

Background: Sperm preparation is essential for isolating high-quality spermatozoa to be used in Assisted Reproductive Technology (ART). Traditional methods, such as Density Gradient Centrifugation and Swim-up, can lead to oxidative stress and DNA damage, particularly in compromised semen samples. Microfluidic devices, which replicate conditions of the female reproductive tract, offer a promising alternative by reducing processing time, avoiding centrifugation, and preserving sperm quality. While studies have shown microfluidic sorting improves sperm quality compared to conventional methods in normozoospermic semen samples, its effectiveness in compromised semen samples requires further investigation.

Aims: To compare two different methods for sperm selection: pellet Swim-up technique (SU) and microfluidic devices (MSS). The study was conducted on 81 semen samples from male subjects undergoing routine semen analysis for couple infertility at the Andrology Laboratory of Careggi University Hospital of Florence. The sample group include 26 normozoospermic, 31 hyperviscous normozoospermic, 15 oligozoospermic and 9 asthenozoospermic samples. Sperm motility, concentration and viability, kinematic parameters and hyperactivated motility (by Computer-Assisted Sperm Analysis, CASA System), sperm DNA fragmentation (sDF, by TUNEL/PI method), oxidative status (by CRO probe) and chromatin compaction (by CMA3), were evaluated before and after selection by both MSS and SU techniques.

Results: As expected, a significant increase in all analysed sperm parameters was observed after selection with both MSS and SU compared to unselected samples in all groups. Notably, MSS selection statistically improved all parameters compared to SU in both non-viscous and hyperviscous samples (n=31) [Figure 1]. In 10 out of 34 normozoospermic samples, SU selection did not improve, and in some cases even increased, sDF, whereas MSS reduced it in all samples [Figure 2]. Additionally, in oligozoospermic samples (n=15) [Figure 3], characterized by a sperm concentration of less than $10 \times 10^6/\text{mL}$, as well as in asthenozoospermic samples (n=9) [Figure 4], characterized by progressive motility of less than 32%, MSS significantly increased the percentage of total motile spermatozoa and improved sperm chromatin integrity compared to SU-selected samples.

Figure 1

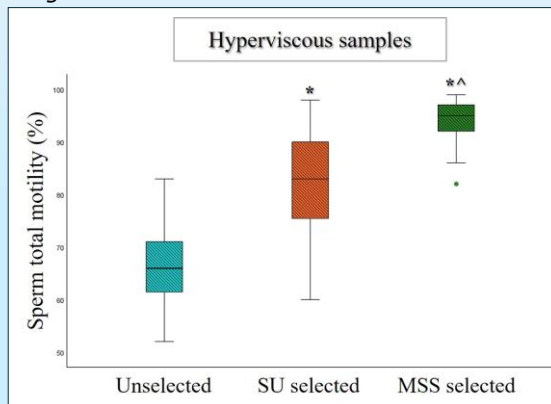


Figure 3

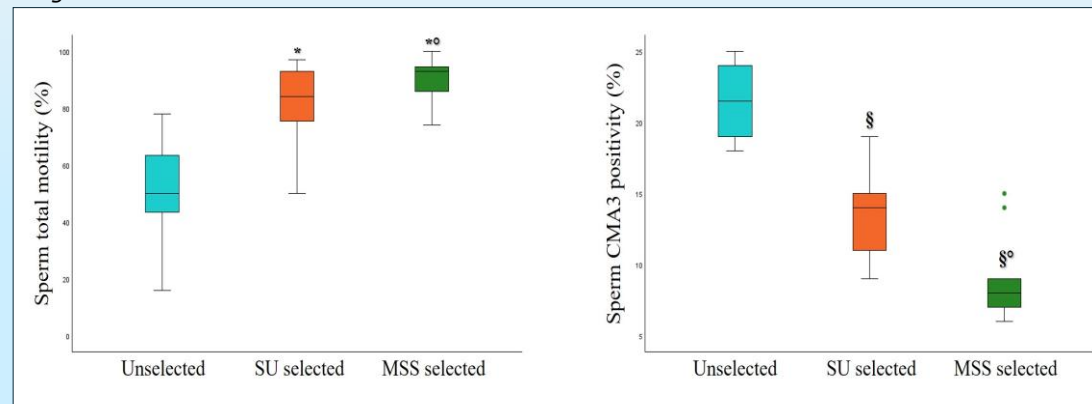


Figure 2

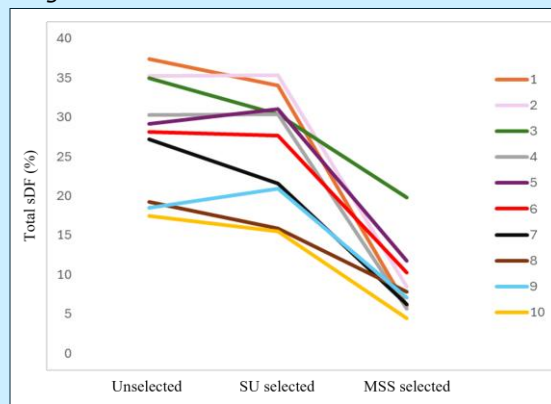
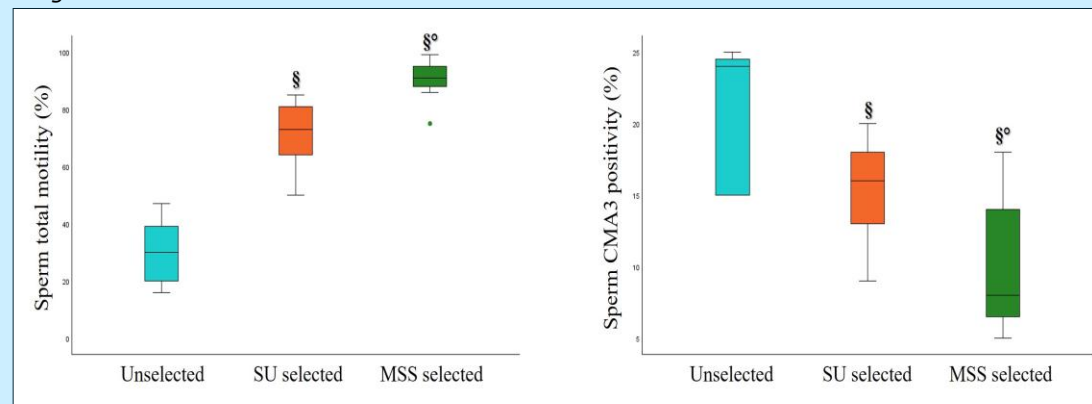


Figure 4



§ p<0.05 vs. Unselected; *p<0.001 vs. Unselected; ° p<0.05 vs. SU selected.

Conclusion: Our study demonstrated that microfluidic sorting is an effective method for selecting the optimal sperm fraction, not only in normozoospermic samples, but also in semen samples exhibiting defects such as hyperviscosity, low concentration, reduced motility, and high sDF levels. This method is faster than traditional techniques and reduces operator variability. Despite its high cost, microfluidic sorting could enhance fertilization success and reproductive outcomes in challenging cases.