

## IMPROVED SPERM CHROMATIN DECONDENSATION RATES IN HAMSTER-OOCYTE ICSI AFTER ANNEXIN-V-MACS IN INFERTILITY PATIENTS

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The depletion of apoptotic sperm using Annexin-V based magnetic cell separation (MACS) has been recently introduced as a mean to improve assisted reproduction outcomes. Annexin-V MACS enhances hamster-oocyte sperm penetration but does not increase sperm chromatin decondensation (SCD) rates following hamster-oocyte ICSI (H-ICSI). Our aim was to evaluate the SCD rates of the annexin-negative (non-apoptotic) sperm fraction of patients with infertility using H-ICSI.

Semen specimens collected from 21 infertility patients with subnormal sperm parameters were subjected to double density gradient centrifugation (DGC) followed by Annexin-V MACS. A non-separated aliquot of each sample served as control. H-ICSI was performed in all aliquots using 20 frozen-thawed hamster oocytes per aliquot. Results were evaluated as the percentage of oocytes showing SCD. In addition, caspase-3 activation (CP3) and disruption of transmembrane mitochondrial potential (TMP) were monitored by FACS to observe the separation effect.

Annexin-V MACS resulted in a significant enrichment of spermatozoa with inactive CP3 and intact TMP in the annexin-negative fraction. Similarly, annexin-negative spermatozoa had the highest SCD rates compared to controls and annexin-positive sperm (table).

In conclusion, semen samples from infertility patients contain high levels of spermatozoa with active CP3 and disrupted TMP. Compared to routine DGC the enrichment of non-apoptotic spermatozoa by Annexin-V MACS resulted in superior sperm chromatin decondensation and may be used to improve the outcome of ICSI procedures in infertility patients.

Parameter	Controls	Annexin-negative	Annexin-positive
active CP3 (% sperm)	43.5 ± 13.8	26.8 ± 12.3*	58.4 ± 11.7*
intact TMP (% sperm)	54.7 ± 23.2	71.6 ± 21.5*	9.8 ± 12.0*
SCD (% oocytes)	31.3 ± 13.1	44.2 ± 15.8*	18.3 ± 6.7*

Values are expressed as mean ± standard deviation. \*p<0.01 in comparison to control. Statistical test: Wilcoxon-test.