Presence of M540 bodies in human semen: techniques to detect them require attention

TO THE EDITOR: We read with interest the paper by Gomez-Lopez et al. published in *Fertility and Sterility* (1), in which the authors investigated the presence of merocyanine 540 bodies (M540 bodies) and their impact on the detection of sperm apoptotic markers, including sperm DNA fragmentation. The authors report that the incidence of M540 bodies in the semen of infertile men is very low (~1%) and that their occurrence does not affect the determination of sperm DNA fragmentation (sDF) by TUNEL coupled to flow cytometry.

These results contrast with data from our group that first described M540 bodies (2) and later demonstrated that they are apoptotic bodies of testicular origin (3). Indeed, we found that M540 bodies can be present in high amount in semen of sub/infertile men (2, 3) and that they cause great error in the determination of TUNEL-positive sperm by flow cytometry (4). We think that the cause of such discrepancies is the technique that the authors used to reveal M540 bodies in TUNEL-processed samples. They first stained by M540 and then washed and processed samples by TUNEL assay. By this procedure, merocyanine labeling is washed away from the bodies, because M540 does not bind in a stable (covalent) manner to bodies. As a consequence, they failed to detect M540-positive elements, not because of their absence but because of the loss of merocyanine staining.

Further, Gomez-Lopez et al. give a definition of M540 bodies that is different from that of the studies first reporting these semen elements (2, 3). They defined as M540 bodies only those elements that are positive for both M540 staining and TUNEL. However, only a small fraction of M540 bodies show detectable DF, whereas most M540 bodies appear devoid of fragmented chromatin (4). In any case, we have clearly shown that their occurrence heavily interferes with the measures of sDF by flow cytometry (4), because such interference does not depend on the possible presence of fragmented DNA within them, but on the fact that they can mask fractions of DNA-fragmented sperm and/or contribute to increase the percentage of global TUNEL-negative events (4).

Finally, Gomez-Lopez et al. claim that the interference of M540 bodies on the flow cytometric measures of sDF was denied by us in a recent comment (5) about a study comparing the levels of sDF between donors and infertile patients and between neat semen samples and sperm samples selected by density gradient centrifugation. Contrary to what was stated by the authors, in that comment we did stress the importance of excluding M540 bodies from the flow cytometric analyses of spermatozoa, especially in the comparisons between samples with a possible difference in incidence of M540 bodies, such as donors and patients or neat semen and selected sperm.

Because M540 bodies can affect any flow cytometric analysis of spermatozoa, and they represent a sign of impaired testis function/apoptosis, we think that the issue of the presence of these elements in semen should not be disregarded.

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