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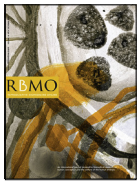
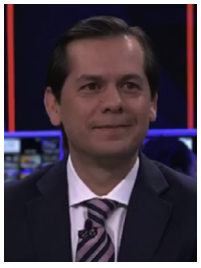


ARTICLE

Computer software (SiD) assisted real-time single sperm selection associated with fertilization and blastocyst formation



BIOGRAPHY

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KEY MESSAGE

Motility characteristics of individual spermatozoa can be traced to IVF treatment outcome through successful fertilization and blastocyst generation. These analyses can be conducted using an automatic tool such as SiD and creates the opportunity of assisting embryologists in selecting the spermatozoon for injection in an intracytoplasmic sperm injection procedure.

ABSTRACT

Research question: Is it possible to explore an association between individual sperm kinematics evaluated in real time and spermatozoa selected by an embryologist for intracytoplasmic sperm injection (ICSI), with subsequent normal fertilization and blastocyst formation using a novel artificial vision-based software (SiD V1.0; IVF 2.0, UK)?

Design: ICSI procedures were randomly video recorded and subjected to analysis using SiD V1.0, proprietary software developed by our group. In total, 383 individual spermatozoa were retrospectively analysed from a dataset of 78 ICSI-assisted reproductive technology cycles. SiD software computes the progressive motility parameters, straight-line velocity (VSL) and linearity of the curvilinear path (LIN), of each sperm trajectory, along with a quantitative value, head movement pattern (HMP), which is an indicator of the characteristics of the sperm head movement patterns. The mean VSL, LIN and HMP measurements for each set of spermatozoa were compared based on different outcome measures.

Results: Statistically significant differences were found in VSL, LIN and HMP among those spermatozoa selected for injection ($P < 0.001$). Additionally, LIN and HMP were found to be significantly different between successful and unsuccessful fertilization ($P = 0.038$ and $P = 0.029$, respectively). Additionally, significantly higher SiD scores were found for those spermatozoa that achieved both successful fertilization ($P = 0.004$) and blastocyst formation ($P = 0.013$).

Conclusion: The possibility of carrying out real-time analyses of individual spermatozoa using an automatic tool such as SiD creates the opportunity to assist the embryologist in selecting the better spermatozoon for injection in an ICSI procedure.

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KEYWORDS

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AI sperm selection assistant
Artificial-vision assisted ICSI
Male factor
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Declaration: ACB, JC, AJD are shareholders at IVF 2.0 LTD. GMR, AFS, RV are employed by IVF 2.0 LTD. IVF 2.0 LTD holds a provisional patent on SID, with a full patent pending. IVF 2.0 LTD holds all copyrights to the software SiD.

INTRODUCTION

Intracytoplasmic sperm injection (ICSI) is the most widely used insemination method (*Palermo et al., 1992*; *Haddad et al., 2021*) and is not just limited to treating male factor infertility (*Rubino et al., 2016*; *Pedrosa et al., 2020*). Sperm quality can affect embryogenesis from an early stage (*Loutradi et al., 2006*; *Mazzilli et al., 2017*), and may reduce blastocyst formation (*Ron-el et al., 1991*; *Janny and Menezo, 1994*).

A range of advanced sperm selection strategies aimed at improving ICSI outcomes has been developed (*Vaughan and Sakkas, 2019*; *Asali et al., 2020*; *Anbari et al., 2021*). This includes swim-up and density gradient centrifugation. Newer techniques include hyaluronic-acid binding, magnetic-activated cell sorting, surface charge Zeta potential, microfluidics and high-resolution morphological sperm selection with or without integration of motile sperm organelle morphology examination (*Bartoov et al., 2013*; *Asali et al., 2020*). Most of these strategies are aimed at improving the quality of injected spermatozoa (*Vaughan and Sakkas, 2019*; *Anbari et al., 2021*; *Baldini et al., 2021*).

Several attempts have been made to improve sperm population analysis with the aim of increasing objectivity and fertility prognosis. Examples include DNA fragmentation and membrane integrity (*Cincik et al., 2007*; *Ribeiro et al., 2017*). Computer-aided approaches have been described for more than 10 years (*Chan et al., 1989*; *Cooper et al., 2010*; *Daloglu and Ozcan, 2017*; *Engel et al., 2019*). Examples of commercially available products include Mojo, LensHook (Bonraybio Corporation, Taichung City, Taiwan) and computer-aided sperm analysis Systems (Hamilton Torne, USA). Further improvement may be achieved by using artificial intelligence to select the single highest quality spermatozoon in real-time.

Standard motility parameters of computer-aided sperm analysis systems include velocity of spermatozoa, i.e. curvilinear velocity, straight-line velocity (VSL) and the average path velocity; the ratios of velocity parameters, i.e linearity of the curvilinear path (LIN), the straightness of the average path and the oscillation of the actual path about the average path; assessments related to the movement of the sperm head reflecting the flagellar wave; the magnitude of lateral displacement of a sperm head about its average path, the average rate at which the curvilinear path crosses the average path and the time-averaged absolute values of the instantaneous turning angle of the sperm head along its curvilinear trajectory (*WHO, 2021*).

Spermatozoa roll as they swim (*Bukatin et al., 2015*). Head movement patterns (HMP) are associated with the flagellum's helicoid beat pattern. The HMP patterns may be related to the quality of spermatozoa (*Subramani et al., 2014*). Translating such criteria in real time during ICSI while observing motile spermatozoa is challenging and subjective without the assistance of automation. This tool should provide instantaneous quantitative information of all spermatozoa in the visual field.

Attempts to improve individual sperm selection in real time include the incorporation of novel computing technologies such as data mining (*Mirroshandel et al., 2016*), deep learning for DNA fragmentation analysis (*McCallum et al., 2019*) and stress-affected sperm identification (*Butola et al., 2020*). Despite the allure of these proposals, their potential effect on fertilization remains unknown as the current methods available for sperm selection during ICSI either do not include, or objectively assess, such features (*Mirroshandel et al., 2016*; *McCallum et al., 2019*; *Ilhan et al., 2020*).

To address this problem, we have developed a novel individual-sperm identification system based on computer vision, and artificial intelligence, termed 'SiD' (Sperm ID or identification). This algorithm assists the embryologist to select sperm during ICSI. SiD detects motile spermatozoa, and individually analyses each spermatozoon in the visual field live using a digitizer attached to an ICSI microscope (magnification of 20x or more). Individual sperm assessment for investigation can also be obtained by analysing prerecorded video. The evaluation of the morphological and motility characteristics of individual spermatozoa is used by SiD to rapidly identify the best spermatozoa from the sample. SiD computes the progressive motility parameters, VSL and LIN, of each spermatozoon's trajectory. Additionally, SiD performs the computation of a quantitative value, which is an indicator of the characteristics of the sperm head movement patterns. The features of each spermatozoon are processed and then evaluated using a mathematical model that determines the quality of each spermatozoon and ranks them accordingly. The result of the ranking is shown to the user in real-time. A block diagram of the processes performed by SiD is shown in FIGURE 1. An example of the graphical user interface of SiD V1.0 is shown in FIGURE 2 and Video 1.

The present study is based on the evaluation of sperm motility patterns, assessed during sperm selection immediately before ICSI, with the assistance of SiD. The purpose of this study was to test the hypothesis that associations exist between individual sperm kinematics measured with SiD, and the spermatozoa selected by an embryologist, with subsequent outcomes, such as normal fertilization and blastocyst formation.

MATERIALS AND METHODS

Study population

Intracytoplasmic sperm injection procedures carried out between March and December 2020 at one fertility clinic in Mexico were video recorded and subjected to analysis using SiD. In total, 383 individual spermatozoa were retrospectively analysed from a dataset of 78 ICSI cycles.

The study was approved by the Institutional Review Board of New Hope Fertility Center (number RPA-2021-03, 12 April 2021). The video files used were anonymized by removing any metadata related to the identity or diagnosis of patients, and by assigning a unique identification number to each file.

The World Health Organization's (WHO) principle for the use of artificial intelligence in healthcare that human autonomy should be protected must be acknowledged; the couples were, therefore, approached for consent before their ICSI videos were used for the study (*WHO, 2021*).

Intracytoplasmic sperm procedure

Standard protocols were used for ICSI, as described elsewhere (*Henkel*

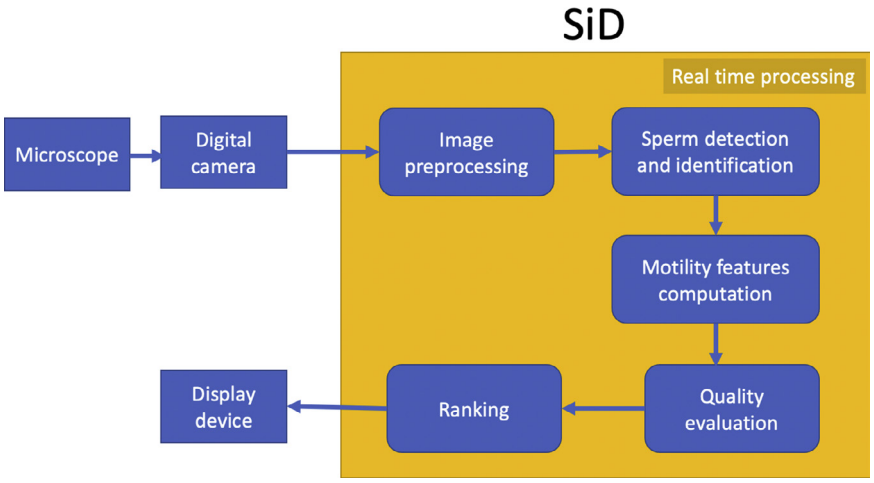

FIGURE 1 Processes performed by SiD.

and Schill, 2003). In brief, the semen sample was prepared using a standard sperm capacitation technique, including centrifugation and swim-up. For manipulation, the spermatozoa were placed in a 10-µl droplet of Multipurpose Handling Medium-Complete (MHM-C) with Gentamicin (Irvine Scientific, Santa Ana, CA, USA). Several spermatozoa were aspirated from the edge of the drop with a pipette and released into a droplet of 0.015 ml polyvinylpyrrolidone (PVP) solution with 7% human serum albumin (Irvine Scientific, Santa Ana, CA, USA) to reduce motility. The embryologist used an inverted microscope (IX71, Olympus), to qualitatively evaluate the released spermatozoa and mechanically immobilize the selected spermatozoon. The immobilized spermatozoon was aspirated into an ICSI needle and injected into a mature oocyte (metaphase II) after spindle identification.

Standard ICSI microscope and camera setting for the ICSI laboratory was used for video recording, which consisted of a digitizer (LYKOS) (Hamilton Thorne, Beverly, MA, USA) attached to a standard brightfield inverted optical microscope (IX71, Olympus), using a magnification of 20x and operating at 15 frames per second, with a resolution of 640 × 480 pixels.

The ICSI outcomes were defined as successful fertilization (presence of two pronuclei and a second polar body) and blastocyst formation. The sample size is limited because the requirement was that sperm samples were on comparable conditions, i.e. the same microscope magnification, same PVP concentration and brand and the technique and criteria used for selecting spermatozoa (two embryologist using the same method in a single clinic).

Analysis with SiD

Each video was analysed from the time the spermatozoa were released into the PVP droplet until the manually selected spermatozoon was immobilized and injected. The software SiD V1.0A was used for quantitative analysis.

SiD is capable of computing the individual values for LIN (with arbitrary units), and VSL (pixels per second) for every spermatozoon in the field.

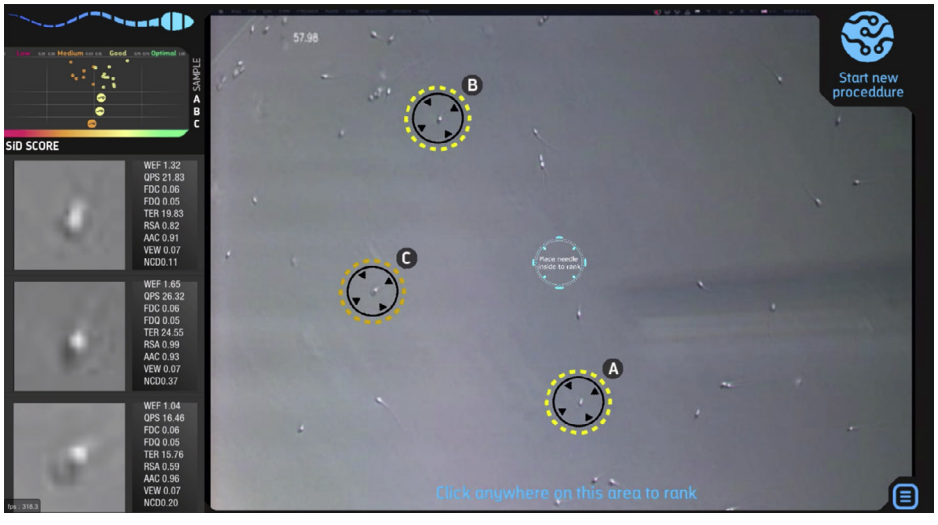

FIGURE 2 SiD's graphical user interface. Version SiD V1.0. The screen shows sperm selection and pick up shortly before intracytoplasmic sperm injection.

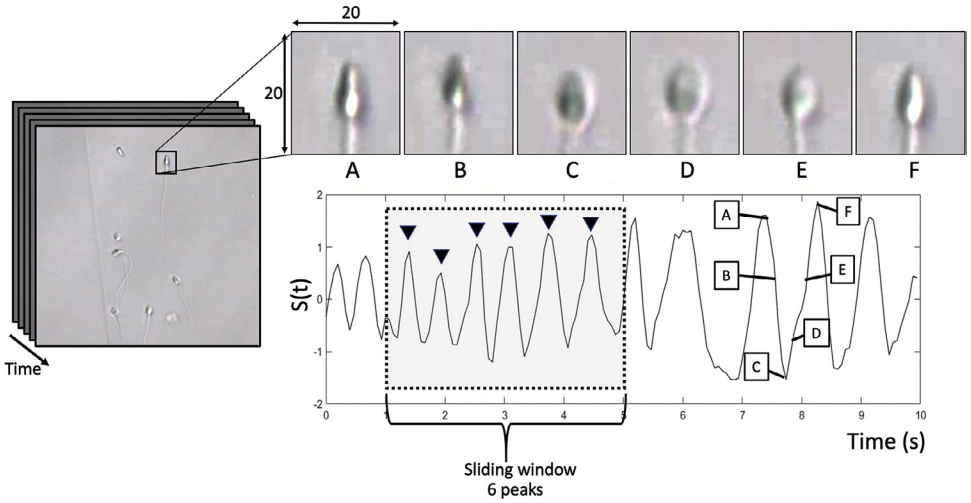

FIGURE 3 Sperm tracking and head movement pattern computation. Depiction of the sub-image generated around the centre of the detected sperm head and the correspondence between the sub-images of the sperm head across time and the mean intensity evolution signal S(t).

Additionally, it computes the HMP index (mean peaks per second [mp/s]), which is a value that is related to the changes of intensity that are observed on the image of the head of individual spermatozoon as they move, mostly related to rotation FIGURE 3. The HMP is computed based on the analysis of a discrete signal S(t), which describes the patterns on the variation of intensity of the sub-image of size w × w around the centre of each sperm head for a number of subsequent frames t∈ $[t_i, t_f]$. The signal S(t) is computed as:

$$S(t)=\frac{1}{3w^2}\left(\sum_{(m,n)} i_R(m,n,t)+\sum_{(m,n)} i_G(m,n,t)+\sum_{(m,n)} i_B(m,n,t)\right) \quad (1)$$

where $i_x(m, n, t)$ is the intensity value of a pixel at position {m,n} in the colour channel x∈ [R, G, B] at frame t. The HMP of a spermatozoon is defined as the mean number of detected peaks (mp/s) per second.

The SiD score σ of a spermatozoon is a value designed to be an index of the quality of its locomotion features. This value is computed as a linear combination of the normalized LIN, VSL and HMP values of each spermatozoon defined by:

$$\sigma=\Omega_{LIN}LIN+\Omega_{VSL}\frac{VSL}{MVSL}+\Omega_{HMP}\frac{HMP}{MHMP} \quad (2)$$

MVSL and MHMP are the maximum observed values for VSL and HMP for all the evaluated spermatozoa, respectively. The used weights were empirically defined as Ω_{LIN} = , Ω_{VSL} = , and Ω_{HMP} = 0.

Statistical analysis

On the basis of the two outcome measures, the mean VSL, LIN and HMP measurements for each set of spermatozoa were compared in three experiments. For the first experiment, two groups were defined: the NI set, consisting of spermatozoa that appeared on the videos, but were not selected by embryologists (n = 305); and the SI set, consisting of all those spermatozoa selected for injection (n = 78).

In the second experiment, two groups were defined: the NF set, consisting of those spermatozoa with negative fertilization (n = 21); and the PF set, consisting of selected and injected spermatozoa with a positive fertilization outcome (n = 57), defined as the presence of two pronuclei (2PN).

In the third experiment, two groups were defined: the NL set, consisting of those injected spermatozoa that were not associated with blastocyst generation (n = 38), and the PL set, consisting of selected and injected spermatozoa that were associated with blastocyst generation (n = 40).

For each experiment, a Shapiro–Wilk test was carried out (*Shapiro and Wilk, 1965*) for assessing the normality of the distribution of the groups to be compared. Whenever the test indicated that the group's data corresponded to normal distributions, the equality of their variances was assessed using the Levene's test (*Levene, 1960*). If, for an experiment, the condition of normality and equality of variance was fulfilled, then a Student's t-test (Microsoft Excel version 16.58) was used to evaluate the significance of the differences between the means of the groups.

In case the normality or equal variance conditions were not fulfilled, a non-parametric Mann–Whitney U test (*Mann and Whitney, 1947*) was used to evaluate the significance of the difference between the distributions of the data in the groups being compared. All the tests were carried out using a confidence interval of α = 0.05.

RESULTS

A total of 383 individual spermatozoa were analysed by SiD's algorithms during 78 recorded ICSI cycles. From this total, 78 spermatozoa were selected for injection, and the remainder (n = 305) were tagged as not-selected spermatozoa (a selected to available sperm ratio of 1:5). Once activated, SiD provided individual assessment and quantitative evaluation for all three parameters on all 383 spermatozoa (100% efficiency rate on parameter evaluation). The normal fertilization rate per ICSI (2PN) was 73% (57/78); with a 48% blastocyst rate (38/78); and 67% blastocyst rate per 2PN (38/57).

For the first experiment, the mean VSL value of the set SI (10.1 ± 3.8 pixels/s) showed a statistically significant difference ($P = 2 \times 10^{-9}$) compared with the mean VSL value of the set NI (6.7 ± 3.8 pixels/s). Similarly, the mean LIN value

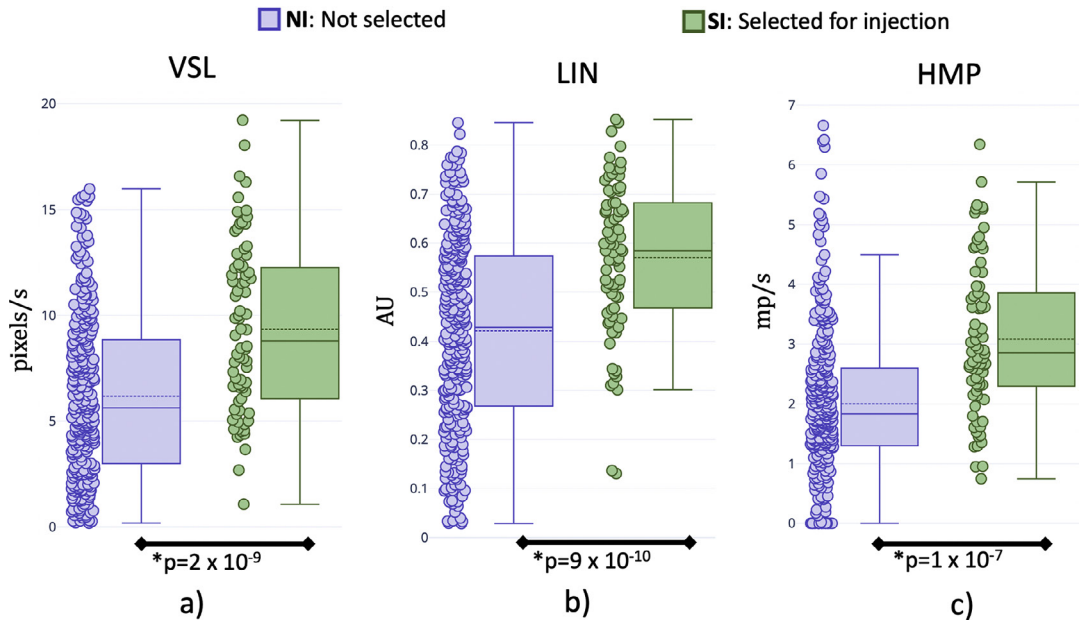

FIGURE 4 Motility parameters by injected and non-injected spermatozoa. Box plots showing the distribution of (a) straight-line velocity (VSL); (b) linearity of the curvilinear path (LIN); and (c) head movement pattern (HMP) values of the spermatozoa in the SI set, consisting of all those spermatozoa that were selected for injection (61 spermatozoa), and the NI set, consisting of all those spermatozoa that appeared on the videos but were not injected (246 spermatozoa). *, significant differences between the means of the two sets using a Student's t-test.

of the set SI (0.58 ± 0.14 AU) showed a statistically significant difference ($P = 9 \times 10^{-10}$) compared with the mean LIN value of the set NI (0.42 ± 0.19 AU). Moreover, the mean HMP value of the set SI (3.0 ± 1.2 mp/s) also showed a statistically significant difference ($P = 1 \times 10^{-7}$) compared with the mean HMP value of the set NI (2.0 ± 1.30 mp/s).

Box plots showing the distribution of VSL, LIN and HMP values for the spermatozoa in each set are presented in FIGURE 4.

For the second experiment, the differences between the mean of the LIN values of the PF set (0.59 ± 0.13) were statistically significant compared with the NF set (0.50 ± 0.18) ($P = 0.038$).

Similarly, the differences between the mean of the HMP values of the PF set (3.27 ± 1.24) were statistically significant compared with the NF set (2.55 ± 1.15) ($P = 0.029$). These results suggest that the spermatozoa in the PF set had more linear trajectories, and larger variations related to head movement patterns. No statistically significant difference,

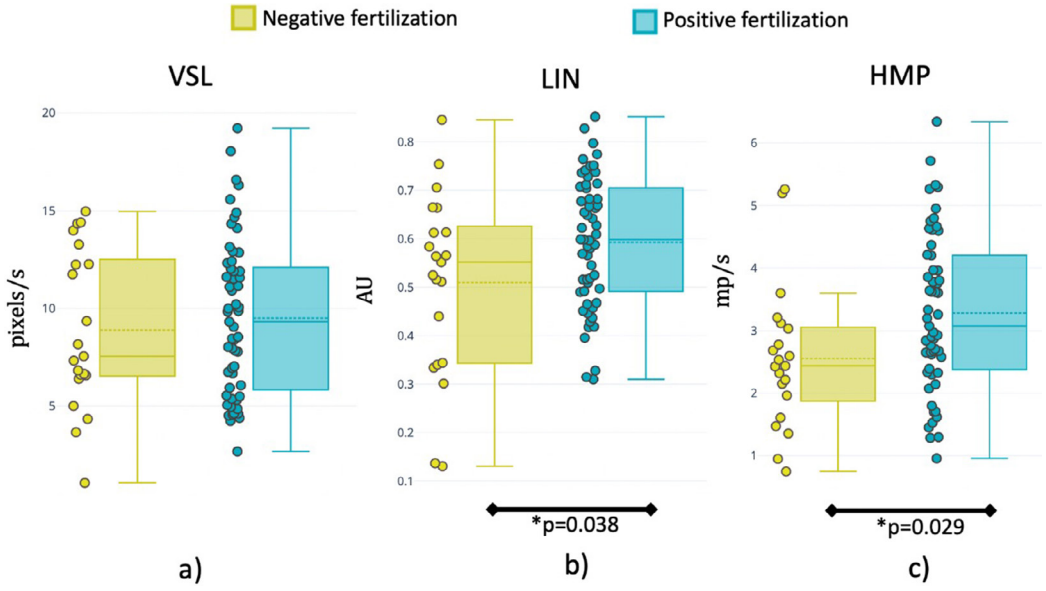

FIGURE 5 Motility parameters by fertilization outcome. Box plots showing the distribution of (a) straight-line velocity (VSL); (b) linearity of the curvilinear path (LIN); and (c) head movement pattern (HMP) values for the PF set, consisting of those selected and injected spermatozoa with a positive fertilization outcome (defined as the presence of two pronucei), and for the NF set, consisting of those spermatozoa with a negative fertilization result. *, significant difference between the means of the two sets using a Student's t-test.

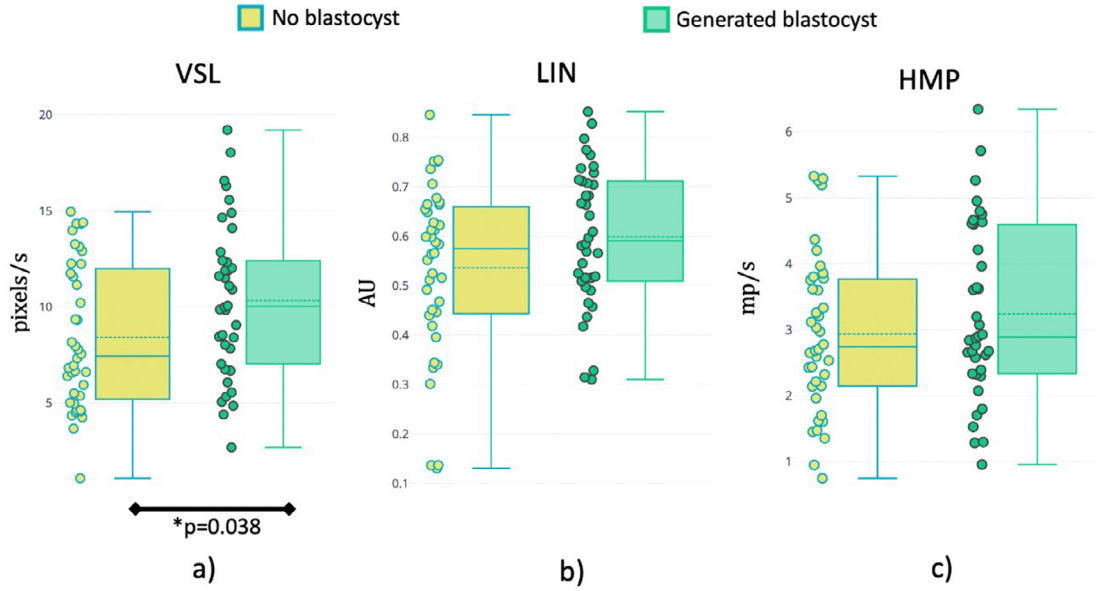

FIGURE 6 Motility parameters by blastocyst formation outcome. Box plots showing the distribution of (a) straight-line velocity (VSL); (b) linearity of the curvilinear path (LIN); and (c) head movement pattern (HMP) values for the PL set, consisting of the selected and injected spermatozoa that were associated with blastocyst generation, and the NL set, consisting of the injected spermatozoa that did not result in the generation of a blastocyst. *, significant differences between the distribution of the two sets using a Mann–Whitney U-test.

however, was found for VSL. Box plots that show the distribution of the VSL, LIN and HMP values for the spermatozoa in the PF and NF sets are presented in FIGURE 5.

In the third experiment, all mean values seemed to be higher for the PL set. Only the differences in the distributions of the VSL value, however, were significant in relation to blastocyst formation using the non-parametric Mann–Whitney U test (P = 0.038). Box plots showing the distribution of the VSL, LIN and HMP values for the spermatozoa in the PL and NL sets are presented in FIGURE 6.

Two box plots showing the distribution of the SiD score obtained by the injected spermatozoa are presented in FIGURE 7. Statistically significant differences (P = 0.004) were observed between the mean SiD scores of spermatozoa with a positive fertilization outcome (0.51 ± 0.10) and those with a negative outcome (0.43 ± 0.12). Similarly, statistically significant differences (P = 0.013) were found between the SiD scores of those spermatozoa that were associated with blastocyst generation

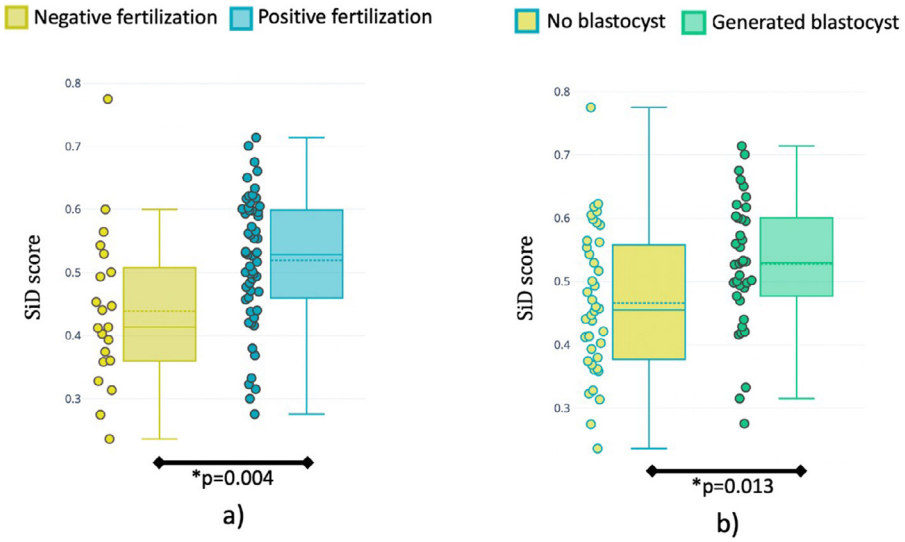

FIGURE 7 SiD score by fertilization and blastocyst formation outcomes. Box-plots showing the distribution of the SiD score values for (a) the PF set, consisting of those selected and injected spermatozoa with a positive fertilization outcome (defined as the presence of two procnuclei), and for the NF set, consisting of those spermatozoa with a negative fertilization result; and (b) the PL set, consisting of the selected and injected spermatozoa that were associated with blastocyst generation, and the NL set, consisting of the injected spermatozoa that did not result in the generation of a blastocyst. *, significant differences between the means of the two sets using a Student's t-test.

(0.52 ± 0.10) and those that did not (0.46 ± 0.11).

DISCUSSION

The motility characteristics of spermatozoa may be linked to the overall function and fertilization potential (*Dubey et al., 2019*). In natural conditions, the spermatozoa's success to fertilize an oocyte is associated with the direction and velocity of displacement. It has been reported that the direction spermatozoa navigate towards the site of fertilization can be associated with the contribution of at least three mechanisms (*Alvarez et al., 2014*; *Simons et al., 2014*; *Ishimoto and Gaffney, 2015*): thermotaxis, chemotaxis and rheotaxis. The velocity of displacement, in addition to being associated with factors that increase or decrease the drag resistance, such as viscosity of the medium, the flow direction and the spermatozoa head and flagellum geometry, also depends on the flagellum's power and beating patterns. Sperm movement patterns, however, seem more complex than originally anticipated (*Gallagher et al., 2019*), with one such example being sperm head movement (*Chan et al., 1989*; *Miki and Clapham, 2013*; *Subramani et al., 2014*).

Miki and Clapham hypothesized that the displacement of spermatozoa requires shear flow, proximity to a surface and a three-dimensional helicoid beat pattern that is also associated with sperm rolling (*Miki and Clapham, 2013*). Under laboratory conditions in which a single spermatozoon is selected for ICSI, spermatozoa are released in a fluid with a different viscosity than its usual environment (Hook, 2020; *Hook and Fisher, 2020*), and its velocity decreases artificially. In addition, the viscous medium is not submitted to any flow. In this environment, sperm movement is mainly associated with the flagellum's power-producing beat patterns that seem to be extensively diverse (*Ishimoto and Gaffney, 2015*). Although the head causes resistance to flow, its diameter is much smaller than that of the oscillation diameter of the flagellum, leading to more significant fluctuations in the pressure around the flagellum compared with the head (*Tian and Wang, 2021*). Consequently, some mathematical models describing the direction and velocity of displacement of spermatozoa neglect the effects of sperm head (*Ishimoto and Gaffney, 2015*). From all these factors, experimental and theoretical evidence strongly suggests that the flagellum's helicoid beat pattern is essential (*Miki and Clapham, 2013*; *Ishimoto and Gaffney, 2015*; *Tian and Wang, 2021*).

In the present study, three positive outcomes, i.e. SI, PF and PL, were related to higher means for all assessed parameters (VSL, LIN and HMP). We should, however, carefully consider the implication of these findings: first, selected spermatozoa had significantly higher means for all parameters evaluated, suggesting that embryologists intuitively selected the fastest spermatozoa with more linear motility and better head movement. Second, the differences between the mean LIN and HMP values were significant for fertilization, indicating that the spermatozoa with more linear trajectories and those with larger variations in head movement patterns may have better chances of fertilization success. Third, when evaluating blastocyst formation, the mean VSL was the parameter that differed significantly, which suggests an association between spermatozoa with higher velocity and improved blastocyst formation. Large HMP or LIN values, however, do not necessarily lead to successful fertilization, nor do large VSL values guarantee blastocyst formation.

Results from the present study suggest that SiD's real-time artificial vision was able to identify beneficial movement patterns of a single spermatozoon in a cohort visualized in PVP immediately before the ICSI procedure. These observations may significantly affect normal fertilization and blastocyst formation during ICSI. One potential benefit is that a digital sperm assistant like SiD could transform sperm selection for ICSI into a more objective process. It would assist embryologists accustomed to qualitative sperm observation by providing a quantitative single sperm analysis.

A point to consider is that the content of PVP and albumin in ICSI media available on the market may differ, which may influence viscosity and consequently velocity and motility patterns (*Hook and Fisher, 2020*). Considering that SiD performs a ranking, however, all spermatozoa will be subject to the same conditions and, therefore, the comparison will allow the identification of optimal candidates from a population.

SiD can work with existing equipment found in any ART laboratory and does not require other assets, such as unique chemical compounds, microfluidic devices or custom-designed Petri dishes (*Teixeira et al., 2013*). It conducts analyses of morphology using artificial intelligence to determine which objects in the sample are spermatozoa and if those spermatozoa are within a focal plane near the needle. The morphology information, however, is not currently used for determining the quality of the spermatozoa, as the resolution of the available ICSI videos is limited and detailed morphological analysis from those images may be challenging. It is planned, however, that future versions will allow morphologic analysis to be carried out by machine learning approaches in real time (*Riordon et al., 2019*; *Iqbal et al., 2020*).

As is common in assisted reproduction studies, other factors, such as oocyte quality or the ICSI technique, which have an effect on short-term outcomes, are likely (*Pool et al., 2012*). We acknowledge that our results should be interpreted carefully considering the high proportion of fertilized oocytes, and blastocyst rate, for which a larger dataset might be needed to fully assess the effect of motility parameters on blastocyst formation.

The present study shows that SiD, a specialized software combining artificial vision and artificial intelligence, can identify, track and quantify individual sperm motility patterns in real time. It can compare these to identify those spermatozoa with the most desirable parameters. To the best of our knowledge, this is the first study to associate quantitative patterns of individual spermatozoa with fertilization and embryo development, differently from other previous population studies.

A larger study including multiple clinics is already being developed to confirm correlations between motility patterns and outcomes. Real-time artificial vision tools such as SiD, which generates a real-time selection choice within a few milliseconds, could effectively assist embryologists during the sperm selection process for ICSI, reducing the time spent during selection and allowing an

objective method for sperm selection and potentially improving fertilization and blastocyst rates.

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SUPPLEMENTARY MATERIALS

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.rbmo.2022.03.036.

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