicating greater pain intensity. The validity and reliability of the MBPS have been supported in studies of infant vaccination pain, including studies conducted in Iran. In an Iranian study, content validity was assessed by 10 faculty members, and inter-rater reliability coefficients were 0.862 before vaccination and 0.853 at 15 seconds after vaccination (14). In the present study, two independent observers assessed the video-recorded responses, and inter-rater reliability was excellent (ICC = 0.852). Pain intensity was assessed at three predefined time points: immediately before vaccination (baseline); during vaccine administration; and 15 seconds after completion of the injection. All vaccination procedures were video-recorded to allow standardized assessment. The recorded videos were subsequently evaluated independently by two observers who were blinded to group allocation. The observers used the same predefined MBPS scoring criteria and were not involved in the intervention or vaccination procedure. The MBPS has demonstrated satisfactory validity and reliability in previous international studies and has also been validated in the Iranian population (15). Inter-rater agreement between the two observers was assessed using the intraclass correlation coefficient (ICC), which was 0.852, indicating excellent agreement. Post-vaccination crying duration was measured using a calibrated digital stopwatch. Timing began at the onset of crying immediately after needle insertion and ended when the infant stopped crying completely. Crying duration was recorded in seconds.

2-2. Statistical Analysis

Statistical analyses were performed using IBM SPSS Statistics. Continuous variables were assessed for normality using the Shapiro–Wilk test, and homogeneity of variances was assessed using Levene's test. Normally distributed continuous variables were summarized as mean ± standard deviation (SD), whereas non-normally distributed variables were summarized as median and interquartile range (IQR). Baseline characteristics were compared between the intervention and control groups using independent-samples t-tests. Pain intensity was assessed at three time points (before, during, and 15 seconds after vaccination) using the MBPS. Changes in MBPS scores over time were analyzed using repeated-measures analysis of variance (RM-ANOVA), with time as the within-subject factor and group as the between-subject factor. The main effect of time and the time × group interaction were evaluated. Mauchly's test was used to assess the assumption of sphericity. Because the assumption of sphericity was violated (P = 0.042), the Greenhouse–Geisser correction was applied to the degrees of freedom. Partial eta squared (η^2p) was reported as the effect-size measure.

In addition, linear and quadratic time-by-group contrasts were examined to characterize the pattern of change in pain scores over time. Crying duration was not normally distributed and was therefore summarized using the median and IQR and compared between groups using the Mann–Whitney U test. All 72 infants completed the study, and no missing outcome data were observed; therefore, no missing-data imputation was required. All statistical tests were two-sided, and a P

value < 0.05 was considered statistically significant.

3-RESULT

A total of 72 healthy two-month-old infants were included in the study, with 36 infants in the intervention group and 36 in the control group. All participants completed the study, and no withdrawals, exclusions, or missing outcome data occurred after enrollment. Baseline demographic and clinical characteristics are presented in Table 1. No statistically significant differences were observed between the intervention and control groups in gestational age at birth (38.5 ± 1.20 vs. 38.6 ± 1.15 weeks, P = 0.750), age at vaccination (64.88 ± 5.32 vs. 63.84 ± 4.60 days, P = 0.359), birth weight (3092.3 ± 425.2 vs. 3190.9 ± 435.9 g, P = 0.335), weight at vaccination (5381.9 ± 595.7 vs. 5443.8 ± 842.9 g, P = 0.720), length (57.60 ± 2.42 vs. 56.82 ± 3.99 cm, P = 0.285), or head circumference (38.67 ± 1.40 vs. 38.48 ± 1.76 cm, P = 0.597). Thus, no statistically significant differences were detected in the measured baseline characteristics between the two groups.

Table-1. Baseline demographic and clinical characteristics.

Variable	Intervention (n=36), Mean ± SD	Control (n=36), Mean ± SD	P value
Gestational age at birth (weeks)	38.5 ± 1.20	38.6 ± 1.15	0.750
Age at vaccination (days)	64.88 ± 5.32	63.84 ± 4.60	0.359
Birth weight (g)	3092.3 ± 425.2	3190.9 ± 435.9	0.335
Weight at vaccination (g)	5381.9 ± 595.7	5443.8 ± 842.9	0.720
Length (cm)	57.60 ± 2.42	56.82 ± 3.99	0.285
Head circumference (cm)	38.67 ± 1.40	38.48 ± 1.76	0.597

Note: P values were obtained using independent-samples t-tests.

Pain intensity was assessed immediately before, during, and 15 seconds after vaccination using the MBPS. As shown in Table 2, the mean baseline MBPS score was 3.22 ± 2.16 in the intervention group and 2.80 ± 1.70 in the control group, with no statistically significant between-group difference (P = 0.376). During vaccination, the mean MBPS score was lower in the intervention group than in the control group (7.13 ± 1.26 vs. 7.77 ± 1.07, P = 0.024). Fifteen seconds after vaccination, the intervention group also had a lower mean MBPS score than the control group (4.58 ± 2.01 vs. 6.38 ± 1.74, P < 0.001).

Repeated-measures ANOVA demonstrated a significant main effect of time on MBPS scores. Mauchly's test indicated that the assumption of sphericity was violated (W = 0.912, $\chi^2(2) = 6.336$, P = 0.042); therefore, the Greenhouse–Geisser correction was applied. The main effect of time was statistically significant (F(1.839, 128.708) = 194.197, P < 0.001, partial η^2 = 0.735).

Importantly, a significant time × group interaction was observed (F(1.839, 128.708) = 12.096, P < 0.001, partial η^2 = 0.147), indicating that the pattern of change in pain scores over the three assessment points differed between the intervention and control groups. Contrast analysis showed a significant linear time × group interaction (F(1,70) = 20.668, P < 0.001, partial η^2 = 0.228), whereas the quadratic time × group interaction was not statistically significant (F(1,70) = 0.024, P = 0.877, partial η^2 < 0.001).

Because crying duration was not normally distributed, it was analyzed using the Mann–Whitney U test. The median crying duration was 15 seconds (IQR: 13–17) in the intervention group and 18.5 seconds (IQR: 15–20) in the control group. The between-group comparison yielded a P

value of 0.050 (Table 3). Given that this value is at the conventional statistical significance threshold, the finding should be interpreted cautiously rather than as definitive evidence of a statistically significant difference.

Table-2. Comparison of pain intensity between the intervention and control groups and repeated-measures analysis

Time point / Effect	Intervention (n=36)	Control (n=36)	F	df	P value	Partial η^2
Before vaccination, Mean ± SD	3.22 ± 2.16	2.80 ± 1.70	—	—	0.376[a]	—
During vaccination, Mean ± SD	7.13 ± 1.26	7.77 ± 1.07	—	—	0.024[a]	—
15 s after vaccination, Mean ± SD	4.58 ± 2.01	6.38 ± 1.74	—	—	<0.001[a]	—
Time	—	—	194.197	1.839, 128.708	<0.001	0.735
Time × Group	—	—	12.096	1.839, 128.708	<0.001	0.147
Linear Time × Group	—	—	20.668	1, 70	<0.001	0.228
Quadratic Time × Group	—	—	0.024	1, 70	0.877	<0.001

Note: Values are presented as mean ± standard deviation.
a: Between-group P values at individual time points were obtained using independent-samples t-tests. Mauchly's test indicated violation of the sphericity assumption ($W = 0.912$, $\chi^2(2) = 6.336$, $P = 0.042$); therefore, the Greenhouse–Geisser correction was applied to the repeated-measures analysis.
Partial η^2 represents effect size. MBPS, Modified Behavioral Pain Scale.

Table-3. Comparison of crying duration between the intervention and control groups.

Variable	Intervention (n=36)	Control (n=36)	P value
Crying duration, median (IQR), seconds	15 (13–17)	18.5 (15–20)	0.050

Note: Crying duration was compared using the Mann–Whitney U test. Because the P value was exactly 0.050, interpret this finding cautiously and do not describe it as definitive evidence of a statistically significant difference.

4- DISCUSSION

The present study investigated the effectiveness of topical sheep oil in reducing procedural pain and crying duration during pentavalent vaccination in two-month-old infants. The findings demonstrated that infants who received topical sheep oil before vaccination experienced significantly lower pain intensity during and after injection and exhibited a shorter crying duration than those who received routine care. These findings suggest that topical sheep oil may represent a safe and effective complementary intervention for alleviating vaccination-related pain in early infancy.

Our findings demonstrate that the application of sheep oil significantly reduces both the duration of crying and the intensity of pain (as measured by the MBPS) compared to the control group. These results are consistent with the broader body of evidence supporting non-pharmacological interventions. For example, sucrose administration has been shown to alleviate pain and crying in neonates receiving Hepatitis B vaccinations (16). Similarly, breastfeeding during vaccination has been reported to reduce pain by strengthening the maternal-infant bond and stimulating the release of cholecystokinin (3). However, oral interventions, particularly breastfeeding, carry a risk of aspiration during the distress caused by injection. In contrast, topical interventions, such as the application of animal oils, offer a safer alternative with fewer risks associated with administration.

The mechanism of sheep oil can be compared to the effects of cryotherapy,

where the reduction of edema and muscle spasms indirectly alleviates pain (1). Combining the routine use of cold compresses with sheep oil may produce a synergistic anti-inflammatory effect, further diminishing the nociceptive experience. When compared to pharmacological agents such as EMLA cream (17), sheep oil offers a more favorable safety profile. Although topical lidocaine is widely recognized as an effective intervention for vaccination-related pain, concerns remain regarding its safety profile, particularly in young infants, because of the risk of methemoglobinemia, local adverse reactions, systemic absorption, and clinically relevant drug interactions (10). In contrast, sheep oil is a natural substance with a long history of safe topical use across all age groups. Although the precise pharmacological mechanism of sheep oil in alleviating vaccination pain is not fully understood, its anti-inflammatory properties are well-documented.

Several studies have also investigated the analgesic potential of topical natural oils in various clinical settings. Herbal oils such as lavender, chamomile, sesame, olive, and coconut oils have been reported to reduce pain, inflammation, and tissue irritation following minor medical procedures, massage, wound care, and musculoskeletal disorders (18).

Evidence from animal models indicates that sheep oil, rich in essential fatty acids and vitamins A and D, accelerates wound healing by suppressing inflammatory pathways and enhancing blood flow (19). Specifically, unsaturated fatty acids in animal oils are known to downregulate the expression of pro-inflammatory cytokines, such as TNF and COX-2(20). Moreover, saturated fatty acids play a crucial role in epithelial cell proliferation and intercellular signaling (21), which contributes to reduced scarring and pain (22). The presence of vitamins A, D (23), E (24), B12 (25), and K2 (26). These effects are further enhanced by the transdermal absorption of vitamins, which inhibit the inflammatory cascade. Since inflammation is a primary driver of pain at the injection site, these bioactive components can effectively reduce the pain response in infants. Although the precise pharmacological mechanism of sheep oil in alleviating vaccination-induced pain is not yet fully elucidated, its anti-inflammatory properties are well-documented. Evidence from animal models suggests that sheep oil, which is rich in essential fatty acids and vitamins A and D, may promote wound healing through anti-inflammatory and tissue-repair mechanisms (27). Specifically, the unsaturated fatty acids present in animal oils are known to down-regulate the expression of pro-inflammatory cytokines, such as TNF-alpha and COX-2 (20). Conversely, saturated fatty acids play a pivotal role in epithelial cell proliferation and intercellular signaling (Perry et al., 2018), which contributes to a reduction in both scarring and pain (21). Furthermore, sheep oil possesses a potent therapeutic profile containing essential vitamins, including A, D (Alsaqr et al., 2015), E (23), B12 (25), and K2 (26). Experimental studies have shown that these compounds possess anti-inflammatory, antioxidant, and tissue-repair properties. Unsaturated fatty acids may suppress the production of pro-inflammatory mediators such as cyclooxygenase-2 (COX-2) and tumor necrosis factor-α (TNF-α), whereas antioxidant vitamins help reduce oxidative stress and inflammatory signaling. In addition, topical application of lipid-rich substances may improve skin hydration, support local microcirculation, and enhance tissue resilience, thereby reducing nociceptive stimulation following intramuscular injection (28). Although these mechanisms remain hypothetical in the context of vaccination pain, they provide biologically plausible explanations

for the beneficial effects observed in the present study.

Compared with topical local anesthetics such as lidocaine–prilocaine cream, sheep oil may offer several practical advantages. Pharmacological topical anesthetics are effective but require additional preparation time, increase healthcare costs, and may occasionally produce local irritation, systemic absorption, or methemoglobinemia in susceptible infants. In contrast, sheep oil is inexpensive, widely available in many communities, culturally acceptable, and has a long history of traditional topical use. These characteristics may facilitate its integration into routine nursing practice, particularly in resource-limited healthcare settings. Nevertheless, direct comparative studies between sheep oil and established pharmacological interventions are required before recommendations for routine clinical implementation can be made.

4-1. Limitations

Several limitations should be considered when interpreting these findings. First, the quasi-experimental, non-randomized design limits causal inference and leaves open the possibility of residual confounding compared with randomized controlled trials. Second, participants were recruited from a single healthcare center, which may limit the generalizability of the findings to other healthcare settings and populations. Third, convenience sampling may have introduced selection bias. Fourth, blinding of participants and healthcare personnel was not feasible, which may have introduced performance or measurement bias. In addition, although major measured baseline characteristics were comparable between the groups, potentially relevant factors such as infant temperament, parental behavior, environmental noise, and individual pain sensitivity could not be fully controlled. The possibility of a Hawthorne effect should also be considered, as parental or healthcare-provider behavior may have been influenced by awareness of study participation. Finally, the outcomes were assessed only during and shortly after vaccination; therefore, the study does not provide information about the persistence of any observed differences over time. An additional limitation is that the control and intervention groups were recruited sequentially rather than concurrently. Because the control group was recruited before the intervention group, temporal changes in the healthcare setting, participant characteristics, or other unmeasured factors may have contributed to the observed between-group differences. Therefore, residual confounding related to the recruitment period cannot be excluded. Future multicenter randomized controlled trials with larger and more diverse samples, appropriate allocation procedures, and, where feasible, blinded outcome assessment are warranted to determine whether topical sheep oil has a causal effect on pain and crying responses following vaccination. Such studies should also incorporate longer follow-up and standardized control of potential behavioral and environmental confounders.

5- CONCLUSION

The findings of this quasi-experimental study indicate that infants who received topical sheep oil one hour before pentavalent vaccination had lower behavioral pain scores during and after vaccination and a shorter duration of post-vaccination crying than infants receiving routine care. Given its low cost, ease of application, and cultural acceptability, topical sheep oil may be a complementary approach for managing vaccination-related discomfort in infancy, particularly in primary healthcare settings.

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7- ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The study was conducted in accordance with the ethical principles outlined in the Declaration of Helsinki. Ethical approval was obtained from the Ethics Committee of Rafsanjan University of Medical Sciences (Approval No. IR.RUMS.REC.1402.066). Written informed consent was obtained from the parents or legal guardians of all participating infants before enrollment.

8- COMPETING INTERESTS

The authors declare that they have no competing interests.

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