

## Review article / Revue

# The fatty acid ethyl esters (FAEE) hair test: emerging technology for the diagnosis of fetal alcohol spectrum disorders (FASD)

## *Dosages des esters éthyliques d'acides gras (FAEE) dans les cheveux : une nouvelle aide pour le diagnostic du syndrome d'alcoolisme fœtal*

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**Abstract – Aim:** Fetal alcohol spectrum disorder (FASD) is the most prevalent cause of neurocognitive handicap among North American children. A serious challenge in the diagnosis of FASD is the need to document excessive maternal drinking during pregnancy, however maternal self-report is often unreliable creating a need for an objective biomarker. **Methodology:** Testing for xenobiotics in hair has been gaining popularity in recent years as a screening method for drug use because of its unique advantage of being non-invasive and providing a stable, long-term record of past and or chronic drug exposure. The recent advent of a hair test to measure excessive alcohol use, the FAEE hair test, has opened the door to exploring its use as a new diagnostic tool for FASD. The current article briefly reviews recent advances in research involving the FAEE hair test in this context. **Conclusion:** Recent advances in research involving the FAEE hair test suggest that FAEE hair analysis may be a powerful tool in detecting heavy alcohol use in the perinatal period and in FASD diagnosis.

**Key words:** Foetal alcoholism spectrum disorder biomarker, FAEE, hair testing

**Résumé – Objectif :** Le syndrome d'alcoolisation fœtal (SAF) est en Amérique du Nord la première cause de handicaps neuro-cognitifs chez l'enfant. Quantifier la consommation excessive pendant la grossesse apparaît crucial pour le diagnostic du SAF, cependant l'information fournie par la mère elle-même apparaît souvent peu fiable, et rend nécessaire le recours à un marqueur biologique fiable. **Méthodes :** La recherche de xénobiotiques dans les cheveux s'est généralisée, ces dernières années, pour détecter l'usage de produits toxiques : elle présente l'avantage de ne pas être traumatique, tout en informant de façon continue, sur de longues périodes quant à l'exposition passée ou chronique à des xénobiotiques. L'arrivée récente d'un test capillaire pour évaluer la consommation excessive d'alcool – recherche et dosage des FAEE – a posé la question de son utilisation comme nouvel outil de diagnostic du SAF. Le présent article passe en revue les avancées récentes concernant l'utilisation du test FAEE dans ce contexte. **Conclusion :** Les recherches relatives au test FAEE montrent que l'analyse des cheveux par ce test peut se révéler un outil puissant pour détecter la consommation d'alcool à hautes doses en période prénatale, et peut être utilisé pour le dépistage du SAF.

**Mots clés :** Syndrome d'alcoolisme fœtal, biomarqueurs, FAEE, analyse des cheveux

Travail présenté au 46<sup>ème</sup> congrès TIAFT, juin 2008, La Martinique

Received 25 September 2008, accepted after revision 12 February 2009  
Published online 12 June 2009

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## 1 Introduction: the fetal alcohol spectrum disorder (FASD)

Prenatal exposure to alcohol can result in a variety of negative outcomes for the child, which can include birth defects, cognitive impairments, as well as social disabilities. Previously, different terms have been used to describe various associated disorders such as fetal alcohol syndromes (FAS), partial FAS, fetal alcohol effects (FAE), alcohol-related birth defects (ARBD), and alcohol-related neurodevelopmental disorder (ARND). However, more recently the term fetal alcohol spectrum disorder (FASD) has been used as an umbrella term to encompass the majority of these disorders. FASD is the most prevalent cause of neurocognitive handicap among children in North America [1, 2]. It has been estimated that approximately one percent of the pediatric population is affected [3, 4], but the majority of cases remain undiagnosed until school age or later, if diagnosed at all [5, 6]. One of the most serious challenges in diagnosing FASD is the need to establish evidence of excessive maternal drinking during pregnancy as one of the major diagnostic criteria [7]. However, maternal self-reports of alcohol use are often unreliable due to fears of stigmatization, embarrassment, shame or guilt [8–11]. Therefore, establishing a biological marker that can detect chronic excessive alcohol use during pregnancy would provide a tremendous advantage in detecting children at risk for FASD, and for diagnostic purposes.

## 2 The use of fatty acid ethyl esters (FAEE) and advantages of hair

The measurement of fatty acid ethyl esters (FAEE) in hair and meconium (the fecal matter that accumulates in the fetus during pregnancy, and which is discharged soon after birth) has demonstrated significant potential as a biomarker for fetal alcohol exposure [12–24]. FAEE are non-oxidative metabolites of ethanol metabolism formed by the conjugation of ethanol to endogenous free fatty acids and fatty acyl-CoA, a reaction catalyzed by microsomal acyl-coA:ethanol O-acyltransferase (AEAT) and cytosolic FAEE synthase [25, 26]. Although FAEE can be detected in hair, meconium, blood, and various organs [15, 16, 26–31], its measurement in hair holds the significant promise for the detection and facilitation of diagnosis of FASD because of its unique advantages. Hair can be obtained from the mother, or infant, is non-invasive, and can measure long-term, past exposure. Furthermore, compared to meconium, hair offers a larger window for detection because neonatal or maternal hair can be collected months, or in some cases years after birth, whereas meconium is only available for the first 2–3 days of infant life. FAEE are also very stable in hair, lasting for long periods of time that can include years, even at room temperature, whereas their stability in meconium may last only days if not properly frozen [17, 18, 32]. Therefore, the FAEE hair test's versatility and robustness make it an attractive candidate for use as a biomarker of excessive alcohol use.

## 3 Hair FAEE using animal models

Since its inception in 2001, the FAEE hair test has been increasingly used in the medical context of alcohol abuse, and the medicolegal context of “driving under the influence” [10, 33] because of its high sensitivity and specificity in the detection of excessive drinking in adults [30, 34]. More recently, however, the Motherisk laboratory has pursued research using animal models to investigate the test's potential as a biomarker for FASD diagnosis. We developed a guinea pig model to study the relationship between alcohol exposure during pregnancy and FAEE levels in the hair of dams and their offspring. It was discovered that dams and offspring exposed to alcohol had significantly higher levels of FAEE (10 and 15-fold respectively) compared to controls [20]. A subsequent study revealed a significant positive correlation between systemic exposure to alcohol and the hair FAEE, ethyl oleate, for both guinea pigs and humans, and that humans on average produced over 10-fold higher levels of FAEE in their hair than guinea pigs for the equivalent exposures [35]. Together, this data suggests that the FAEE hair test should be very sensitive in detecting alcohol exposure in mothers and neonates.

As important in the development of any new hair test, the issue of a potential hair colour bias was also investigated using an animal model. Although still in hot debate [36], many studies have investigated and reported the existence of a potential hair colour bias, based on interactions between melanin granules and drugs with primarily cationic, or basic properties [37–41]. In order to test the hypothesis that FAEE do not preferentially accumulate in pigmented hair, the Motherisk laboratory developed an experimental model using Long Evans rats; rats that contain naturally occurring black and white patches of hair. Results show that FAEE are detectable in this model post-treatment with ethanol, and that there is no statistical difference in levels of cumulative FAEE between white and black hair, respectively [42]. Therefore, these results indicate the FAEE hair test is not likely susceptible to a hair colour bias.

## 4 Alcohol use in the family and FASD

Excessive alcohol use by parents puts families at risk for having children with FASD and contributes to the propagation of this disorder across generations. Clearly maternal alcohol use during pregnancy is a necessary factor in the etiology of FASD, however, several studies have discussed the role of paternal alcohol use both as a direct teratogenic factor, and more importantly, as a contributing factor towards maternal alcohol use before and during pregnancy [43–50]. For example, having a stable relationship with strong emotional and social support, and a planned pregnancy can help to reduce alcohol use during pregnancy, whereas having an alcoholic partner who is not supportive of alcohol abstinence efforts or perhaps abusive can have the opposite effect [43, 50]. Alcohol use in the family, paternal use in particular, has been linked to increased risk of child abuse, as well as future alcohol abuse by children themselves [51–53]. This can predispose children to behaviors that can perpetuate the occurrence of FASD as they grow into child bearing years [43, 51–57]. Therefore, a familial approach in

the detection of alcohol use is important for advances in FASD research.

Furthermore, alcohol use has been demonstrated to be a major problem in many families involved in the child welfare system, putting this population at high risk for having children with FASD [58–60]. A recent Canadian study revealed that out of children with disabilities in the care of child welfare services in Manitoba, 34% were diagnosed with FASD, representing 11% of all children in care [61]. Therefore, this population is an at-risk population for FASD and in need of targeted research efforts.

## 5 Hair FAEE in families at-risk for FASD

Since October 2005, the Motherisk laboratory in Toronto, Canada, in collaboration with the Pragst's laboratory in Berlin, Germany, opened the FAEE hair test for clinical use to Canadian children's aid societies. Since that time the overall level of excessive alcohol use in this cohort was determined by FAEE hair analysis to be 33% ( $n = 324$ ) and 18% in pregnant women ( $n = 28$ ) [62]. The high prevalence of excessive alcohol use in this cohort greatly exceeds Canadian norms for the general adult population. The Canadian Addiction Survey of 2004 reported that although 79.3% of Canadians said they consumed alcohol in the past 12 months, only 7.9% consumed alcohol more than 4 times a week, and only 12.7% said their typical usage pattern of alcohol was more than 5 drinks on occasion [63]. The high prevalence of positive tests found in parents and pregnant women involved with children's aid societies corroborates social workers' clinical suspicions of heavy alcohol use and confirms that this population is indeed at greater risk for having children with FASD.

## 6 Combined use of hair FAEE with hair ethyl glucuronide

A recent pilot study, involving the measurement of hair FAEE in pregnant women of a general obstetric population in Sweden, found that 3% of women were positive for excessive alcohol use by FAEE hair analysis, however, a total of 7% were identified by the combined use of FAEE and ethyl glucuronide, EtG, hair analysis [10]. EtG is minor metabolite of alcohol metabolism produced when alcohol is glucuronidated with activated glucuronic acid (UDP-GA) [64]. Several studies have investigated the measurement of EtG in hair [33, 65–76]. Hair analysis of EtG has been shown to be highly specific (100%) in the detection of heavy alcohol users, however there have been variable reports with regards to the test's sensitivity, ranging between 50 and 100%. This variation is likely due to the large range of analytical limits of detection (LOD) reported between laboratories [64–68] moreover, a previous study showed that in a cohort of heavy drinkers both FAEE and EtG hair tests were able to identify all alcoholic patients, therefore, the tests acted redundantly [68]. Consequently, it remains possible that the use of both tests may have added value for the detection of pregnant alcohol abusers; however, more research is required to determine the cost versus the benefits.

## 7 Conclusion

Fetal alcohol spectrum disorder is a devastating and preventable condition. Detection and diagnosis are key to intervention in reducing the incidence and severity of FASD. Establishing maternal alcohol use during pregnancy is a necessary criterion for the diagnosis of FASD. The FAEE hair test provides a unique advantage of being able to confirm or refute clinical suspicions of chronic alcohol abuse in an objective manner. Furthermore, the FAEE hair test can be used to assess excessive alcohol use in the family in order to mobilize resources to decrease the occurrence and propagation of this disorder. Recent advances in research involving the FAEE hair test suggest that FAEE hair analysis may be a powerful tool in detecting heavy alcohol use in the perinatal period, and in FASD diagnosis.

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