

# Paternal aging and associated intraindividual alterations of global sperm 5-methylcytosine and 5-hydroxymethylcytosine levels

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**Objective:** To evaluate the relative intraindividual changes in sperm 5-methylcytosine (5-mC) and 5-hydroxymethylcytosine (5-hmC) levels associated with age and to compare the levels of 5-hmC in mature human sperm to blood DNA.

**Design:** Prospective research study.

**Setting:** University-based andrology and in vitro fertilization (IVF) laboratory.

**Patient(s):** Fifteen known fertile sperm donors, 22 other known fertile controls, and 41 male blood donors from a general population tissue bank.

**Intervention(s):** None.

**Main Outcome Measure(s):** Measurements of global 5-mC and 5-hmC levels via an enzyme-linked immunosorbent assay (ELISA)-based assay.

**Result(s):** Global sperm 5-mC levels exhibit a statistically significant increase with age, and a similar trend was seen in 5-hmC levels. On average, in the age ranges we analyzed, 5-mC increased by 1.76% per year, and 5-hmC, though more variable, increased by approximately 5% per year. Additionally, we found that 5-hmC levels in sperm are 32.59% of that found in blood DNA.

**Conclusion(s):** Global sperm DNA methylation patterns are stable over short periods of time but increase with age, which raises important questions regarding the risks of advanced paternal age. Additionally, as we would predict for a transcriptionally quiescent cell type, 5-hmC levels are statistically significantly lower in human sperm than in blood DNA. (Fertil Steril® 2013; ■: ■-■. ©2013 by American Society for Reproductive Medicine.)

**Key Words:** Advanced paternal age, global DNA methylation, 5-hydroxymethylcytosine, 5-methylcytosine, sperm DNA methylation

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**A**ging is known to severely affect female fertility, including elevating the risk of spontaneous abortion, chromosomal defects in the offspring, preterm delivery, and intrauterine growth restriction (1). In contrast, the effect of advanced paternal age on fertility, pregnancy outcome, and offspring health has received far less

attention. However, recent studies linking older fathers to an increased prevalence of several neuropsychiatric disorders in the offspring has increased interest on the effect of advanced paternal age on offspring health. Specifically, various reports have linked increased paternal age at conception with bipolar disorder, schizophrenia,

and autism in humans as well as decreased social and exploratory behavior in animal models (2–5). Additionally, studies have shown an association between advanced paternal age and increased DNA damage and decreased chromatin integrity in addition to adverse birth outcomes, including alterations in weight, premature delivery, and various other offspring abnormalities (6–9).

The etiology of the increased frequency of these various disorders in the offspring of aged males remains poorly defined, though epigenetic mechanisms are an obvious possibility. It has been demonstrated that DNA

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methyltransferase 3a (DNMT3a) and 3b (DNMT3b) expression increases with the age of the tissues investigated (10). Additionally, it is known that DNA methylation is altered in many somatic cell types with age, but similar changes in the sperm remain uncharacterized (11, 12). Despite this, the age-related DNA methylation modifications that occur in virtually every other tissue in the body suggest that similar patterns exist in sperm. In further support of this idea is work demonstrating that frequently dividing cells have more striking methylation changes associated with age than do cells that divide less often (13). Thus, alterations in sperm DNA methylation represent an undefined but highly plausible mechanism contributing to the increased incidence of neuropsychiatric disorders seen in the offspring of older fathers.

Our understanding of the paternal epigenetic landscape has increased dramatically in the recent past. Among the important epigenetic marks in sperm is DNA methylation at cytosine residues (5-mC). These marks are typically associated with gene silencing by inhibiting access of transcription-related binding proteins to the DNA. Recently, there has been increased interest in the role of DNA demethylation intermediates such as 5-hydroxymethylcytosine (5-hmC), which is formed by the 10-11 translocase (TET) family of enzymes. These marks are thought to play a regulatory role in gene expression and/or poisoning. The current literature describes unique epigenetic marks in sperm that are capable of facilitating proper function in the mature gamete as well as marks that are uniquely capable of poisoning specific genes in the early embryo for activation (14, 15). These data establish the importance of the paternal germline for embryogenesis in a way that was never previously understood.

It is clear from the available evidence that the inheritance of DNA methylation alterations is a plausible candidate for the etiology of many heritable disorders previously considered idiopathic in nature. Additionally, aberrations in the sperm methylome may contribute to the elevated risk of certain disorders associated with advanced paternal age. We describe the relative changes in global DNA methylation that occur through the process of aging by comparing semen samples collected by sperm donors many years apart. This study involved three basic aspects. First, we sought to determine the effect of aging on sperm 5-mC and 5-hmC levels. Second, we analyzed the intraindividual variability of 5-mC and 5-hmC levels in samples collected within 14 days of each other. Finally, we evaluated the difference in 5-hmC levels in sperm and blood DNA.

## MATERIALS AND METHODS

### Study Participants

Our laboratory has a research tissue bank that includes sperm and blood samples from previous sperm donors. Fifteen semen donors of known fertility who had previously banked samples at our laboratory were asked to return and collect a sample in 2008 under a protocol approved by the institutional review board. The time between the sample collections varied between 9 and 21 years. The basic semen analysis data for the two collections are available in [Supplemental Table 1](#) (available online). The age at early collection for the donors in our sample set was between 27 and 56 years ([Fig. 1](#); [Table 1](#)). To

**FIGURE 1**

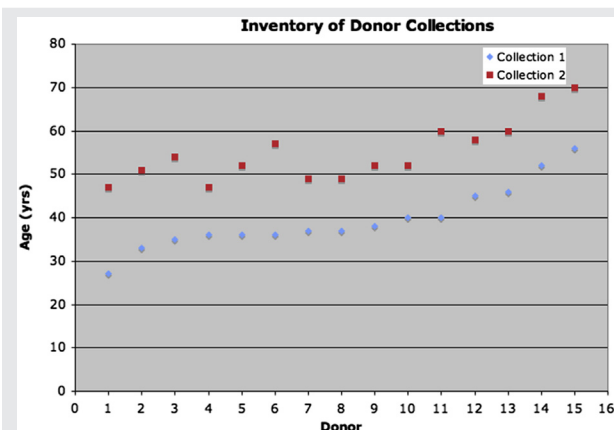


Illustration of the ages at which donors collected their samples. Nine of the 15 donors collected three samples, and the remaining six donors only collected two samples.

Jenkins. Sperm DNA methylation and aging. *Fertil Steril* 2013.

determine the intraindividual variability of sperm DNA methylation levels, 17 sperm donors collected three samples during a period of less than 14 days. These samples were used to test the consistency of sperm DNA methylation over a very short period of time.

To compare the levels of 5-hmC in sperm and blood DNA, we analyzed 52 sperm samples and 41 blood samples from our general population tissue bank. These donors are from a nonselected pool of men ([Table 1](#)).

### Sample Collection and DNA Extraction

The men provided both semen and blood samples. The donors were required to strictly follow the University of Utah Andrology Laboratory collection instructions, which include abstinence time of between 2 and 5 days. After collection, the samples were frozen with test yolk buffer (TYB; Irvine Scientific) and were stored in liquid nitrogen before DNA isolation. No sperm selection method was employed as it was our aim to observe changes to all sperm in the ejaculate.

The samples were thawed and DNA extracted simultaneously to decrease batch effects. Sperm DNA was extracted using a sperm-specific extraction protocol used routinely in our laboratory. Before DNA extraction, we employed a

**TABLE 1**

Patient demographics of semen and blood donors.

| Sample set         | No. of patients | Body mass index ( $\pm$ SEM) | Age ( $\pm$ SEM)    |
|--------------------|-----------------|------------------------------|---------------------|
| Young              | 15              | 27.05 ( $\pm$ 0.93)          | 39.33 ( $\pm$ 2.02) |
| Aged               | 15              | 28.32 ( $\pm$ 1.37)          | 53.27 ( $\pm$ 1.92) |
| General population |                 |                              |                     |
| Blood samples      | 41              | 27.16 ( $\pm$ 0.88)          | 37.64 ( $\pm$ 1.65) |
| Sperm samples      | 52              | 26.44 ( $\pm$ 0.46)          | 38.20 ( $\pm$ 1.18) |

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