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## P-029 Centrifugation-free sperm separation device offers an efficient and standardized protocol to select high quality spermatozoa

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**Study question:** Can centrifugation-free sperm separation device be used to simplify and standardize the selection of high-quality spermatozoa?

**Summary answer:** Centrifugation-free CA0 sperm separation device minimizes inter- and intra-operator variability and yields spermatozoa with comparable fertilizing properties in a variety of semen conditions.

**What is known already:** Centrifugation-based sperm separation methods have been used in assisted reproductive technology (ART) for many decades. However, the conventional methods are criticized for harmful effects due to centrifugation. To overcome the disadvantage, more noninvasive technologies have been developed and attempted to improve the sperm separation process, e.g., the formation of capillary bridge to select motile spermatozoa, migration-sedimentation technique to sort out functional spermatozoa, microfluidic sorting chip to isolate healthy sperm. While these methods provide alternatives for noninvasive sperm separation, the limitations such as inconsistency of semen quality improvement and lack of standardized procedure remain to be resolved.

**Study design, size, duration:** A randomized controlled trial of 76 men who sought ART treatment (Lee Women's Hospital, Taichung, Taiwan) from June to October 2022 was carried out. Seventy-six neat semen specimens were categorized into 27 normozoospermic specimens, and 49 non-normozoospermic samples (semen quality below any of the WHO 5th Edition lower reference values: concentration <15 million/mL, total motility <40%, or normal morphology <4%).

**Participants/materials, setting, methods:** Neat semen samples were separated for three replicates (replicate 1-3) using LensHooke® CA0 sperm separation device (Bonraybio, Taichung, Taiwan). Three operators (operators 1–3) performed CA0 procedures on each sample to test inter-technician variability. Interclass-correlation coefficient (ICC) between replicates as well as operators were evaluated. Pre-selection and post-selection semen quality were evaluated. The parameters included total motility, progressive motility, rapid progressive motility, morphology, DNA fragmentation index (DFI), and acrosome reaction rate (AR).

**Main results and the role of chance:** CA0 selects self-propelling spermatozoa within a microenvironment created by a microporous filter membrane. The procedure involves three pipetting steps: loading semen sample, adding sperm washing medium, and recovering the processed sample. Following the standard procedure, CA0 resulted in a low intra- and inter-operator variability and ICC values between replicates and different operators were all greater than 0.9, indicating an excellent reproducibility of CA0. In addition, significant higher levels of motility and normal morphology were observed in post-selection specimens either of normozoospermic or non-normozoospermic samples (pre-selection vs. post-selection, all $p < 0.0001$). In paired analysis of the advanced semen parameter, our study showed noteworthy results that CA0 significantly improved DFI from 18.2% to 2.6% for normozoospermic samples; such reduction was also found in non-normozoospermic sample processing, from 13.4% to 4.2% (both, $p < 0.0001$). The levels of AR were significantly reduced in normozoospermic samples (from 14.4% to 5.4%) and non-normozoospermic samples as well (from 12.8% to 5.0%) (both groups $p < 0.0001$). In conclusion, CA0 provides an efficient, noninvasive, standardized, and reproducible sperm separation model that CA0 diminishes the variations and ensures sperm quality.

**Limitations, reasons for caution:** The presented study was a pilot trial examining the sperm quality improvement. Follow-up analysis on IUI/IVF outcomes associated with the improvement in semen quality utilizing CA0 will be assessed in future studies.

**Wider implications of the findings:** CA0 provides multifaceted benefits covering consistent clinical outcomes, simplified and standardized procedure, user-friendliness, and cost reduction. We believe CA0 not only allows noninvasive sperm separation of clinically usable but also gives the possibility of standardization on sperm separation procedure.

**Trial registration number:** CS2-22039