


LETTER TO THE EDITOR

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Epimutations in human sperm from patients with impaired spermatogenesis



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In this Letter to the Editor, we comment on the recent publication by Leitão et al. *The sperm epigenome does not display recurrent epimutations in patients with severely impaired spermatogenesis* (Clinical Epigenetics 2020, DOI: 10.1186/s13148-020-00854-0), where concerns about the validity of our studies reporting imprinting errors in human sperm from infertile patients have been raised.

We read with great interest this recent publication describing genome-wide methylation in human sperm from oligozoospermic patients, which could be an important resource to understand the extent of methylation defects and whether these are restricted to imprinted genes.

However, we were surprised to find that the Authors attributed imprinting methylation errors to contamination of somatic cells and even stated that they “suspect that other studies also suffer from DNA contamination issues”.

Thereby, we would like to clarify that, in our studies, in order to eliminate somatic cell contamination, we have prepared sperm by density gradient centrifugation (DGC) (using density gradients) prior to swim-up separation, as we previously described [1, 2]. The sequential method of DGC swim-up is the elective method for sperm preparation to be used in assisted reproductive treatments (ART) [3]. Additionally, we visually inspected, by optical inverted microscopy with Hoffman modulation contrast, a 20- μ l droplet of all the samples that were included in our studies, before using sperm samples in experiments.

We noticed that in the study by Leitão and collaborators [4], density gradient separation was not performed and direct swim-up was employed, thereby increasing the

likelihood of having somatic cells contamination in their sperm samples, as is suggestive from the author’s WGBS data, including for *H19* methylation values (ranging from 75 to 82% methylation in normal controls in their study vs 95% in our study [2]). For density gradient centrifugation, we used Puresperm gradients (Nidacon, Gothenburg, Sweden) which contain silane-coated silica particles that enable motile sperm to be separated from non-germinal cells and seminal plasma. The advantages of the density gradient method is the attainment of an excellent yield of highly motile normal spermatozoa, whereas leukocytes, bacteria, epithelial cells, cell debris, and most abnormal sperm are eliminated, while sperm DNA fragmentation and reactive oxygen species are significantly reduced [3, 5]. In fact, our team has more than 20 years of experience in preparing semen samples for ICSI and to date sperm samples obtained after gradient centrifugation followed by swim-up were never found to be contaminated by leukocytes and, in ICSI, purified sperm are selected individually.

Moreover, in our studies conducted on testicular sperm, that were individually isolated by micromanipulation from testicular biopsies [6, 7], and therefore not contaminated with somatic cells, we have also observed imprinting errors, namely *H19* DMR hypomethylation and *MEST* DMR hypermethylation.

Nevertheless, we agree with the Authors that it is necessary to reassure patients undergoing IVF treatments and our works contributed to this purpose by showing a very low frequency of sperm with completely altered methylation patterns, in infertile patients.

Furthermore, in most of the cases presented in our results, the patient producing sperm with imprinting errors also carried sperm with correct methylation patterns. In the future, we hope to understand how these imprinting errors occur, in order to contribute to improve the safety and efficacy of assisted reproduction techniques.

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Author's response to Marques et al.

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We appreciate the comments by Marques et al. and their efforts to purify spermatozoa, which go far beyond what most researchers in this field do. Most of the previous studies were performed on swim-up samples, and we have found that samples prepared in this way can be contaminated with somatic DNA. Of course, we could not reanalyze the samples that were used by others, but our lack of finding any evidence for an epimutation in 93 sperm samples, which make our study one of the largest in its field, justifies our conclusion that “the prevalence of aberrant methylation in swim-up purified sperm of infertile men has likely been overestimated” [4]. Irrespective of the purification procedure used, the DNA should always be prescreened for somatic DNA contamination by bisulfite sequencing of at least the four genes suggested in our paper (*H19*, *MEST*, *DDX4*, and *XIST*) or the four genomic loci suggested by Jenkins et al. [8].

Unfortunately, Marques et al. have not considered genetic variation as another confounder in this type of studies. As shown by us, a common genetic variant at the *H19* locus is associated with DNA hypomethylation, which should not be confused with an infertility-associated epimutation [4].

Abbreviations

DGC: Density gradient centrifugation; ART: Assisted reproductive treatments; WGBS: Whole-genome bisulfite sequencing; ICSI: Intracytoplasmic sperm injection; DMR: Differentially methylated region; IVF: In vitro fertilization

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Competing interests

The authors declare that they have no competing interests.

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