Title
Reduction of sperm DNA fragmentation using the magnetic activated cell sorting (MACS) with annexin V microbeads in assisted reproduction.

Study question
To evaluate sperm DNA fragmentation rates of the non-apoptotic sperm fraction of patients before ICSI.

Summary question
Using the MACS, in the most patients received improvement in sperm DNA fragmentation, but there are cases when the level of sperm DNA fragmentation has not been modified or decrease was obtained.

What is known already
Sperm DNA integrity is an important paternal factor that has been shown to influence fertilization rates, embryo development and pregnancy rates. To reduces the number of apoptotic spermatozoa many technics are used before ICSI treatment (e.g. using of antioxidants, testicular spermatozoa). Recently, the use of annexin V microbeads, as a non-invasive method to reduce high levels of sperm apoptosis, could be a new tool to optimize sperm selection in assisted reproduction.

Study design, size, duration
From July 2011 to December 2012, a total of 171 ICSI patients with sperm DNA fragmentation 20.84 ± 3.47% (range 15-30%) were recruited to this study.

Participants/materials, setting, methods
The basic semen analysis of sperm count, motility and DNA fragmentation before and after MACS were evaluated. DNA fragmentation was measured by the terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assay.

Main results and the role of Chance
Reduction sperm DNA fragmentation was achieved in 153 patients (89.47%), in 7 patients (4.1%) there was no difference before and after MACS, while in 11 patients (6.43%) was obtained increase. The average level of DNA fragmentation before MACS in the group of 153 patients was 20.84 ± 3.47% and after MACS was 9.76 ± 5.44%. In the group of 11 patients respectively 23.09 ± 0.03% and 30 ±0.07%. No improvement in the level of DNA fragmentation may be associated with an early stage of apoptosis, where was no presentation phosphatidylserine (PS) residues yet or other cause apoptosis not linked with DNA fragmentation. The increase can be explained by advanced process of apoptosis, when additional preparation could intensified damage of spermatozoa.

Limitations, reasons for caution
MACS of sperm samples with elevated count of leucocytes can be performed incorrectly. PS-binding site can be occupied by other cells, therefore apoptotic spermatozoa could flow through the column.

Wider implications of the findings

In spite of the MACS significantly improves the results of sperm DNA fragmentation, patients especially with high levels of DNA fragmentation should undergo a trial MACS before IVF. Even if MACS does not change result of sperm DNA fragmentation it still should be recommended, because it could eliminate PS positive apoptotic spermatozoa.

Study funding/competing interest(s)

None of the authors has any competing interest. The study was not supported by an external grant.

Keywords:

annexin V microbeads, apoptosis, DNA fragmentation, MACS, spermatozoa,

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