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Live Motile Sperm Sorting Device Improves Embryo Aneuploidy: A Retrospective Cohort Study

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ABSTRACT

Background: Conventional sperm selection methods, involving centrifugation, exert a detrimental effect on sperm DNA integrity due to mechanical stress. The recent noninvasive sperm selection device, CA0, based on live sperm sorting technology, facilitates the retrieval of highly motile sperm and minimizes sperm DNA fragmentation (SDF). This study was to investigate the impact of various sperm separation methods, with and without centrifugation, on embryo ploidy status.

Methods: The retrospective study comprised 82 intracytoplasmic sperm injection (ICSI) cycles involving preimplantation genetic testing for aneuploidy (PGT-A) cases, with a focus on recruiting egg donation cycles to thoroughly investigate the impact of male factors. Two populations are classified based on semen quality: normozoospermic (n = 33) and non-normozoospermic (n = 49). Subjects were allocated to either swim-up (SU) or CA0. Preimplantation genetic testing results were recorded.

Results: When comparing male characteristics between subgroups, no significant differences were observed except for a lower normal morphology rate in the CA0 group compared to SU (SU: 3 [3–4] vs. CA0: 2 [2–2.8], $p < 0.0001$) in the non-normozoospermic cohort. There were no differences in female factors such as age and mature oocyte count (MII) number between subgroups, indicating that this model is ideal for assessing the impact of male factors on clinical outcomes. In the normozoospermic cohort, euploidy rates were similar between SU and CA0 (SU: 71.9% vs. CA0: 64.2%). However, in the non-normozoospermic cohort, CA0 showed a significantly higher euploidy rate compared to SU (SU: 53.6% vs. CA0: 74.2%) and a lower aneuploidy rate (SU: 37.1% vs. CA0: 25.8%). Additionally, CA0 minimized the incidence of mosaic embryos, whereas a mosaicism rate of 9.3% was observed with SU. This trend highlights CA0's distinct advantage in optimizing outcomes for non-normozoospermic cases.

Conclusions: CA0 is a reliable intervention to optimize paternal genetic quality before assisted insemination, thereafter effectively reducing the incidence of embryo aneuploidy associated with male factors.

***Keywords*:** Sperm Isolation; Sperm DNA Fragmentation; Paternal Effect; PGT-A.

INTRODUCTION

With the advent of intracytoplasmic sperm injection (ICSI), embryologists select the "best" sperm based on motility and morphology, enabling men diagnosed with oligozoospermia and/or asthenozoospermia to conceive genetically related children (Tozour et al., 2024). However, the average live birth rate per ICSI cycle is only 22%, and it does not exceed 40% after multiple attempts (Oseguera-López et al., 2019). It points out an emerging need to understand the plausible reasons for ICSI failures and improve the success rate by addressing them.

Sperm selection in ICSI versus natural conception highlights significant differences. In natural conception, sperm must overcome a series of physiological and geographical obstacles in the female reproductive tract, such as the acidic environment of the vagina and the narrow uterotubal junction; ultimately, only a small number of sperm cells is capable of reaching the fertilization site. In contrast, sperm selection used prior to ICSI primarily relies on motility, such as swim-up (SU) or density gradient centrifugation (DGC) with an additional gradient challenge. The discrepancy in sperm selection efficiency may contribute to the poorer success rate of ICSI compared to natural conception. Second, during ICSI manipulation, embryologists solely focus on basic sperm phenotypic characteristics, which underestimates the complexity of sperm fertilization competency. Sperm DNA fragmentation (SDF) is

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prevalent in male factor infertility. Up to 50% of men with idiopathic male infertility have abnormal SDF rates, and 20% of men with normal semen parameters also exhibit high SDF levels (Erenpreiss et al., 2008; Esteves, 2021). High SDF significantly elevates embryo aneuploidy, accounting for 28.8% of cases due to chromosomal abnormalities of paternal origin (Gao et al., 2023). Additionally, SDF is associated with miscarriage as well (Asgari et al., 2022; Ping et al., 2023). Collectively, improvement of SDF can be an effective strategy for enhancing the ICSI success rate.

Three strategies are available to address the SDF effect. First, testicular sperm extraction can be effective for managing high SDF. However, this approach carries surgical risks and potential complications. Moreover, although testicular sperm often show a lower DNA fragmentation index (DFI), the biological benefit may be offset by an increased likelihood of sperm aneuploidy due to the incomplete maturation of spermatozoa (Zhao et al., 2023). Egg donation is an alternative treatment in mitigating a mild degree of SDF due to ooplasmic repair mechanisms, while the repairability of donor eggs is limited in cases of severe male infertility (Beguería et al., 2014; Gao et al., 2023). Third, advanced sperm selection methods based on a variety of technical principles have been proved to reduce the levels of SDF, such as electrophoresis-based technology (Razavi et al., 2010), PICSI dish (Parmegiani et al., 2010), microfluidic sorting device ZyMot® (Gode et al., 2019; Rao, 2022; Tatsumi et al., 2020), and migration gravity sedimentation method (Miglis®) (Kiratli et al., 2018). There is a very recent development, live motile sperm sorting device called LensHooke® CA0 (CA0). The CA0 device is bioinspired by the sperm journey during natural conception, with its columnar joints mimicking the uterine tubule microenvironment and a built-in microchannel membrane that simulates the narrow uterotubal junction for final sperm selection. It is demonstrated that CA0 resulted in the highest sperm motility and the lowest SDF among infertile men, when compared to other sperm selection methods, including DGC and ZyMot® (Hsu et al., 2023). Although CA0 shows promise in selecting the "best" sperm based on its bio-inspired design, there is limited clinical data on its use in the field.

In this cohort study, egg donation ICSI cycles are considered as an ideal population to elucidate the effects of paternal risk factors. We incorporated egg donation with preimplantation genetic testing for aneuploidy (PGT-A) in this retrospective cohort study. Our objective was to evaluate the efficiency of CA0 in improving embryonic aneuploidy compared to SU. Furthermore, we aimed to assess the impact of sperm selection methods on various male infertility cases.

METHODS

Study population and design

We conducted a retrospective cohort study at the Center for Reproductive Health, Chachava Clinic, Tbilisi, Georgia. Couples attending our center for infertility between April 2021 and May 2023 were recruited. Given that the main intervention of the study involved in routine sperm preparation, clinical trial registration was not required. Inclusion criteria were egg donation ICSI cycles with PGT-A. Cycles were excluded if male chromosomal abnormality was reported or surgical sperm or frozen-thawed testicular sperm were used.

Basic semen analysis

Semen samples were collected after 2–5 days' sexual abstinence and deposited into sterile cups by masturbation. After liquefaction at room temperature (RT) within 30 min, an aliquot of the semen sample was loaded into a 10 μm deep chamber slide. Semen parameters, including sperm concentration (M/mL), total motility (%), and progressive motility (%), were measured using the CEROS II device (Hamilton-Thorne, Danvers, MA, USA), according to the WHO 5th edition manual (*WHO laboratory manual for the examination and processing of human semen,* 2010). Normozoospermic was defined if total motility above 40% and a normal morphology rate above 4%; non-normozoospermic was grouped if total motility less than 40% or a normal morphology rate less than 4%.

Sperm morphological evaluation

The sperm smears were prepared using 10 μL liquefied semen specimen. Air-dried slides were stained using Asur-Eosin by Romanowsky (Ecolab, Russia). In brief, air-dried slides were immersed in Asur-Eosin Fixative for 50 s and sequentially immersed in Asur-Eosin staining I and staining II for 20 s each. The stained slides were washed by distilled water and air-dried. Sperm morphology is determined at 1000× magnification under a bright-field light source according to the WHO 5th edition guideline. All slides were blindly read by experienced embryologists, and at least 200 sperm were evaluated in each test.

Conventional swim-up

One milliliter semen samples were transferred to a clean 15-mL centrifuge tube and gently layered with 1.2 mL of sperm wash medium G-IVF™ PLUS (Vitrolife). The tubes were inclined at a 45° angle and incubated at 37°C for 60 min. After incubation, 1 mL of uppermost was collected in a new tube. Two milliliters of sperm wash medium were added and centrifuged at 500 × g for 5 min. The supernatant was discarded, and the sperm pellet was resuspended in a 0.5–1 mL of medium for further use.

Live motile sperm sorting device

Separation procedure for LensHooke® CA0 (Bonraybio, Taichung, Taiwan) involved: (1) 1 mL raw semen sample was filled into the lower chamber, (2) the upper chamber was attached to the lower chamber, (3) 0.9 mL sperm washing medium G-IVF™ PLUS (Vitrolife) was added into the upper chamber, (4) the cover piece was placed over the two-chamber device, (5) the assembly was incubated at 37°C for 30 min, and (6) 0.5 mL sperm suspension was aspired from the upper chamber for further used.

Ovarian stimulation

Controlled ovarian stimulation was applied using the GnRH antagonist protocol, and medications used included follitropin alfa (Gonal-F, Merck Serono, Germany), human menopausal gonadotropin (Menopur, Ferring Pharmaceuticals, Saint-Prex, Switzerland), and cetrorelix acetate (Cetrotide, Merck Serono). Final oocyte maturation was triggered by either human chorionic gonadotropin (Ovitrelle, Merck Serono) or GnRH agonist (Decapeptyl, Ferring Pharmaceuticals), and ultrasound-guided oocyte retrieval was performed approximately 36 h after oocyte maturation trigger injection.

Embryo culture and determination of embryonic ploidy status

ICSI was performed according to the standardized protocol described previously. Briefly, ICSI was conducted in a fertilization medium G-MOPS + (Vitrolife) with supplementation of 15% serum protein substitute G-MOPS + (Vitrolife). At 16–18 h after inseminations, the oocytes were examined for the presence of pronuclei. At 70–72 h after inseminations, embryos were moved to a dish equilibrated with cleavage medium G2 (Vitrolife)

containing 15% SPS. Embryos with more than seven cells are indicated as cleaving embryos. On the morning of Day 5, blastocyst morphology was assessed using the grading system established by Gardner and Schoolcraft (Gardner and Balaban, 2016). All embryo grading was reviewed in real-time by two senior embryologists for verification and consistency. The blastocysts with at least grade B of inner cell mass and at least grade C of trophectoderm (TE) were selected for TE biopsy. Biopsy procedures were performed with micromanipulation tools Polar Body Biopsy Pipettes 30'C (Florida, USA) and a microscope (Leica, USA). Five to eight TE cells were carefully aspirated into a biopsy pipette and prepared for shipment to the genetics laboratory. The mosaic levels of biopsied samples were determined according to the manufacturers' instructions for the high-resolution next-generation sequencing (hr-NGS) platform. The ploidy status of each sample was determined according to the following criteria: mosaic levels ≤20% (euploidy); mosaic levels between >20% mosaic and ≤80% (mosaicism); and mosaic levels >80% (aneuploidy).

Statistical analysis

The dataset with a normal distribution verified by the Shapiro–Wilk test was analyzed by one-way ANOVA and presented as means ± standard deviations. The results with a skewed distribution were analyzed by the Kruskal–Wallis test and presented as the median (interquartile range). Summary statistics were computed on the basis of the chi-square. Different alphabets indicate a significant difference between two methods when p-value less than 0.05. Statistical analysis was performed by Prism software, version 6.01 (GraphPad software, Inc.).

RESULTS

Participants flow

Between April 2021 and May 2023, 82 eligible couples were enrolled in this study (Fig. 1). We stratified the couples into two groups: normozoospermic (n = 33) and non-normozoospermic (n = 49), based on a total motility lower reference limit of 40% and a normal morphology rate of 4%. Both subgroups underwent sperm selection

Fig. 1. Flow chart of the retrospective cohort study comparing embryo ploidy outcomes following swim-up and CA0 sperm separation.

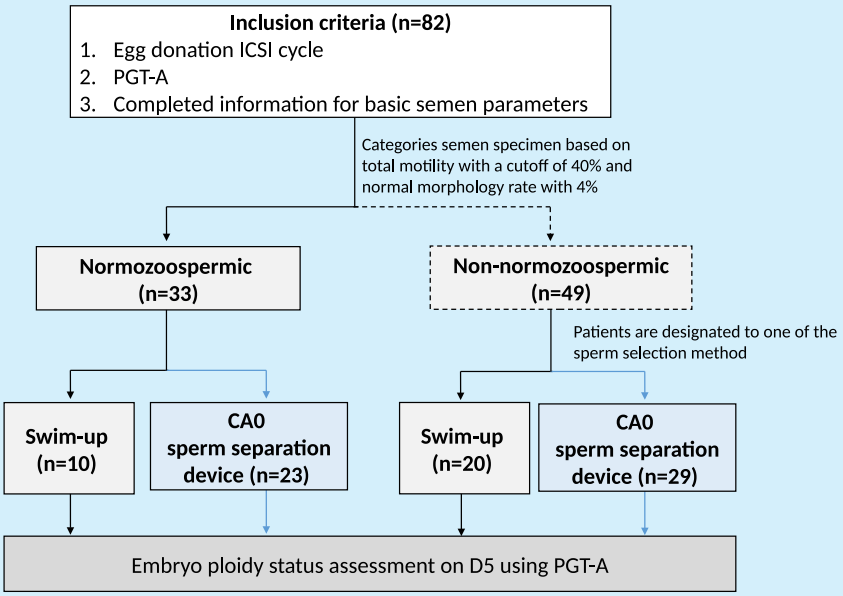


Table 1. Demographic and cycle characteristics.

	Normozoospermic			Non-normozoospermic		
	SU	CA0	*P* value	SU	CA0	*P* value
Male age (years)	40.5 (39.8–46)	41 (36–49)	1	47 (41.3–50.5)	46.5 (41.3–51)	0.95
Sperm concentration (M/mL)	95 (41.5–140)	90 (50–130)	0.784	50 (36.5–75.5)	30 (8.5–84)	0.106
Total motility (%)	52.5 (43–60.3)	60 (54–63)	0.13	37 (22–53.5)	41.5 (31.3–53)	0.349
Morphology (%)	4 (4–5)	5 (4–5)	0.214	3 (3–4)	2 (2–2.8)	<0.0001
Female age (years)	28.5 (24–30)	26 (24–29)	0.58	28 (24.5–30)	24.5 (23–28)	0.063
MII (n)	12 (11.5–13.3)	11 (9–13)	0.277	11 (9–12)	12 (7.5–15.8)	0.278

Values are presented as median (interquartile). *P* values were derived from the Kruskal–Wallis test.

using either SU or CA0. Our primary outcome was the embryo ploidy status, assessed using PGT-A on Day 5 of embryo culture.

Demographic and cycle characteristics

Male physiological characteristics included male age, sperm concentration, total motility, morphology, and SDF. Characteristics of egg donors comprised female age and mature oocyte count (MII). In the normozoospermic cohort, no differences were observed between SU and CA0 for any of these characteristics. However, in the non-normozoospermic cohort, the normal morphology rate was lower with CA0 compared to SU (SU: 3% vs. CA0: 2%, $p < 0.0001$), while other parameters were comparable between the two methods (Table 1). By designating egg donation cycles, we are able to assess paternal effects with minimal influence from female confounding factors.

Ploidy results of embryo under male risk factors

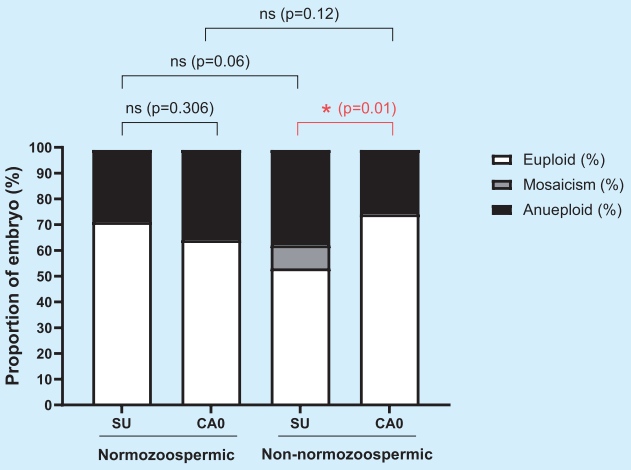
First, we compared ploidy results (euploid, mosaicism, aneuploidy) between SU and CA0. In the normozoospermic cohort, embryo proportion did not differ between SU and CA0, with SU compared to CA0 (SU: 53.6% vs. CA0: 74.2%), and the aneuploidy rate was higher with SU compared to CA0 (SU: 37.1% vs. CA0: 25.8%). No mosaic embryos were found with CA0, whereas 14 out of 151 embryo specimens (9.3%) were diagnosed as mosaic with SU (Table 2; Fig. 2). In summary, the ploidy distribution varied significantly between the different sperm selection methods.

Table 2. Ploidy results of embryos according to seminal parameters and sperm preparation methods.

	Normozoospermic		Non-normozoospermic	
	SU	CA0	SU	CA0
Case number/ biopsied number	10/57	23/120	20/151	29/89
Euploidy rate (%)	71.9% (41/57)	64.2% (77/120)	53.6% (81/151)	74.2% (66/89)
Mosaicism rate (%)	0% (0/57)	0% (0/120)	9.3% (14/151)	0% (0/89)
Aneuploidy rate (%)	28.1% (16/57)	35.8% (43/120)	37.1% (56/151)	25.8% (23/89)

Data are presented as a proportion of categorical variables (%) and numbers. *P* values were obtained by the Chi-square test.

Fig. 2. Ploidy results of embryos according to seminal parameters and sperm preparation method. Comparison of subjects according to seminal parameters and sperm preparation swim-up (SU) and CA0 methods. Asterisks indicate a significant difference ($p < 0.05$) based on the Chi-square trend test.



DISCUSSION

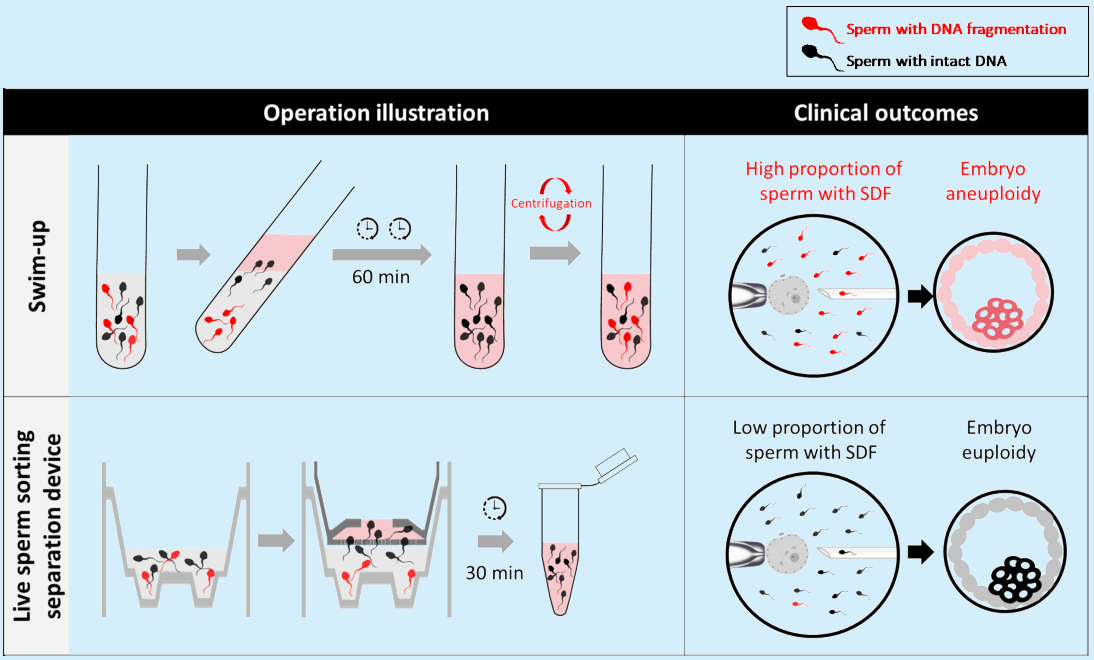
In this study, we highlighted the distinct clinical associations arising from various sperm selection methods. Comparing the euploidy rates between the conventional sperm selection method (SU) and the live motile sperm sorting device (CA0), CA0 resulted in a higher embryo euploidy rate and a lower incidence of mosaic embryos than SU.

Both SU and CA0 primarily rely on sperm motility; however, several discrepancies between these methods can be identified. In terms of geometric differences, CA0 is equipped with a filter that has a porosity of approximately 10%, compared to SU 100% effective area for sperm selection. The restricted effective area in CA0 limits the number of sperm that can pass through, but it enhances the selection of sperm with the highest physiological properties. On the other hand, conventional SU without centrifugation is effective in reducing SDF (Zini et al., 2000). However, a modified SU protocol involving brief centrifugation is more commonly used in andrology laboratories due to the insufficient recovery rate when processing severe oligozoospermic specimens (Dai et al., 2020; Yamanaka et al., 2016). Our clinic used centrifugation-involved SU protocol as well. Mechanical stress generated from centrifugation processes increases reactive oxygen species (ROS), and an excessive ROS associates with impaired motility and DNA damage due to oxidative stress-induced mutagenesis (Chianese and Pierantoni, 2021; Hussain et al., 2023). While the causal mechanism of paternal embryo aneuploidy, whether due to SDF or the ROS-dependent pathway, remains to be elucidated, the impact of centrifugation should not be overlooked. Although mild centrifugation has minimal impact on normozoospermic samples, abnormal semen specimens appear to be more vulnerable to mechanical stress and DNA damage (Muratori et al., 2016; Takeshima et al., 2017). This may explain our observation that the centrifugation-free CA0 improves embryo aneuploidy rates, particularly when processing non-normozoospermic samples. However, CA0 clinical benefits are observational. Further comparative study should also be carried out to determine whether other centrifugation-free sperm selection methods or microchannel-based, such as ZyMot, would achieve the same results on PGT-A and clinical outcome as CA0.

Besides the difference in selection efficiency, operational reproducibility and consistency may be a potential variable affecting the PGT-A outcomes. CA0, which involves only three pipetting steps—loading the semen specimen, adding sperm washing medium, and recovering the sperm suspension—improves consistency in selection effectiveness (Wang et al., 2023). In contrast, centrifugation-based protocols demand skills related to the isolation of various proportions of sperm suspension. Poor aspiration can disrupt the layer between the isolated sperm and the raw semen sample, discounting the selection effectiveness. López et al. indicated that the high inter-operator variation in centrifugation-based sperm selection is an inevitable factor causing controversial clinical benefits (López-Fernández, 2013). Accordingly, the improvement in embryo euploidy rates using CA0 may be attributed to the practical simplification and standardized procedure.

PGT-A represents a significant advancement in the current era of ART medicine. By carefully screening and selecting embryos with

Fig. 3. Proposed model of swim-up and CA0. CA0 can prevent sperm DNA fragmentation (SDF), provide high operational reproducibility, and facilitate embryo euploidy rate subsequently. Sperm with SDF are indicated in red, while sperm with intact DNA are illustrated in black. This model is derived from findings in this study and adapted from published validation (Hsu et al., 2023).



genetic integrity, PGT-A has demonstrated notable increases in first-transfer pregnancy rates and live birth rates (Ma et al., 2023; Masbou et al., 2019). However, it is noted that PGT-A does not function as a preventive solution against embryo aneuploidy; its benefits and effectiveness in enhancing the ART success rate are constrained, particularly for patients with a limited number of blastocysts available for testing. This highlights the need for a preventative solution for embryo aneuploidy. As many female factors, such as advanced age, are difficult to change, optimizing paternal factors holds greater potential for preventing embryo aneuploidy. Practical solutions rooted in male factors have been developed, such as shorter abstinence periods (Scarselli et al., 2019) and advanced microfluidic sperm selection (Kocur et al., 2023). Both interventions can improve sperm chromatin integrity and subsequently increase the euploidy rate. In this study, we upgraded sperm quality using CA0, and a significant reduction of embryo aneuploidy rate was found compared to SU in the non-normozoospermic cohort. Collectively, our findings and previous studies implicate that optimizing andrology lab preparation has the potential to improve embryo aneuploidy.

The findings in this report are subject to two limitations. First, due to the retrospective nature of the study, DFI results corresponding to pre-processing and post-processing semen are not available. Additionally, we have limited information regarding variables related to SDF, such as alcohol consumption, long abstinence periods, smoking habits, and other lifestyle factors. Second, these data cannot be directly correlated with clinical outcomes after embryo transfer, considering the intervention of PGT-A embryo selection. Collectively, further randomized controlled trials exploring the long-term clinical outcomes with a larger sample size could provide valuable insights into ART procedures.

Figure 3 illustrates the model of CA0. CA0 showed a higher euploidy rate compared to SU in processing non-normozoospermic specimens, attributed to the noninvasive sperm selection microenvironment. The centrifugation-free CA0 can serve as an effective prenatal intervention to prevent embryo aneuploidy linked to paternal risk factors.

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CONFLICT OF INTEREST

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and publication of this article.

AUTHOR CONTRIBUTIONS

K.G., N.M., and T.J. contributed to the design and draft of the manuscript. M.M. acquired and analyzed data. K.G., N.M., T.J., and M.M. reviewed the manuscript for intellectual content, approved the final version to be published, and agreed to be responsible for all aspects of the work.

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