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P-028 Microfluidics as a methodology for sperm selection. A prospective functional study

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Study question: Is microfluidics an optimal technique to improve the sperm selection process in comparison with standard techniques like Density Gradient Centrifugation or Swim-up?

Summary answer: A significant increase in sperm quality was obtained when microfluidics was compared to density gradient centrifugation but improvement evidence regarding swim-up is not yet demonstrated.

What is known already: Assisted reproduction clinics for in vitro fertilization treatments have developed several techniques to perform sperm selection, being density gradient centrifugation (DGC) and swim-up the most widely used. However, sperm selection is a procedure that requires bulky and expensive equipment, long waiting times and gamete manipulation, which results in cellular stress. The SwimCount Harvester is a microfluidic-based device capable of performing sperm selection and overcoming the problems of other sorting systems. In our study, we analyzed several sperm quality parameters between these three sperm selection techniques.

Study design, size, duration: This was a prospective, cohort and observational study including 111 semen samples from patients and donors (mean age 33,7±9,3 years) between February 2021 and January 2022. The semen sample from each patient or donor was divided into two volumes, one part, the sperm selection was performed using the SwimCount Harvester and the other part using DGC or Swim-up. These sperm selection techniques were used to isolate sperm based on fluid dynamics and cell motility.

Participants/materials, setting, methods: Fresh ejaculate and sperm selected samples from each patient were analyzed according to the 2010 WHO-criteria to assess concentration, motility, morphology and vitality, using automatic image analysis. The excessive histone retention indicating defective chromatin compaction was assessed using aniline blue staining. Sperm chromatin fragmentation (SCF) was assessed by TUNEL on at least 20.000 sperm using flow cytometry. Kruskal-Wallis test was performed in order to assess statistical differences of the variables between the sperm selection methods.

Main results and the role of chance: The SwimCount Harvester was compared to DGC (n = 95). Ejaculated sperm yielded an average concentration of $53,2\pm34,2x10^6$/mL. After DGC and SwimCount Harvester, the sperm concentration was $11,1\pm8,8$ and $12,5\pm11,2x10^6$/mL, respectively. The motility of fresh sperm sample improved from $41,9\pm10,4\%$ to $71,6\pm10,6\%$ after DGC and $76,8\pm10,0\%$ after SwimCount Harvester (P < 0,05). The percentage of normal sperm increased from $2,1\pm1,2\%$, for the fresh samples, to $3,5\pm1,4\%$ and $4,2\pm1,6\%$ for the samples processed by DGC and microfluidics, respectively (P < 0,05). The percentage of live sperm increased from $74,0\pm8,1\%$ and $77,5\pm8,7\%$ in fresh sperm and after DGC, respectively to $85,9\pm9,0\%$ after using microfluidics (P < 0,05). In the same way, the normal sperm chromatine structure percentage increased from $67,4\pm7,5\%$ to $75,4\pm7,9\%$ for the sperm samples selected by DGC and $77,7\pm8,9\%$ when the SwimCount Harvester was used (P < 0,05). A decrease in SCF was observed from $12,9\pm8,4\%$ in samples selected by DGC to $10,4\pm5,1\%$ in raw samples (P > 0,05). However, after sperm selection using SwimCount Harvester, SCF fell to $4,6\pm4,1\%$, showing significant differences between both sperm selection methods (P < 0,05). Similar results were obtained for oligozoospermic samples (n = 6). When the SwimCount Harvester was compared with the Swim-up (n = 10), non-significant improvements were observed for all the parameters studied due to the reduced sample size.

Limitations, reasons for caution: The database of samples processed using swim-up and oligozoospermic samples is too small to draw reliable conclusions. Although significantly better results are obtained in sperm samples selected by the SwimCount Harvester with respect to DGC, a clinical study using the microfluidic device in assisted reproduction cycles has to be performed.

Wider implications of the findings: The SwimCount Harvester, in addition to significantly improving sperm selection and quality, is a reliable alternative to integrate numerous laboratory steps into a single automated procedure, reducing workload, the amount of culture media and equipment used, gamete handling and the stress that produces. Moreover, microfluidics may eliminate inter-laboratory variability.