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# Effects of different sub types and timing of childhood maltreatment on sperm small non-coding RNA levels

Advanced studies thesis

Spring semester 2025

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Spring semester 2025

TURKU UNIVERSITY
Faculty of Medicine

ILIADOU SOFIA: Effects of different sub types and timing of childhood maltreatment on sperm small non-coding RNA levels

Advanced studies thesis 16 p
Psychiatry
February 2025

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**Background:** Childhood maltreatment (CME) consisting of various forms of abuse, including physical and emotional abuse and neglect, and has been extensively linked to harmful effects on psychological and physiological well-being. For instance, maltreatment has been shown to be associated with increased levels of cortisol, a hormone critical to stress response, and alterations in growth hormone levels, which can have cascading effects on overall health (Jensen et al., 1991). Furthermore, emerging studies highlight the impact of CME on reproductive health, including altered sperm epigenetics, such as small non-coding RNA (sncRNA) profiles and DNA methylation patterns, which may influence fertility and offspring health (Gapp et al., 2014; Rodgers et al., 2013). This thesis focuses on two key sncRNAs identified in animal studies to have intergenerational effects, the miR-34c and miR-449a. This intersection of psychological trauma and physiological outcomes highlights the need for a comprehensive understanding of the mechanisms by which childhood maltreatment affects individuals across their lifespan.

**Methods:** This study investigates the associations between childhood maltreatment experiences (CME), measured via the Trauma and Distress Scale (TADS), and sperm small non-coding RNAs (sncRNAs) miR-34c and miR-449a. It builds upon data from the FinnBrain Birth Cohort Study and subsequent follow-ups. Data were derived from 30 male participants aged 30–48 years (mean: 39,1 ± 5,0). CME exposure, including five factors: emotional neglect, emotional abuse, physical neglect, physical abuse, and sexual abuse, was assessed across three age periods: 0–6, 7–12, and 13–18 years. TADS scores were used to calculate cumulative and factor-specific CME exposure for each age range separately (5 factors x 3 age ranges) and due to high number of variables were entered into stepwise regression models that aimed to predict relative abundance of miR-34c. Key health variables were included as covariates in statistical testing; including BMI, depressive and anxiety symptoms, smoking,

alcohol consumption, and sperm quality metrics. Statistical analyses were performed with stepwise linear regression models with a significance threshold of $p < 0.05$.

**Results:** When examining the age window of maltreatment on the sperm miRNA-levels, it was found, that CME during puberty (7–12 years) was associated negatively with miR-34c levels (ß = -0,498, p = 0,010). In wider analysis studying the combination of type and age of maltreatment, physical neglect during early childhood (0–6 years) was negatively associated with decreased miR-34c levels (ß = -0,602, p = 0,001). When examining each type of maltreatment throughout childhood (0–18 years), emotional abuse has the strongest association with reduced miR-34c levels (ß = -0,500, p = 0,009). Unlike miR-34c, no statistically significant associations were found between CME and miR-449a levels, except for a potential link with sperm concentration (ß = -0,408, p = 0.039).

**Conclusion:** There is growing evidence that CME has implications for reproductive and overall health outcomes in later life. Our findings emphasize the CME sensitive periods in childhood for epigenetic alterations in sperm sncRNAs, particularly miR-34c, to be puberty for overall maltreatment and early childhood in a combination with physical neglect. No significant associations were identified between CME exposure and miR-449a levels, suggesting that miR-449a may be less sensitive to early-life stress or influenced by other factors. Despite the identified associations, further research is necessary to clarify the underlying mechanisms and the extent to which these findings can be generalized beyond the studied population, and to develop interventions targeting the psychological and physiological effects of childhood maltreatment to mitigate long-term health risks.

**Key words:** childhood maltreatment, epigenome, sperm cell, sncRNA

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# 1 Introduction

Adverse childhood experiences (ACE) increase the risk of mental health illness, and specifically childhood maltreatment (CME, independently of genetic risk (Baldwin et al., 2023). Recent studies have shown that paternal ACE affects not only the exposed individual but can also have intergenerational effects by affecting the sperm epigenome, particularly the levels of sperm small non-coding RNA (sncRNA), such as micro-RNAs (miRNA), and DNA methylation. These changes may, in turn, modify the gene expression of the offspring and consequently, affect the phenotype and health of the offspring (Dickson et al., 2018; Gapp et al., 2014; Roberts et al., 2018; Rodgers et al., 2013). DNA methylation, histone modifications and the function of sncRNAs play an important role in the epigenetic regulation of gene expression, which is critical for the control of all cellular functions. These also act as epigenetic markers and can be mediated by environmental factors (Bure et al., 2022). A link between environmental stressors and lifestyle with the development of progeny has already identified. However, the exact pathways are not completely understood (De Castro Barbosa et al., 2016; Donkin and Barrès, 2018).

While multiple animal studies show evidence of the effects of early life stress experiences on the individual's sperm epigenome and the likelihood of affecting the next generations (Bohacek et al., 2015; Gapp et al., 2014; Rodgers et al., 2013), only a few studies have been performed on humans (Dickson et al., 2018; Jawaid et al., 2020; Roberts et al., 2018; Tuulari et al., 2025). In 2018 twelve DNA methylation profile changes in sperm were found between individuals with high and low childhood abuse levels measured by childhood Trauma Questionnaire (CTQ) and Conflict Tactics Scales (CTS) (Roberts et al., 2018). The same year another study was published showing a negative association between the levels of multiple sperm miRNAs of the miR449/34 family and scores received through an ACEs screen (Dickson et al., 2018). Children who had experienced paternal loss and maternal separation were reported to have both higher serum levels of miR-16/375 and reduced levels of high-density lipoproteins (HDL) when studied amongst both children of age 7-12 and adults of age 18-25 years. Additionally, the levels of miR-16/34/375 in sperm of adults of age 21-50 years, previously exposed to CME, surveyed with CTQ, were found to be lower than within the control group (Jawaid et al., 2020). Tuulari et al. replicated the previous findings of two prior studies by Dickson et al. and Jawaid et al. on lower miRNA has-miR-34c-5p levels in sperm

of individuals with high CME exposure, assessed by the Trauma and Distress Scale (TADS) questionnaire (Tuulari et al., 2025).

In animal studies, the effects of early life stress are shown to affect not only the epigenome of the individual, but also the development and health of the progeny. In two separate studies, mice exposed to early-life stress showed multiple miRNA alterations. In the F1 generation, these changes were observed in sperm, serum, hippocampus, and hypothalamus, while in the F2 generation, alterations were detected in serum and hippocampus (Bohacek et al., 2015; Gapp et al., 2014). Male mice exposed to chronic stress in their adolescence or adulthood for the duration of the entire spermatogenesis cycle were measured to have higher levels of sperm miR-193-5p, miR-204, miR-29c, miR-30a, miR-30c, miR-32, miR-375, miR-532–3p and miR-698, which was linked to hypothalamic–pituitary–adrenal (HPA) axis dysregulation of their progeny (Rodgers et al., 2013). Some representative examples from previous studies on associations found between stress exposure (animal studies) and CME (human studies) and sperm epigenome are summarized in Table 1.

**Table 1.** Examples of previous studies on the association of CME and sncRNA levels and/or sperm DNA methylation.

| Study | Year | Studied sperm | Method of CME determination | Main finding |
|---|---|---|---|---|
| Roberts et al. | 2018 | Humans | childhood Trauma Questionnaire (CTQ) and Conflict Tactics Scales (CTS) | 12 DNA methylation profile changes between high and low CME levels |
| Dickson et al. | 2018 | Humans | Adverse Childhood Experiences (ACEs) screen | negative association between levels of multiple miRNAs of the miR-449/34 family and ACE scores |
| Jawaid et al. | 2020 | Humans | Childhood Trauma Questionnaire (CTQ) | decrease in miR-16/34/375 amongst adults of age 21-50 years exposed to CME |
| Tuulari et al. | 2025 | Humans | Trauma and Distress Scale (TADS) questionnaire | lower miRNA hsa-mir-34c-5p in individuals with high CME |
| Wang et al. | 2021 | Mice | 5 weeks of unpredicted mild stress exposure before breeding and blocked transmission of | depression-like symptoms but no sperm sncRNA alterations in offspring at two months of age |

| | | | | |
|---|---|---|---|---|
| | | | the stress-induced phenotype by miRNA inhibitor injection | |
| Bohacek et al. | 2015 | Mice | postnatal traumatic stress | decreased gene expression in neuronal signaling pathways and altered synaptic plasticity in adulthood in F1 generation |
| De Castro Barbosa et al. | 2015 | Rats | metabolic stress through high-fat diet | altered expression of miRNA let-7c in metabolic tissues of F1 generation in addition to metabolic changes such as reduced body weight and glucose tolerance |
| Gapp et al. | 2014 | Mice | unpredictable maternal separation combined with unpredictable maternal stress (MSUS) | multiple miRNA alterations in sperm, serum, hippocampus and hypothalamus of F1 generation and in serum and hippocampus but not sperm of F2 generation |
| Rodgers et al. | 2013 | Mice | 6 weeks of chronic stress before breeding (during complete spermatogenesis) either in puberty or adulthood | increase of miR-193-5p, miR-204, miR-29c, miR-30a, miR-30c, miR-32, miR-375, miR-532–3p and miR-698 in the sperm of individuals exposed to chronic stress |
| Carone et al. | 2010 | Mice | low-protein diet | altered gene methylation and histone modification H3K27me3 in the sperm of the exposed individuals |

Intergenerational transmission is linked in animal studies to the function of different classes of sperm RNAs including miRNAs, transfer RNA derived RNA fragments (tRFs), PIWI-interacting RNAs (piRNAs), long linear RNAs, and circular RNAs (circRNAs) (Kretschmer and Gapp, 2022). The role of most sncRNAs in the sperm epigenome remains partially unclear. Multiple studies have reported an association between alterations in the sperm epigenome and metabolic, behavioral, and phenotypic changes (Bohacek et al., 2015; Carone et al., 2010; Gapp et al., 2014; Wang et al., 2021). However, the specific effects of sperm miRNA level alterations in humans, particularly their impact on progeny, remain uncertain. The levels of miR-34b/c and miR-449a/b/c were found to be associated with adverse childhood experiences, (ACEs) in previous studies. These miRNAs are present in sperm, but not in oocytes, and are associated with the regulation of spermatozoa maturation and

functionality and play a role in male fertility in mice (Bao et al., 2012; Pantos et al., 2021; Yuan et al., 2015). Specifically, miR-34c is also found to affect preimplantation embryonic development in mice (Cui et al., 2023). MiR-449 plays a role in the multiciliogenesis of the vertebrate (Marcet et al., 2011). Simultaneous inactivation of the two groups miR-34b/c and miR-449a/b/c are reported to regulate genes involved with cell-fate control, brain development and microtubule dynamics, which are essential in brain development, motile ciliogenesis, and spermatogenesis (Wu et al., 2014).

miR-449 is expressed at high levels in spermatocytes and spermatids of murine male germ cells, where they target the cell proliferation regulating pathway of retinoblastoma tumor suppressor gene product pRb and the transcription factor E2F (Bao et al., 2012; van den Heuvel and Dyson, 2008). Mutations in E2F weaken G1–S-phase transcription and significantly reduce DNA replication and cell proliferation. Furthermore, deregulation of E2F, primarily E2F1, can cause lethality, ectopic pregnancies, and apoptosis (van den Heuvel and Dyson, 2008). Demonstrating epigenetic inheritance is challenging, and requires the simultaneous quantification of the exposure, sperm epigenome, and offspring phenotype. While epigenetic inheritance has been demonstrated in animal models as presented in figure 1, it has not been comprehensively shown to exist in humans. This thesis focuses on associations between CME and sperm epigenome and does not directly assess epigenetic inheritance.

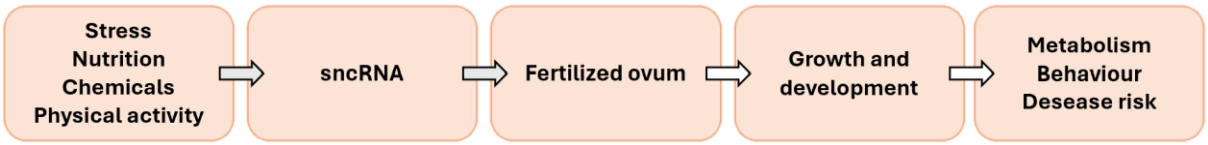


**Figure 1.** Epigenetic inheritance from the father to the offspring has been shown in animal models and is explained with environmental factors of the parent affecting the sperm sncRNA and later the gene expression of the embryo. (Raitakari et al., 2021).

The role of age and the developmental stage should be considered when studying the sperm epigenetic profile. Spermatogenesis begins during puberty and continues throughout life decreasing at older age (Dong et al., 2022; Holstein et al., 2003). The puberty in males begins typically at the age of 9,7 – 14,1 and continues until the age of 13,7 – 17,9 (Lee, 1980). In Finland, the typical range for the beginning of puberty, also called G2 growth phase for males, is from 9 to 13,5 years (Renko et al., 2023).

The structures of the male reproductive system, containing the testis, accessory ducts (epididymis, vas deferens and ejaculatory duct), accessory glands (seminal vesicles, bulbourethral glands, and prostate glands), and supportive structures (scrotum and penis), are developed by the 40th week of pregnancy at full term. Embryonic development of the testis is initiated by the SRY gene on the Y chromosome, which leads to testicular differentiation. This differentiation stimulates androgen production by Leydig cells in response to human chorionic gonadotropin (hCG) from the placenta. Testosterone, developed by the Leydig testis cells, affects male secondary sexual characteristics, sperm development, and fertility (Wu, 2022).

Spermatogenesis starts around puberty and occurs within the seminiferous tubules of the testis and steroidogenesis, more specifically testosterone synthesis occurs within the interstitial compartment of testis. The male germ cell precursors, embryonic primordial germ cells (PGCs) enter mitosis and are referred to as prospermatogonia. Prospermatogonia remain in the G0/G1 phase of the cell cycle until birth, as testis development occurs during embryogenesis. After birth, they transition into undifferentiated spermatogonia, which during puberty finish mitosis and generate differentiated spermatogonia (Kubo et al., 2015). Spermatogenesis can be divided into three stages, the first being mitotic cell division where spermatogonia multiply. In the second stage, the meiotic cell division occurs where diploid primary spermatocytes form haploid secondary spermatocytes, and eventually spermatids. Finally, in a process called spermiogenesis, spermatids differentiate into mature spermatozoa (Clermont, 1972). Spermatozoa become functionally mature as they migrate through the epididymis (Wu, 2022). The spermatogenetic cycle takes at minimum 86 days in human including the development of spermatids and the transportation of spermatozoa through the epididymal duct system (Holstein et al., 2003). The maturation process of primordial germ cells into functional spermatozoa is schematized in figure 3.

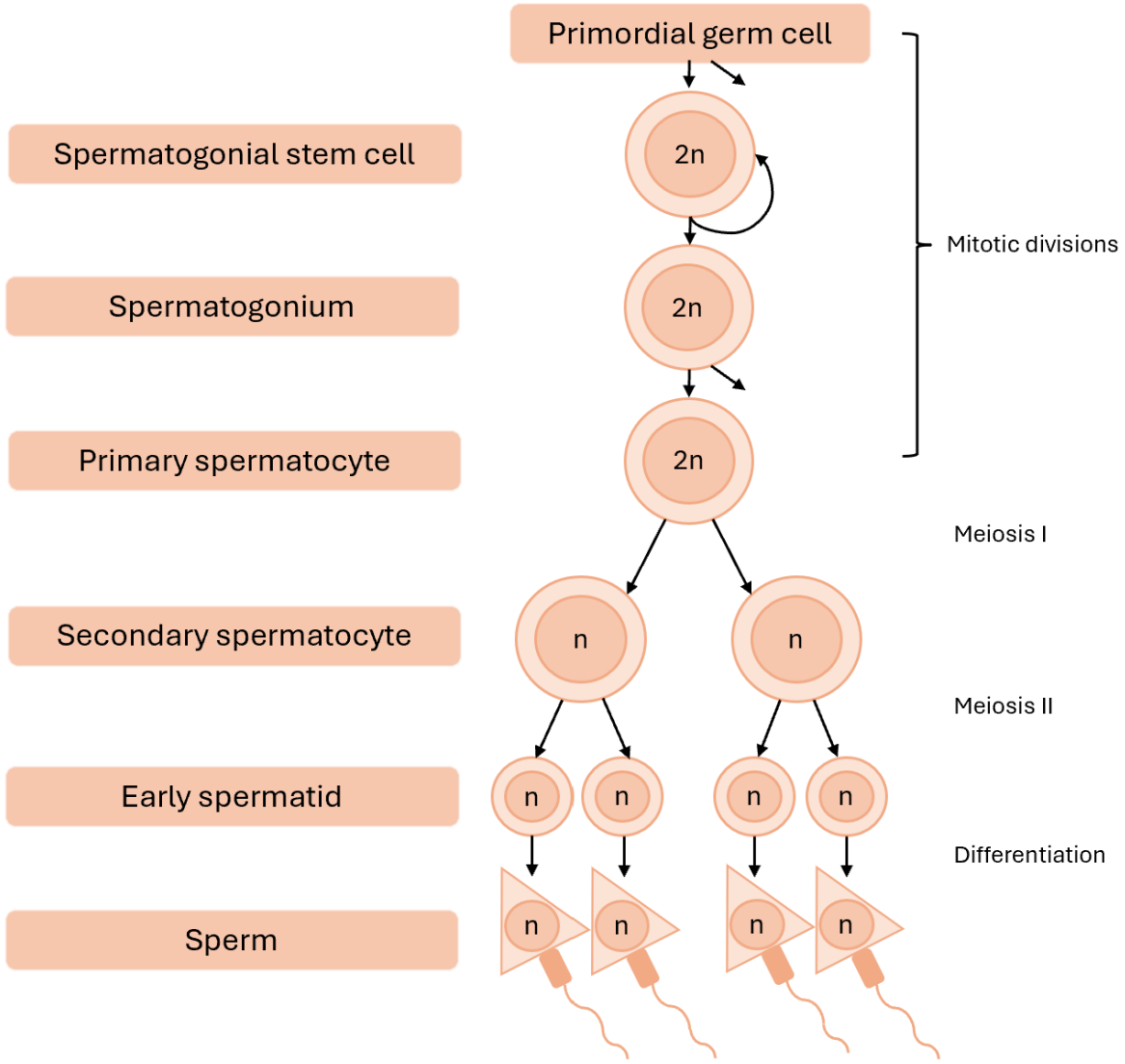


Figure 3. Schematic of sperm cell maturation (Wu, 2022).

Epigenetic gene regulation is defined as a process that regulates gene expression without affecting the DNA nucleotide sequence. The best-known epigenetic mechanisms include DNA methylation and post-translational modifications of histone tails (e.g. acetylation, methylation, and phosphorylation). In addition, ncRNAs have a critical role in epigenetic gene regulation (Loscalzo and Handy, 2014). Male germ cell differentiation is regulated by such epigenetic processes. PGCs during embryonic development undergo dramatic epigenetic resetting, during which the DNA methylation marks are erased and re-established (Carrell, 2012; Kubo et al., 2015). The second round of epigenetic resetting takes place right after fertilization in early embryos (Kubo et al., 2015). To be able to transmit epigenetic information from one generation to the next, the organisms have to cope with these processes of epigenetic resetting.

Multiple studies demonstrate that various epigenetic marks are associated with the inheritance of phenotypes and diseases. DNA methylation is the most extensively studied form of epigenetic modification, with significant evidence linking it to paternal epigenetic inheritance (Bird, 2002; Duan et al., 2019; Hackett et al., 2013; Illum et al., 2018; Zhu et al., 2021). Additionally, histone modifications (Carone et al., 2010; Skvortsova et al., 2018) and sncRNAs (Chen et al., 2016; Quarato et al., 2022; Yan and Zhai, 2016) have also been implicated in this process.

The process of spermatogenesis is sensitive to environmental fluctuations such as hormones and temperatures as well as nutritional deficiencies including insufficient A, B, and E vitamin intake, anabolic steroids, metals, X-ray exposure, dioxin, drug toxicants, and diseases of pathogens (Wu, 2022). The environmental factor of special interest in this thesis is (early life) stress, which can be triggered by traumatic events experienced in childhood or adulthood. Prolonged traumatic stress can impact brain functioning, especially the HPA-axis, and related brain structures such as the amygdala, hippocampus and frontal cortex, and other signaling pathways such as mineralocorticoids and the balance between oxytocin and arginine vasopressin. (Fares-Otero et al., 2025; Jawaid et al., 2018). Furthermore, there is evidence that testosterone levels are increased amongst individuals who have experienced CME and higher levels of testosterone can be associated with emphasized amygdala responses, aggression, violence, and risk of child abuse and negative parenting, resulting in the potential perpetration of childhood maltreatment. (Fares-Otero et al., 2025). However, the role of the quality of the traumatic event on the effects of epigenetic modifications in gametes and on the health of the individual has not been extensively studied.

This study focuses on the possible association of the age and type of CME exposure, measured by the Trauma and Distress Scale (TADS) questionnaire on the sperm miR-34c and miR-449a levels. This study is based on the same cohort data used in the case-control research of Tuulari et al. where the overall association between previous maltreatment experiences and sperm non-coding RNA levels were examined (Tuulari et al., 2025). The hypothesis is that the earlier CME exposure occurs, the greater the effects on the miR-34c and miR-449a levels are. No hypothesis was set for the possible difference in miR-34c or miR-449a levels based on the form of maltreatment.

# 2 Materials and methods

## 2.1 Participants

The data of the model is collected from the FinnBrain Birth Cohort Study between 2011 and 2021. The FinnBrain study was initially performed between 2011 and 2015 to study prospectively the effects of early life stress (ELS), prenatal stress (PS) and paternal health on the health and development of the brain of the offspring as well as identifying biomarkers for ELS and PS exposures. The participants were recruited at gestational week (gwk) 12 from maternity clinics in southwest Finland and the data was collected at the first prenatal assessment at gestational week (gwk) 14. 3808 mothers, 2623 fathers or partners and 3837 children took part in the initial prospective study, while 2270 (59,6 %) of the participants continued to the cohort follow-up. (Karlsson et al., 2018).

A follow-up visit focusing on paternal health was performed between 2019 and 2021 (report includes partial data of the total data collection), and the study has been continued into a cross-sectional study. From the 2270 participants, 75 males also participated in the case-control study 2023 by determining their CME exposure by the Trauma and Distress Scale (TADS) questionnaire. From these 75 males recruited to the visits, 55 sperm samples were analyzed, from which 30 were subject to sperm sncRNA quantification. (Tuulari et al., 2025). The data used in this study was collected during the FinnBrain cohort study and its follow-up study. Thus, the number of participants in this study is 30 due to the restricted availability of sncRNA information among the participants in the previous studies. The age of the participants varied from 30 to 48 years with the mean age being 39,1 years and standard deviation (SD) 5,0 years. The flow chart of the participant selection for this study is presented in figure 4, and the demographics are provided in Table 2.

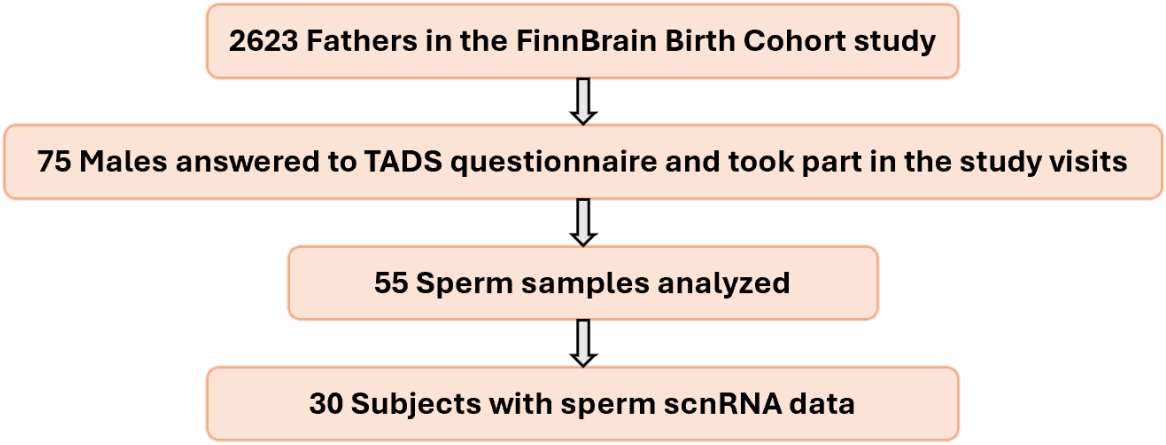


**Figure 4.** Flow chart of the selection of participants in this study.

**Table 2.** Descriptive statistics of demographic characteristics. The variables that are overruled were not used in the regression models based on their low variance that precluded their inclusion into the regression models and also made them unlikely determinants of the effects of the CME exposure.

| Characteristic | Mean | SD | Min | Max |
|---|---|---|---|---|
| Age (years) | 39,1 | 5,0 | 30 | 48 |
| Body mass index (kg/m2) | 26,2 | 4,5 | 19,8 | 37,8 |
| EPDS score | 3,7 | 4,6 | 0 | 24 |
| SCL-90 score | 4,1 | 5,4 | 0 | 25 |
| miR-34c-5p | 992,3 | 934,9 | 107,6 | 4408,8 |
| miR-449a | 63,5 | 86,7 | 0 | 349,4 |
| Semen volume (ml) | 3,1 | 1,4 | 1 | 7,7 |
| Sperm concentration ($10^6$ ml) | 101,6 | 68,6 | 8,3 | 231,3 |
| Smoking | 1,7 | 1,2 | 1 | 5 |
| Average daily alcohol consumption | 2,1 | 0,8 | 1 | 4 |
| TADS sum worst | 25,2 | 23,0 | 0 | 78 |
| TADS direct sum worst score | 13,2 | 14,1 | 0 | 43 |
| TADS direct sum score 0-6 years | 13,9 | 14,9 | 0 | 52 |
| TADS direct sum score 7-12 years | 19,0 | 18,8 | 0 | 67 |
| TADS direct sum score 13-18 years | 21,5 | 20,0 | 0 | 62 |
| TADS factor sum score 0-6 years | 7,4 | 9,0 | 0 | 29 |
| TADS factor sum score 7-12 years | 10,1 | 11,8 | 0 | 39 |
| TADS factor sum score 13-18 years | 11,7 | 12,6 | 0 | 35 |
| TADS emotional neglect worst score (0-18 years) | 4,8 | 5,2 | 0 | 15 |
| TADS emotional neglect 0-6 years | 3,4 | 4,2 | 0 | 13 |
| TADS emotional neglect 7-12 years | 4,1 | 4,7 | 0 | 13 |
| TADS emotional neglect 13-18 years | 4,6 | 5,1 | 0 | 15 |
| TADS emotional abuse worst score (0-18 years) | 3,0 | 4,0 | 0 | 14 |

| | | | | |
|---|---|---|---|---|
| TADS emotional abuse 0-6 years | 1,3 | 2,3 | 0 | 9 |
| TADS emotional abuse 7-12 years | 2,3 | 3,3 | 0 | 13 |
| TADS emotional abuse 13-18 years | 2,8 | 3,9 | 0 | 14 |
| ~~TADS physical neglect 0-6 years~~ | ~~2,3~~ | ~~2,0~~ | ~~0~~ | ~~6~~ |
| ~~TADS physical neglect 7-12 years~~ | ~~3,2~~ | ~~3,3~~ | ~~0~~ | ~~11~~ |
| ~~TADS physical neglect 13-18 years~~ | ~~3,6~~ | ~~3,5~~ | ~~0~~ | ~~11~~ |
| TADS physical neglect worst score (0-18 years) | 3,3 | 3,3 | 0 | 10 |
| TADS physical neglect 0-6 years | 1,7 | 1,7 | 0 | 5 |
| TADS physical neglect 7-12 years | 2,5 | 2,8 | 0 | 9 |
| TADS physical neglect 13-18 years | 2,8 | 3,0 | 0 | 10 |
| ~~TADS physical abuse 0-6 years~~ | ~~0,5~~ | ~~1,3~~ | ~~0~~ | ~~6~~ |
| ~~TADS physical abuse 7-12 years~~ | ~~0,5~~ | ~~1,3~~ | ~~0~~ | ~~6~~ |
| ~~TADS physical abuse 13-18 years~~ | ~~1,2~~ | ~~2,3~~ | ~~0~~ | ~~10~~ |
| TADS physical abuse worst | 2,0 | 3,0 | 0 | 12 |
| TADS physical abuse 0-6 years | 1,0 | 2,2 | 0 | 9 |
| TADS physical abuse 7-12 years | 1,2 | 2,2 | 0 | 9 |
| TADS physical abuse 13-18 years | 1,5 | 2,5 | 0 | 11 |
| TADS sexual abuse worst score (0-18 years) | 0,1 | 0,5 | 0 | 3 |
| TADS sexual abuse 0-6 years | 0,0 | 0,0 | 0 | 0 |
| TADS sexual abuse 7-12 years | 0,1 | 0,5 | 0 | 3 |
| TADS sexual abuse 13-18 years | 0,0 | 0,0 | 0 | 0 |

## 2.2 Childhood maltreatment exposure

The participants reported their level of exposure to CME in three age ranges; 0-6 years, 7-12 years and 13-18 years. For each age range, the CME consists of the following forms of abuse and neglect: emotional neglect, emotional abuse, physical neglect, physical abuse, and sexual abuse. To determine the individual level of exposure to each of these items, the participants were surveyed with the TADS questionnaire with questions related to their previous life experiences. There were in total 43 questions in the TADS questionnaire, of which 41 assess the childhood traumatic experiences and two the reliability of the answers. For each TADS question, there are answer options from 0 = never to 4 = almost always (Salokangas et al., 2016).

The TADS points for each CME factor were then summarized to form a direct sum score and sum of within factor sores (TADS factor sum score). TADS sum is used for summarizing the

direct points from the questionnaire. In the first analysis, the association of both TADS sum and TADS factor sum are studied in relation to the miR-34c-5p and miR-449a. The TADS sum scores ranged from 0 to 78 (mean 25,2 and SD 23,0). The level of TADS scores is used as an indicator to the level of CME as high TADS points indicate higher adverse life experiences.

The participants who reported any type of CME reported mostly at least one other type of CME (Figure 5). Most participants who reported experiencing emotional neglect reported a relatively stable level of neglect for all age ranges. For emotional abuse, 10 % of participants only reported exposure at part of their childhood, mostly at age higher than 6 years. Physical neglect was similarly mostly reported to be continuous during childhood. However, 10 % reported neglect only at age 13-18 years while 3 % reported neglect only at age 0-6 years. While similar continuity was typical in physical abuse, 13 % only reported physical abuse for ages 7-12 and 13-18 years, 3 % only for age 0-6 years, 3 % for ages 0-6 and 7-12, and 3 % for ages 0-6 and 13-18. Only 3 % of the participants reported sexual abuse and only at age 7-12.

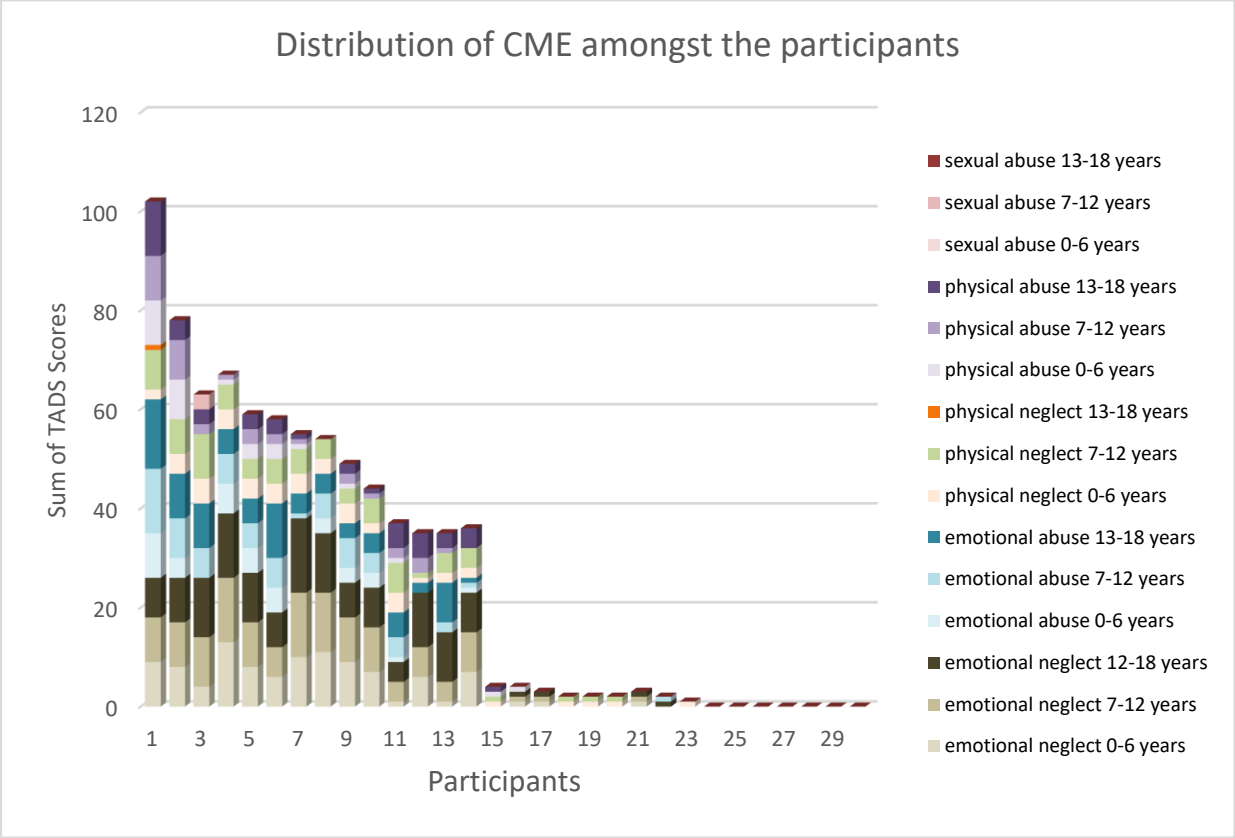


**Figure 5.** Breakdown of forms and timing of CME experienced for the participants of the study; each column represents one participant and the components on the y-axis represent the magnitude of each type of CME for the participant presented as a sum of TADS scores.

## 2.3 Micro-RNA quantification

The sperm miR-34c-5p and miR-449a expression levels have been reported as relative expression levels. Amongst the studied participants miR-34c-5p levels ranged from 107,6 to 4408,8 (mean 992,3, SD 934,9) and mir-449a levels ranged from 0 to 349,4 (mean 63,5, SD 86,7). The quantification of miRNA expression levels in sperm was performed using sncRNA sequencing (sncRNA-seq). RNA was extracted from sperm samples using TRIzol LS, with tris(2-carboxyethyl)phosphine (TCEP) added as a reducing agent to enhance sperm nucleus lysis. The RNA was then precipitated with isopropanol in the presence of GlycoBlue and subjected to DNaseI treatment to remove potential DNA contamination. Following this, the quality of the extracted RNA was assessed using the Agilent Bioanalyzer with the RNA 6000 Pico Kit to verify purity and ensure the absence of somatic cell contamination (Tuulari et al., 2025).

Libraries for small RNA sequencing were constructed using the NEB Next® Multiplex Small RNA kit, and sequencing was performed on the NovaSeq 6000 system (Illumina). Read quality was evaluated with FastQC, and adapter trimming was conducted using cutadapt (v3.5). The SPORTS1.1 pipeline was employed for reading alignment and mapping, with reads mapped to the human genome (hg38) and then classified based on known sncRNA databases, including miRBase for miRNAs. The analysis pipeline ensured accurate identification and quantification of small RNAs, including miR-34c-5p and miR-449a (Tuulari et al., 2025).

## 2.4 Health and lifestyle variables

Environmental toxins and drugs may affect the epigenome of the gamete (Rajender et al., 2011). Therefore, for all models, smoking and high alcohol consumption have been considered as possible cofactors in the alteration of miR-34c levels in sperm and have thus been standardized in all analyses. Smoking and alcohol consumption of the participating fathers was surveyed regarding the duration of their child being 0-6 years old. Smoking was reported on a scale of 1 to 5, where 1 is no consumption and 5 is a weekly consumption of 20 cigarettes. Similarly, alcohol consumption was reported on a scale of 1 to 5, 1 being no consumption and 5 being over 21 portions weekly. 30 % of the participants reported smoking at least once a week, while 83 % reported alcohol consumption at a minimum of one portion

weekly, from which 80 % reported a low level of alcohol consumption (1 – 7 portions weekly).

Additionally, the age of the participants, body mass indicator (BMI), semen volume and concentration as well as the sum of EPDS (Edinburgh Postnatal Depression Scale) questionnaire scores, and SCL-90 (anxiety subscale of Symptom Checklist 90) questionnaire scores were included as covariates. The BMI varied from 19,8 kg/m2 to 37,8 kg/m2 (mean 26,2 kg/m2, SD 4,5 kg/m2), and was normally distributed as the age. 60 % of the participants were at least slightly overweight, 43 % were slightly overweight (BMI 25-29.9 kg/m2), 0,1 % were moderately overweight (BMI 30-34.9 kg/m2) and 0,07 % were significantly overweight (BMI 35-39.9 kg/m2). The semen volume and concentration varied more amongst the participants. Semen volume varied from 1,0 to 7,7 ml (mean 3,1 ml, SD 1,4 ml) and concentration from 8,3 106/ml to 231,3 106/ml (mean 101,6 106/ml, SD 68,8 106/ml).

The EPDS questionnaire was used to determine depressive symptoms. EPDS scores ranged from 0 to 24 (mean 3,7, SD 4,6) with the maximum possible score being 30. SCL-90 is a questionnaire with 90 questions regarding somatization, obsessive-compulsive disorder, interpersonal sensitivity, depression, anxiety, hostility, phobic anxiety, paranoid ideation and psychoticism (Holi et al., 1998). The SCL-90 questionnaire was used here to assess anxiety symptoms (Tuulari et al., 2025). Its scores vary from 0 to 25 (mean 4,1, SD 5,4).

## 2.5 Statistical methods

The data used is from a prospective cohort study. The study design was fundamentally a case-control study with regards to CME, but we chose to model the CME scores as continuous variables. Six ‘forward and backward’ stepwise linear regression models (three for miR-34c and three for miR-449a) were built to identify possible predictors in type and age of CME with the levels of sperm miR-34c and miR-449a used as primary variables and marked as hsa-miR-34c-5p and hsa-miR-449a. Additionally, secondary variables related to health and sperm quality characteristics (age, BMI, smoking, alcohol consumption, depressive and anxiety symptoms, sperm volume, and concentration) were included as covariates (of no interest).

The stepwise regression model variable addition and exclusion were performed based on the p-values. The significance level alpha used in all the models was set to 0,05 with 95 % confidence limits. The regression models were deemed appropriate by the visual inspection of the normality of the residuals and Q-Q plots. We used JASP 0.18.3 for statistical analyses.

### 2.5.1 Model 1: Associations between timing of CME and miR-34c expression

Three stepwise linear regression models were used to determine the possible predicting factors of miR-34c expression in sperm, analyzing the three age groups (0-6 years, 7-12 years, 13-18 years) of cumulative CME, the five forms of TADS groups of abuse and neglect (emotional neglect, emotional abuse, physical neglect, physical abuse, and sexual abuse), and other identified health-related and sperm quality measures (age, BMI, smoking, alcohol consumption, depressive and anxiety symptoms, sperm volume, and concentration). While most of these variables have non-normal distributions, this does not prevent linear regression modelling. We carefully inspected the normality of the regression model residuals of each model. The demographics of the participant data are presented in Table 1.

To study age-related prediction factors, the first model was created using information on the age of CME, with CME exposure treated as total TADS score and factor score for each age group (0-6 years, 7-12 years, 13-18 years).

### 2.5.2 Model 2: Associations between timing and type of CME and miR-34c expression

A second regression model was conducted to examine the associations between the type and age of CME exposure with miR-34c expression. Participants were divided into groups based on their TADS factor scores, corresponding to emotional neglect, emotional abuse, physical neglect, physical abuse, and sexual abuse. However, only one participant reported sexual abuse at 7-12 years of age. Due to insufficient data, sexual abuse as a TADS group was left out of the study. For all other types of abuse and neglect, participants reported various exposure levels at different ages, allowing for meaningful statistical analysis.

### 2.5.3 Model 3: Associations between type of CME over childhood and miR-34c expression

The third model focused on assessing the cumulative effect of different types of CME across childhood on miR-34c expression. This model examined whether the type of exposure, regardless of age, was associated with miR-34c levels. The worst reported exposure for each CME type was considered the key predictor variable for this assessment. The significant findings from the three miR-34c models have been visualized in Table 3.

### 2.5.4 Models for miR-449a expression

A similar set of three models was created to examine associations between type and/or age of CME and miR-449a levels. However, no significant associations were identified for hsa-miR-449a, and it was excluded from subsequent analyses.

# 3 Results

## 3.1 CME during early puberty is associated with lower levels of miR-34c in sperm

When analyzing the associations of TADS direct sum scores and factor sum scores of each age group to the levels of miR-34c, no significant association was found for the groups of early childhood (0-6 years) and late puberty (13-18 years). The only statistically significant association was a negative association found between miR-34c and CME at the age of 7-12 years (ß = -0.498, $F(1, 24) = 7{,}91$, $p = 0{,}010$).

This finding is interesting as the age range of 7-12 lies at the beginning of puberty (Renko et al., 2023), which is an important time period regarding hormonal regulation and maturation of the reproductive system. Secretion of gonadotropin-releasing hormone from the hypothalamus during puberty leads to maturation of various brain regions and the reproductive system, which is therefore also potentially vulnerable to epigenetic alterations during this period. (Morrison et al., 2014). Furthermore, spermatogenesis is initiated during puberty (Holstein et al., 2003), and preadolescence and early adolescence can therefore have unique relevance to the profile of adult sperm epigenome.

## 3.2 Exposure to physical neglect by the age of 6 years is moderately associated with lower levels of sperm miR-34c

The wider regression model including each of the TADS factor score groups (emotional neglect, emotional abuse, physical neglect, physical abuse, and sexual abuse) for each age range (0-6 years, 7-12 years, and 13-18 years) and the levels of sperm miR-34c were studied and the only statistically significant negative association was found for physical neglect by the age of 6 years (ß = -0,602, $F(1, 24) = 13{,}66$, $p = 0{,}001$) indicating possible association between physical neglect experienced in early childhood and miR-34c mediated epigenetic changes in sperm during adulthood. The groups reporting sexual abuse at all age stages were left out of the study due to the low level of participants having this exposure (complicating the stepwise regression models).

This finding is intriguing as prior studies have shown that physical neglect at an early age affects brain health and can cause impairment in learning and memory (Wang et al., 2016).

Furthermore, physical abuse and neglect in early childhood affect general health through development and brain-mediated hormone balance (Bayat, 2019). It can therefore result in multiple later effects, including changes in sperm epigenome.

## 3.3 Emotional abuse during childhood is highly associated with lower levels of miR-34c in sperm

Lastly, each type of CME (emotional neglect, emotional abuse, physical neglect, physical abuse and sexual abuse) was evaluated throughout the entire childhood by its highest TADS score to determine the possible association of each CME type with the levels of sperm miR-34c. Interestingly, the only statistically significant association was a negative association found between the highest TADS score in emotional abuse and miR-34c levels (ß = -0,500, $F(1, 24) = 8,00$, $p = 0,009$).

While the association of childhood emotional abuse and sperm sncRNA has not previously been studied, emotional abuse and neglect, including exposure to interpersonal violence, have been shown to harm the child's brain functioning even more than physical and sexual abuse (Benoit et al., 2008). Thus, emotional abuse during childhood may have an indirect association with testicular development or sperm maturation.

Table 3. Regression with levels of miR-34c-5p as the dependent variable.

| **Age and type of exposure** | **ß** | **df** | **F** | **p** |
|---|---|---|---|---|
| **Model 1 Age of exposure** | | | | |
| 7-12 years | -0,498 | 1/24 | 7,91 | 0,010 |
| **Model 2 Age and type combined** | | | | |
| physical neglect at 0-6 years | -0,602 | 1/24 | 13,66 | 0,001 |
| **Model 3 Type of exposure** | | | | |
| emotional abuse | -0,500 | 1/24 | 8,00 | 0,009 |

## 3.4 Type and age of exposure are not associated with changes in sperm miR-449a levels

While all the forms of CME reported through TADS groups were studied for each age group, no association was found with levels of sperm miR-449a. The only association was found between sperm concentration and the levels of sperm miR-449a (ß = -0,408, $p = 0,039$). However, the association is negative, indicating that as the levels of sperm miR-449a increase, sperm concentration decreases. MiR-449a is known to regulate genes involved in

spermatogenesis, particularly in sperm differentiation and motility (Khawar et al., 2019; Pantos et al., 2021). Moreover, it is known from animal models that miR-449a has been implicated in the E2F-pRb pathway, which controls cell cycle progression and apoptosis in germ cells (Bao et al., 2012). Lower concentration in sperm in turn likely decreases the probability of fertilization. Consequently, sperm miR-449a could be a marker of infertility, but it was not influenced by CME in our analyses.

# 4 Discussion

This study investigated the long-term impacts of different types of childhood maltreatment (emotional and physical abuse and neglect, as well as sexual abuse) occurring in different age windows (0-6 years, 7-12 years, 13-18 years), specifically exploring their association with sperm epigenome in adulthood. We analyzed data from the FinnBrain cohort, utilizing a retrospective questionnaire and sperm samples to understand potential connections between early life experiences and sperm epigenome. Our findings suggest that physical neglect during early childhood and overall CME during early puberty have the strongest associations with miR-34c levels, and they could tap distinct developmental processes occurring at these times. Moreover, across all possible CME variables, emotional abuse showed the most significant negative association with miR-34c levels. Unlike miR-34c, miR-449a levels had no significant association with any studied CME type or age but had a negative correlation with sperm concentration.

## 4.1 The Role of miR-34c and its association with CME

During the past two decades associations between CME and sperm sncRNA levels as well as DNA methylation profiles have been identified in various studies (Dickson et al., 2018; Gapp et al., 2014; Roberts et al., 2018; Rodgers et al., 2013). Early life stress, especially CME, is shown to affect the sperm epigenome, more specifically miR-34 (Dickson et al., 2018; Jawaid et al., 2020; Tuulari et al., 2025). Moreover, physical, cognitive and emotional abuse during childhood are found to be associated with the methylation of 39 miRNAs and 997 gene promoters, typically resulting in corresponding gene silencing (Suderman et al., 2014).

In addition to miR-34c, miR-449a, which shares functional overlap with the miR-34 family, is implicated in regulating spermatogenesis. Both miRNAs influence the epigenetic modification of genes crucial for sperm motility and DNA integrity. Dysregulation in their expression is associated with male infertility, highlighting their role in maintaining gamete quality and reproductive potential (Khawar et al., 2019; Pantos et al., 2021). These findings emphasize the regulatory importance of miR-34c and miR-449a in the male germline and early embryonic development of the progeny. Thus, abnormal levels of these two miRNAs can serve as biomarkers for infertility and may provide therapeutic targets for addressing reproductive dysfunction.

In this study, we further investigated possible associations between specific age or type of CME to the levels of two sperm sncRNAs: miR-34c-5p and miR 449a. While previous studies have already demonstrated reduced sperm miR-34 levels amongst individuals exposed to childhood maltreatment in general (Jawaid et al., 2020; Tuulari et al., 2025), in this study, physical neglect, specifically during early childhood, was found to be associated with lower levels of miR-34c. However, the effects of emotional abuse showed overall the greatest association. Furthermore, the most sensitive time period for miR-34c level mediated epigenetic changes in sperm were observed with CME during early puberty.

Interestingly, while associations between specific ages and types of maltreatment to miR-34c levels were identified, no significant associations were found with miR-449a. However, as miR-449a levels (mean 63,5) were significantly lower than those of miR-34c (mean 992,3) in the samples, drawing reliable conclusions about its potential associations is challenging. Nevertheless, the difference in the response of sperm miR-34c and miR-449a to CME may result from their distinct biological roles, expression patterns, and regulatory pathways. While both miRNAs are essential in spermatogenesis, miR-34c has a critical function in early embryonic development and appears more vulnerable to stress-related epigenetic modifications that disrupt the HPA-axis (Cui et al., 2023). On the other hand, miR-449a may be less directly impacted by systemic stress signals due to possible compensatory mechanisms involving other miRNAs. This could make its expression more resilient to environmental factors, thereby mitigating the influence of stress-induced changes.

## 4.2 Physical Neglect during early childhood: Endocrinological mechanisms

Early childhood is a critical period for the establishment of foundational neural and physiological systems, including the HPA and hypothalamic-pituitary-gonadal (HPG) axis (Gunnar et al., 1996; Gunnar and Donzella, 2002). The stress-response system of HPA-axis is one of the multiple hormone-mediated information pathways regulated by the central nervous system (CNS). Kaess et al. suggest that childhood maltreatment affects the development of the HPA-axis through initial chronic hyperactivity during childhood leading to attenuation of

the system during adolescence and physical enlargement of the pituitary gland volume (2018). Hormonal changes, especially related to HPA, have been identified in individuals with CME. Levels of adrenocortical produced and released cortisol were reported to be increased in maltreated individuals (Hart et al., 1996). Additionally, differences in levels of growth hormone were identified between maltreated children and their peers (Jensen et al., 1991). Moreover, maltreated children have a higher likelihood of developing endocrine diseases, especially hypothyroidism, hyperthyroidism, type 2 diabetes, hyperparathyroidism, and hypofunction of the pituitary gland (Wen et al., 2024).

Chronic stress is known to cause also chronic inflammation (Chiang et al., 2022). Danese et al. explored key inflammation markers in adults and discovered a strong link between experiencing childhood maltreatment, particularly in the first ten years of life, and elevated levels of inflammatory markers like C-reactive protein (CRP) later in life, proposing thus that inflammation could play a central role in connecting early-life stress to negative health outcomes in adulthood (2007). These findings build on prior knowledge that high levels of inflammatory markers are associated with health issues such as cardiovascular disease, diabetes, and chronic lung disease (Arora et al., 2019; Schram et al., 2005; Wang et al., 2013). Furthermore, a direct association has been found between CME and elevated blood pressure, which is linked i.e. to cardiovascular disease (Al-shoaibi et al., 2024).

The significance of physical presence during early childhood is known to be crucial for the general mental and physical development of a child. Multiple associations between physical neglect during early childhood and deficiencies in brain health, memory, learning, and communicating abilities have been identified (Culp et al., 1991; Grassi-Oliveira et al., 2008; Wang et al., 2016)**.** Considering the wide and yet partly under-examined effects of early physical neglect in brain and endocrinological health, it is possible that the regulative effects would also impact the epigenetic regulatory components of the gamete. Moreover, it can provide an explanation of why physical neglect during early childhood seemed to have the greatest impact on levels of sperm miR-34c. However, even though multiple physiological changes accompany CME, the exact pathways on how CME affects the sperm epigenome are not known.

## 4.3 Effects of CME and the window of early puberty: Endocrinological and gamete related mechanisms

Overall, maltreatment and the prolonged stress response it causes, are linked to multiple deleterious factors such as sleep deficiency, elevated blood pressure, chronic inflammation, and endocrinological disturbances affecting the development and functioning of the reproductive system (Colten, 2006; Danese et al., 2007; Hart et al., 1996; Jensen et al., 1991; Jessen et al., 2015; Kaess et al., 2018; Li et al., 2024; Wen et al., 2024). Furthermore, previous studies in animals have demonstrated that pubertal testis development and testosterone production are suppressed by prolonged stress-related chronically elevated cortisol levels (Consten et al., 2001; Fernandino et al., 2013; Honda et al., 2008).

Stress prioritizes survival functions over reproduction, diverting energy and resources away from the reproductive system. This is reflected in the downregulation of the HPG axis during prolonged stress or chronic HPA activation (Li et al., 2024). As a result, secretion of gonadotropin-releasing hormone (GnRH), luteinizing hormone (LH), and follicle-stimulating hormone (FSH) are reduced, leading to decreased androgen production by Leydig cells. The reduced availability of testosterone disrupts the function of Sertoli cells and germ cells in the seminiferous tubules, impairing spermatogenesis. Furthermore, lower androgen levels from reduced testosterone impair Sertoli cell function, which is critical for germ cell support and maturation. This further disrupts spermatogenesis (Caroppo, 2009). However, the correlation between maltreatment and testosterone levels remains in need of further research**.**

Chronic elevation of growth hormone and testosterone in animal models are also known to have significant impact on testis development by the downstream effects of insulin-like growth factor 1 (IGF-1) (Griffeth et al., 2014; Xu et al., 2022). Based on these pathways, cortisol and growth hormones are likely to play a crucial role in the physiological changes observed in people subject to CME, moreover, in the development of testis and the male reproductive system as well as the maturation process of spermatozoa.

Early puberty is critical in gonadal activity and hormonal changes, alongside the remodeling of brain regions involved in emotional regulation (Gatta et al., 2021). Emotional abuse during this sensitive window may strengthen stress responses and disrupt spermatogenic pathways mediated by the HPG-axis. These disturbances could lead to specific epigenetic changes in

sperm, particularly in miR-34c. Additionally, pubertal timing is associated with the HPA-axis controlling stress response and development of the androgen-producing adrenarche. Chronic stress may result in hypoactivity of HPA, especially during adolescence and early childhood. (Mendle et al., 2016). Downregulated HPA, in turn, disturbs normal development of testis and spermatogenesis, mediated by androgen production and regulatory pathways (Rodgers et al., 2013). The association of CME specifically during early puberty with levels of sperm miR-34c complies with the previous findings of spermatogenesis disturbance as a result of chronic stress response in the HPA- and HPG-axis during puberty.

CME, like stress exposure in general, is known to affect also sleep quality and hours of sleep (Schønning et al., 2024). Sleep is vital for normal growth, hormonal balance and general homeostasis, immune system functioning and maintenance of brain functionality through the glymphatic system. Maltreated individuals are thus at greater risk of deleterious effects of sleep loss, such as obesity, diabetes, cardiovascular disease and hypertension, anxiety symptoms and substance use. (Colten, 2006; Jessen et al., 2015).

Indirect effects of chronic early life maltreatment exposure, such as stress-related, cortisol, testosterone or growth hormone-mediated outcomes and reduced sleep quality may explain the physical changes in the male reproductive system and gamete epigenome. Metabolic syndrome for example, which is highly associated with chronic stress, elevated cortisol levels, reduced sleep and obesity, is found to be more common among people with CME (Lee et al., 2014; Osode et al., 2024). Furthermore, in animal models metabolic syndrome is associated with testis abnormalities and declined spermatogonia differentiation and damaged spermatogenesis (Marchiani et al., 2015; Zhang et al., 2022). Thus, stress-related inflammation resulting from childhood maltreatment can contribute to the changes in gamete epigenome.

## 4.4 Intergenerational transmission in CME and implications on the brain of the offspring

As gamete transfers information from the parents to the progeny, is interesting to understand, whether parental CME can affect the health of their children. In previous animal studies, associations between early-life environmental stressors and the development of the progeny have been identified (Bohacek et al., 2015; De Castro Barbosa et al., 2016; Donkin and

Barrès, 2018; Gapp et al., 2014; Rodgers et al., 2013). Therefore, a similar outcome can be expected to be in humans as well.

MiR-34c, a sperm-enriched miRNA, is important in post-fertilization events. It has been shown to facilitate the degradation of maternal mRNAs in zygotes, a process essential for the activation of the embryonic genome and normal preimplantation development. Experimental studies in mice demonstrated that inhibition of miR-34c in zygotes significantly reduced the rate of two-cell and four-cell embryo formation, as well as blastocyst development (Cui et al., 2023)**.** This indicates its critical function in early cell division and embryo viability. Moreover, in addition to miRNAs, intergenerational transmission of CME in animal studies has been linked to multiple sperm sncRNA subgroups such as transfer RNA derived RNA fragments (tRFs), P-element Induced Wimpy testis interacting RNAs (piRNAs), long linear RNAs and circular RNAs (circRNAs) (Kretschmer and Gapp, 2022). However, the possible mechanism of inheritance is not comprehensively understood, neither in animals nor in humans. Therefore, while associations were found between CME and sperm epigenome, no conclusions can be drawn to the possible inheritance of qualities to the next generation of humans based on this or the previous studies. This would necessitate measuring offspring outcomes in addition to parental exposure and sperm epigenome.

In rodent studies, changes in sperm miRNA resulting from exposure to moderate or high stress, have been also shown to result in changes in behavioral, metabolic, and phenotypical changes in the progeny and in some cases also changes in the sperm epigenome of the F1 generation (Gapp et al., 2014; Rodgers et al., 2013; Wang et al., 2021). Further studies amongst humans are needed to determine whether the changes in sperm RNA obtained through early life stressors are shown in F1 and F2 generations, as well as to determine the impact on metabolism and regulation of the HPA-axis. On the contrary, the plasticity of the inherited epigenome needs to be further studied both in animal and human groups to understand the longitudinal effects of the sperm miRNA changes occurred and the overall inheritance of environment-induced epigenetic changes.

In addition to environmentally mediated damage to the individual, there is evidence that genetics can affect the risk for direct mental health effects of childhood maltreatment (Chen et al., 2025). Furthermore, genetic linkage has been demonstrated by observing overlap in genes

in individuals with similar maltreatment backgrounds (Warrier et al., 2021). However, there is yet little study on this area and the impact on physical effects remains unresolved.

## 4.5 Limitations

The most significant limitation of this study is the low number of participants, which was only 30. The previous studies on humans shown in Table 1 had also relatively few participants, varying from 28 to 72. To ensure the reliability of the results, further studies are needed to replicate the findings and if possible, to test the linkages in larger cohorts of participants. Since this study had a low number of participants, it is prone to sampling bias and type I (false positive) and II (false negative) errors in findings. We did not formally control for multiple comparisons, but the p values of the main findings would survive Bonferroni correction across the three main models (corrected $p = 0.05 / 3 = 0.0167$).

Prospective study on maltreatment and its health and physiological impacts is challenging to perform as it is unethical to cause CME to participants intentionally. Therefore, in this study, as in the previous ones, we relied on retrospective questionnaire material combined with physiological samples. However, this kind of data is prone to multiple errors. Firstly, the individuals that participate in such studies represent only partly the entire population which poses important limitations to the generalizability of the findings as the participants don't fully represent neither the entire population or the part of the population that has been exposed to more extreme levels of CME. Children exposed to various forms of maltreatment have a higher tendency of brain dysfunctions and health issues leading to memory deficiency, social challenges, autoinflammatory diseases, and higher mortality (Benoit et al., 2008). The part of individuals with difficult stages of such symptoms are unlikely to attend such studies and may have reduced likelihood of having offspring, which was not the case for the participants in this study.

Furthermore, the questionnaire data that is the most common way of assessing CME is prone to subjective estimating bias, intentional and unintentional misreporting, and low accuracy. Additionally, the age of each participant may affect the reported information. It should be noted, for example, that individuals with psychological symptoms may remember traumatic events differently than individuals whose experiences occurred a long time ago. Similarly, the subjective experience of traumatic events may be different for different generations.

Within this study, the level of overall CME slightly increased with age, which can also result from the loss of memories of traumatic events from which a significant time period has passed. Reported emotional and physical abuse also increased with age. However, emotional and physical neglect was reported mostly at ages of 0-6 years and 13-18 years and being lowest at early puberty at age 7-12 years. Only one participant reported sexual abuse, and this CME type has thus been left out of this study.

In this study, the number of participants reporting each type of maltreatment was unequal leaving only a few samples to study for part of the CME types. According to Scannapieco et al. neglect is the most common type of maltreatment (2005). Similarly, in this study emotional neglect was the most common form of reported maltreatment (mean TADS-score 4,8) and physical neglect second common (mean TADS-score 3,3), followed by emotional abuse (mean TADS-score 3,0) and physical abuse (mean TADS-score 2,0). Sexual abuse was only reported once and therefore does not provide reliable information. It is notable, however, that most participants who reported CME, reported having experienced more than one type of maltreatment. Moreover, it was common to have experienced the same type of maltreatment during the entire childhood, or at least at two different age groups. Thus, separating the impacts of each age and maltreatment type, was not fully possible.

The co-occurrence of physical and emotional maltreatment often results from overlapping risk factors and dynamics within the abusive environment. Perpetrators of maltreatment frequently engage in multiple forms of abuse due to factors like impaired emotional regulation, unresolved trauma, or substance abuse (Felitti et al., 1998). Additionally, social and environmental stressors, such as poverty or intergenerational patterns of violence, worsen these behaviors. Emotional maltreatment often occurs alongside physical abuse as a means of control or as a consequence of emotional dysregulation in caregivers, creating a cycle where both forms of abuse reinforce each other. (Felitti et al., 1998; Scannapieco and Connell-Carrick, 2005). Thus, examining each age group and maltreatment type as fully independent variables is not possible, as they correlate with each other. The associations obtained can therefore be taken only as indicative.

One of the strengths of the study is that its participants had a wide age range. The ages of the participants varied from 30 to 48 years, the mean age being 39,1 years. Another strength of

this study is that it is based on a large cohort study enabling a sampling from a versatile population that has been fertile. Additionally, the study is performed on a relatively healthy population that is not suffering from infertility, showing that childhood maltreatment does not just affect psychiatric and IVF clinic patients but has notable associations with the epigenome of healthy individuals as well.

Finally, there are multiple factors that can affect the outcome of both forms of data used, the samples and the questionnaire results, as well as the actual situation of the individuals. The state of the physiological environment, experienced culture, norms and beliefs, personality traits and protective psychosocial and physical factors could not be considered in this study but are likely to have an impact on the results. Further study is needed to expand the sample size and geographic covering, and to specify further details such as the source of maltreatment, mother/father/other caregiver/other not close person.

## 4.6 Conclusions

The differential timing of the critical periods of early childhood and adolescence reflects the interaction between biological maturation processes and environmental influences. Early childhood experiences shape the baseline functioning of regulatory systems, while experiences during puberty amplify or alter these systems as they undergo further development. Moreover, epigenetic changes in sperm linked to early life CME are likely related to the importance of this period on the CNS and the endocrinological systems it regulates. In contrast, epigenetic changes observed when CME was experienced during early puberty, may stem from the spermiogenesis-related regulatory role of sperm miRNAs. Emotional abuse during early puberty may also coincide with the heightened activity of stress-responsive pathways like the HPA-axis, further influencing epigenetic markers like miR-34c.

From all types of maltreatment emotional abuse had the strongest association with levels of miR-34c. While specific studies directly linking emotional abuse to gamete abnormalities are limited, the stress and systemic inflammation caused by maltreatment, as well as the HPA-axis dysfunction and hormonal imbalances, could indirectly impair gametogenesis. However, further research is needed on the specific physiological impacts of childhood emotional abuse.

Chronic stress from CME affects hormonal balance, notably cortisol and testosterone, and can lead to metabolic syndrome, inflammation, and sleep disturbances. These factors collectively impact reproductive health and epigenetic markers such as miRNA. To date, similar research on the associations of specific types and ages of CME to the sperm epigenome has not been published. Further studies are needed to evaluate the findings of this study. While the study is based on a large cohort, the number of participants that have also participated in sperm sampling is limited to 30, which underlines the need for similar studies to comprehensively understand the associations and possible causality.

Understanding the associations between the experienced maltreatment and changes that are likely to occur in sperm sncRNA levels allows further research on possible epigenetic inheritance of CME-related changes in humans. Furthermore, this enables understanding of whether possible epigenetic changes in sperm resulting from CME can result in changed gene expression and gene-mediated behavioral changes in the next generations.

Considering the diverse indirect effects of CME, the complexity of the possible causality of various forms and ages of CME to the epigenetic changes in gamete leaves gaps in understanding the outcomes of this study. However, this study can be seen as further evidence of the importance of age and quality of maltreatment to the health and development of the individual and their gamete, as well as the suspect of its intergenerational physiologically mediated effects. Furthermore, possible impacts on the progeny would require further research.

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