

9th Yazd International Congress and Student Award on Reproductive Medicine with 4th Congress of Reproductive Genetics

Oral Presentations

O-20

Annexin-V MACS sperm selection method could be effective on separation of sperm with high expression of *PLCZ1* gene and development of high blastocyst rate in male factor patients with high DNA fragment

Salehi Novin M, Zandieh Z, Bakhtiari M, Aflatoonian R.
Department of Anatomy, Iran University of Medical Sciences, Tehran, Iran.
Email: maryam.salehi74@gmail.com

Background: Sperm selection according to morphology and motility in assisted reproduction technologies (ART), is not sufficient for selecting the best sperm especially in patients who have male factor problems. Apoptotic sperm and non-apoptotic ones are distinguished from each other by negative selection in Annexin-V magnetic-activated cell sorting (MACS) technique. So, compaction rates in embryo quality are enhanced in this method. *PLCZ1*, which is one of the oocyte activating factors, starts oscillations of Ca^{2+} in oocyte and it has a significant impact on the fertilization and implantation process.

Objective: In this study, hypostasis of sperm selection relying on motility and morphology in ART techniques, not be sufficient for selecting the most qualified sperm especially in male factor patients are shown. Apoptotic sperm cells and non-apoptotic ones are distinguished from each other in the Annexin-V MACS-DGC technique. Therefore, this method enhanced the quality of embryo compaction rate.

Materials and Methods: Semen samples of 30 infertile couples who have male factor problem with DNA fragmentation index (DFI) above 30% were selected for this study. The samples of each patient were divided into two groups, control and experimental. The control group was washed with the routine density gradient centrifugation (DGC) method and the experimental group was selected by magnetic-

activated cell sorting combined isolate density gradient centrifugation (MACS-DGC). Similarly, eggs in each female of patients were divided into 2 mentioned groups. Control was injected by DGC, on the other hand, the experimental group was injected by MACS-DGC. On both before and after processing sperm parameters were evaluated. DFI was reported based on the halo sperm method both before and after processing. Embryo quality, blastocyst formation rate, and fertilization rate were estimated, after ICSI. Expression of *PLCZ1* was evaluated by real-time PCR. Comparison between results of two groups was determined by SPSS software.

Results: The research reported that sperm morphology and motility after the MACS-DGC method (1.7%, 45%) were significantly higher in comparison with the DGC method (1.1%, 40%) and before washing (0.9%, 35%). The percent of DFI in the MACS-DGC group (36%) was significantly reduced in comparison to DGC (45%) and primitive group (55%). The number of oocytes injected was 93 and 111 in DGC and MACS-DGC group, respectively. The fertilization rate in both groups was approximately equal (73.11 in DGC versus 72.07% in MACS-DGC). The rate of day 3 embryos with good grade was significantly higher than in the MACS-DGC group (72.5%) in comparison to the DGC group (51.47%) ($p < 0.05$). The blastocyst rate in the MACS-DGC group (69.69%) was significantly higher than the DGC group (48%). *PLCZ1* gene expression in MACS-DGC was significantly increased compared to the DGC group ($p = 0.046$).

Conclusion: Results show that sperm selection based on the MACS-DGC method can enhance morphology, motility, and decrease sperm DFI. No significant difference was observed in fertilization rate, but the percent of the high-quality embryo on days 3 and 5 was significantly higher by this method. According to the mechanism of the MACS-DGC method, it can be suggested as a good choice for patients with high DFI.
Key words: MACS, *PLCZ1*, High DFI, Male factor.