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Version Accepted Manuscript

Citation Xiao, S.; Riordon, J.; Simchi, M.; Lagunov, A.; Hannam, T.; Jarvi, K.;
Nosrati, R.; Sinton, D. FertDish: Microfluidic Sperm Selection-in-a-Dish
(published version) for Intracytoplasmic Sperm Injection. Lab Chip 2021, 21 (4), 775–783.
<https://doi.org/10.1039/D0LC00874E>.

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FertDish: Microfluidic Sperm Selection-in-a-Dish for Intracytoplasmic Sperm Injection

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Electronic supplementary information (ESI) available: Fig. S1 shows the concept of the Sperm Chromatin Dispersion (SCD) assay for assessing sperm DNA quality.

Abstract

The selection of high quality sperm is critical for intracytoplasmic sperm injection (ICSI), a prevalent assisted reproduction technology. However, standard selection methods are time-consuming and fail to recover the most viable sperm, thereby limiting the ICSI success rate. Microfluidics enables rapid selection of viable sperm in a manner representing *in vivo* processes, however, existing platforms lack clinical applicability. Here, we present FertDish, which integrates the clinically established ICSI petri dish with a film featuring an array of sperm-selecting microchannels for selection of sperm directly from semen. The FertDish format mimics the clinician-familiar ICSI dish setup, and provides rapid (< 10 min) single stage sperm preparation that circumvents standard labour-intensive multi-stage sperm processing steps. Tests with human donor and patient semen samples show that FertDish enables the selection of a high quality sperm sub-population, featuring improvements in DNA fragmentation index of more than 91% (donor) and 74% (patient) versus raw semen and 50% (donor) and 63% (patient) versus standard methods, and a distribution of more than 97% sperm with viable and high level DNA. The FertDish enables a high sperm recovery rate ($> 3.3 \times 10^5$ sperm/mL), and is readily adaptable to the clinical workflow with potential to improve ICSI outcomes.

Introduction

Human infertility is a global health concern, affecting at least 50 million couples and 186 million individuals worldwide, and causing significant social, physiological, psychological, and economic consequences.¹⁻³ Male infertility accounts for 50% of all infertility cases, and is caused by low semen quality (*i.e.* low sperm DNA quality, motility, and concentration) that is often insufficient for natural fertilization.^{4,5} Intracytoplasmic sperm injection (ICSI) is a prevalent (implemented in ~66% of clinics worldwide) infertility treatment technique.⁶⁻⁸ In ICSI, a single sperm cell is injected into an oocyte for fertilization *in vitro*. The success of ICSI and sperm-oocyte fertilization potential relies considerably on the selection of high quality sperm, which are largely characterized by high DNA quality and motility, from the cohort of damaged and unviable sperm and debris in semen.^{9,10}

Standard methods used for sperm selection for ICSI involve multiple semen purification and sperm isolation steps. Viable sperm from semen are first isolated based on their densities *via* density gradient centrifugation (DG), and then motile sperm are separated out of the viable population *via* the sperm swim-up assay (SU).¹¹ In the last step, following DG and SU, viable and motile sperm are introduced into a pipetted media droplet in a petri dish, and individual sperm are selected from the drop for injection. This standard selection approach has several disadvantages: (i) DG and SU require multiple stages and protocols, and are time-consuming (2-3 h); (ii) DG and SU can induce sperm damage and produce inadequate sperm concentrations;¹¹⁻¹³ and (iii) the formation of the pipetted media droplet is a non-standardized step that involves freely pipetting a bulk fluid droplet, and droplet shapes vary by user and clinical setting. These established selection methods fail to select the top sperm candidates in terms of quality and fertilization potential – a key factor limiting the success rate of ICSI (~33%).¹⁴⁻¹⁶

Microfluidics provides promising *in vitro* opportunities for mimicking the *in vivo* environment to support cells and their innate cellular functions.¹⁷ For infertility applications, there has been the development of microfluidic sperm selection platforms. Such platforms provide confined microgeometries resembling, and on the scale of, the structural microenvironment that sperm encounter during natural fertilization.^{18,19} High quality sperm can be selected based on their migration behavior in these confined microgeometries.¹⁸⁻²¹ The process of sperm selection can support preparation procedures that are considerably more efficient than standard methods (*i.e.* DG and SU), with sufficient recovery of selected sperm at various clinically relevant times (minimum 20 min; maximum 3 h). The minimal preparatory time gives opportunities for adaptability within the clinical workflow for both female and male processes. The dynamic nature of oocyte

preparation and maturation significantly influences the overall ICSI timeline. As such, the time taken for sperm selection can be readily tuned to coincide sperm-oocyte schedules, and employ better patient-oriented testing.

There have been several notable microfluidic sperm selection platforms to date. Nosrati *et al.* introduced a radial platform comprised of 500 parallel microchannels (9 mm length $\times$ 100 μ m width $\times$ 75 μ m depth) that lever sperm boundary following – a behavior in which sperm travel along, and are directed by, a surface (*e.g.* microchannel walls) – to select for motile sperm.^{21,22} Riordon *et al.* developed a platform comprised of 3250 microchannels (400 μ m length $\times$ 2 μ m depth), which select sperm based on their ability to swim in the two-dimensional planar mode – a behavior that is exhibited in sperm with superior DNA quality.^{23,24} Chinnasamy *et al.* demonstrated a design featuring a periodic array of micropillars (10 μ m diameter $\times$ 50 μ m height) that only allows sperm with normal morphology and motility characteristics to traverse through and be selected.²⁵ Another design, by Asghar *et al.*, incorporates a membrane with confined micropores (8 μ m diameter) and that selects sperm based on their ability to migrate through the pores.²⁶ For all these microfluidic platforms, considerable improvements were reported in the quality of selected sperm, in particular DNA quality, when compared to raw semen, indicating that microconfinement can enable the selection of a high quality sperm sub-population.^{22,24-26}

Success of microfluidic sperm selection platforms (including those introduced by Nosrati *et al.* and Riordon *et al.*) on the lab bench, has not translated to clinical settings. The issue is clinical applicability.^{18,22,24} The manufacturing of these platforms involves infrastructure-intensive fabrication methods (*e.g.* clean-room), which lack the ability for mass production, and the use of polydimethylsiloxane (PDMS).^{18,20} In addition, the sperm selection process focuses solely on the sperm preparation and separation steps, aiming to replace DG and SU, and thus requires additional clinical steps including further sample processing and material transfers (to culture tubes or ICSI petri dishes) – all of which introduce costs and risk. Clinical application is further burdened by unfamiliar preparation and operating procedures, with associated training and personnel costs.^{18,20} These barriers to the adoption highlight the need for a platform that fully integrates the existing clinical requirements, protocols and materials with microfluidic sperm selection. Such a platform would include only clinically applicable materials, require no additions to current clinical workflow, and prioritize the experience of the clinician.^{27,28}

Here, we focused on the clinically familiar and ubiquitous ICSI petri dish platform and integrated microfluidic capabilities that enable high quality sperm selection. This microfluidic

sperm selection-in-a-dish platform, FertDish, incorporates a two-layer film with an array of confined microchannels that select sperm, *via* boundary-following behavior, directly from raw semen. Preparation of the FertDish is representative of the clinical ICSI petri dish setup, rapid (< 10 min), and single stage, supporting all sperm preparation and processing steps and ICSI in-dish. The performance of the FertDish is evaluated using human donor and in-clinic patient semen samples, and quantified by selected sperm DNA quality – expressed *via* DNA fragmentation index (DFI) and the distribution of sperm with non-fragmented DNA and intact DNA strands. Sperm selected by the FertDish, at clinically relevant processing times, exhibit significant improvements in DNA quality over the initial raw semen, including > 91% (donor) and > 74% (patient) in DFI, and > 46% (donor) and > 10% (patient) in the distribution of sperm with non-fragmented and intact DNA. Moreover, the FertDish provides sufficient throughput, recovering $2.3 \times 10^5 - 3.3 \times 10^5$ sperm/mL and $1.2 \times 10^5 - 1.5 \times 10^5$ sperm/mL in donor and patient tests, respectively, which comfortably meet the ICSI standard. When tested alongside standard selection methods (*i.e.* DG and SU), the FertDish shows significant advantages in operating efficiency and streamlining preparatory stages, and DNA quality, providing DFI improvements of > 50% (donor) and > 63% (patient). The FertDish effectively selects high quality sperm in a clinically applicable and adaptable manner, with potential to improve both the economics and the effectiveness of ICSI.

Results and discussion

Platform design and operation

The FertDish was designed based on clinical relevancy and hence only materials and protocols used in clinical settings were considered. The design was inspired by the setup of the standard ICSI petri dish as an open system concept, allowing accessible loading and recovery of samples. The platform features a two-layer film, comprised of a patterned film and a cover film, which is incorporated within a petri dish (Fig. 1a and b). The patterned film consists of an array of 60 open microchannels that converge to an open center region, while a cover film overlays a section of the patterned film, forming closed channels in that section. The cover film establishes open inlet and outlet regions, and prevents mixing. A cutting plotter was used to cut the films and pattern (or etch) the channels, and allowed for rapid (< 5 min) and inexpensive (< \$5 CAD per platform) fabrication, while supporting the necessary channel resolutions for proper sperm confinement. Each channel had a cross-section resembling a trapezoid, with widths of 10 μ m and 50 μ m in the bottom and top channel planes, respectively, effectively confining sperm and providing a continuous boundary for

sperm to follow. The length of each channel was 10 mm, ensuring sufficient distance for motile sperm to migrate between the inlet and outlet without fatigue or loss of quality.^{22,29}

The FertDish enables a rapid (requiring < 10 min technician time) and single stage preparatory procedure for sperm selection that mimics the setup process of the standard ICSI petri dish, and replaces current multi-stage semen processing and sperm washing protocols (requiring > 2 h technician time). The procedure involves loading the outlet with polyvinylpyrrolidone (PVP) media (70 μ L), which also simultaneously fills the connecting channels *via* capillary action, followed by loading the inlet with raw semen (100 μ L), and then overlaying the entire platform with paraffin oil (2 mL) to prevent evaporation (Fig. 1c). PVP exhibits a relatively high viscosity of > 20 mPa-s, similar to that of the mucus in the female reproductive tract, and has high flow resistance. Hence, only motile sperm with boundary-following behavior are able to migrate radially inwards through the channels to reach the outlet during the incubation time (at 37°C), whereas non-motile sperm and debris remain at the inlet (Fig. 1e). Conceptually, ICSI can be performed following the incubation time for sperm selection (Fig. 1d). Droplets containing oocytes can be introduced, *via* a holding capillary, into the designated region opposite to the films. Subsequently, individual selected sperm can be injected, *via* a transfer capillary, into the oocyte for fertilization *in vitro*.

The time-on-task demanded of technician to complete the process, and the total time required for the process are important timescales in the clinical application of sperm processing and selection. A low technician time-on-task (here ~10 min) reduces cost. The time required for the sperm selection process, however, must be compatible with the overall clinical workflow, which is generally governed by the female side. Current clinical practice requires ~2 h total duration to perform ICSI, and thus the FertDish was tested with incubation periods of 1 and 2 h to ensure compatibility with the clinical workflow.

Fig. 1 FertDish sperm selection. a) Assembly of the FertDish, comprising of a two-layer film (a combination of a cover film and a patterned film) and a standard ICSI petri dish. The inset shows a schematic of the channel cross-section, with widths between 10 μ m (bottom plane) and 50 μ m (top plane). b) The FertDish platform, showing open inlet and outlet regions, along with closed microchannels formed by the cover film overlay. Scale bar: 10 mm. c) Process flow diagram of the FertDish operation, which consists of dish preparation and incubation steps for sperm selection. d) The FertDish ICSI process, including oocyte loading, selected sperm injection, and

sperm-oocyte fertilization steps. e) Conceptualization of the FertDish sperm selection, illustrating the migration of motile sperm within the channels *via* boundary-following swimming, with images of sperm at the (i) top and (ii) bottom channel planes. Scale bars: 15 μ m.

Assessing selected sperm DNA quality with donor semen samples

Sperm DNA quality is an important measure of the overall sperm quality and plays a critical role in fertilization rate, embryo development, and ICSI success.³⁰ To quantify sperm DNA quality, we used the sperm chromatin dispersion (SCD) assay. SCD provides a simple, reliable, and reproducible approach to examine sperm DNA at both the population and single cell level based on the ratio between the produced “halo” of dispersed DNA (termed dimension ‘B’) around the sperm head (termed dimension ‘A’).^{31,32} The halo size represents the intactness of double stranded DNA (dsDNA) and is negatively correlated to the level of sperm DNA fragmentation – larger halos are present when dsDNA are intact (higher DNA integrity) and smaller or no halos appear when there are many DNA strand breaks (lower DNA integrity). A B/A ratio threshold of 0.34 indicates whether an individual sperm has non-fragmented or fragmented DNA (Fig. S1 in ESI). Sperm DNA quality based on SCD was quantified by two metrics: 1) the percent of sperm with fragmented DNA within the sample population, as represented by percent DNA fragmentation index (DFI), and 2) the distribution of sperm B/A values, as determined by the peak (mean) sperm B/A value and percent of sperm with intact DNA of the sample population.

The selection performance of the FertDish was first characterized with human donor semen samples. The DNA quality of selected sperm was quantified over testing times of 1 h and 2 h, and compared to that of the initial raw semen and standard DG+SU selection. On average, the DFI of sperm selected by FertDish at 1 h and 2 h were 2.8% and 2.6%, respectively (Fig. 2a), indicating improvements of > 91% over the initial raw sample (33% DFI). The selected sperm at 1 h and 2 h featured distributions that were largely composed of sperm with non-fragmented DNA (> 97% of the population) and high peak sperm B/A values (~0.87), which translated to improvements of 46% and 75% when compared to raw, respectively (Fig. 2b and c). The FertDish outperformed DG+SU selection by > 50% in DFI in all cases. The outcomes between 1 h and 2 h testing were similar (no significant difference, $p > 0.05$), demonstrating that the effect of testing time on selected sperm DNA quality was minimal.

Notably, the FertDish was able to effectively select a cohort of sperm with non-fragmented DNA and high B/A values, regardless of the range of donor semen quality (18-50% DFI) or testing time, displaying a consistent, reliable, and clinically adaptable sperm selection process.

Fig. 2 Sperm selection outcomes with human donor semen samples. a) DFI of sperm selected by the FertDish at 1 h and 2 h testing times as compared to sperm from the initial raw semen and DG+SU selection. b) Distribution of sperm B/A levels from raw, DG+SU selection, and FertDish at 1 h and 2 h. The dashed line shows the threshold value of 0.34 separating fragmented and non-fragmented DNA. c) Peak sperm B/A levels from raw, DG+SU selection, and FertDish at 1 h and 2 h. Given three donor samples, for each test sample, $N = 1$ replicate for raw and DG+SU, and $N = 2$ replicates for FertDish at 1 h and 2 h, with $n = 1000-2000$ sperm analyzed for each replicate. * $p < 0.05$ for DG+SU and FertDish 1 h and 2 h when compared to raw. • $p < 0.05$ for FertDish 1 h and 2 h when compared to DG+SU. Error bars represent standard error of the mean in a, b, and c.

Clinical assessment of selected sperm DNA quality with patient semen samples

To examine the applicability of the FertDish in a clinical setting, we tested the FertDish in-clinic and with human semen samples from patients undergoing real-time fertility evaluations. The selection performance of the FertDish was quantified by the DNA quality of selected sperm at 1 h and 2 h testing times, and the outcomes were compared to that of the initial raw semen and clinical DG+SU selection.

The DFI of sperm selected by FertDish at 1 h was 3.1% and at 2 h was 2.5%, improvements over that of the initial raw semen (12% DFI) by 74% and 79%, respectively (Fig. 3a). The distribution of B/A values for both FertDish at 1 h and 2 h were similar (no significant difference, $p > 0.05$) and featured a considerable amount of sperm with non-fragmented DNA (> 97% of the population) and a high peak sperm B/A level (~0.83) – improvements of more than 10% and 11%, respectively, when compared to raw (Fig. 3b and c). The performance of the FertDish exceeded that of DG+SU selection (8.4% DFI, distribution of 92% non-fragmented sperm, 0.76 peak sperm B/A). Notably, the DFI of sperm selected by the FertDish at 1 h and 2 h were lower by 63% and 70%, respectively. In addition, for both FertDish testing times, the distributions consisted of a relatively higher percentage of non-fragmented sperm (> 5%), while the peak sperm B/A value was higher by > 10%.

A slight discrepancy was observed in the DFI between the 1 h and 2 h testing times of the FertDish (~20% difference). This outcome suggests that testing time may influence the population numbers of selected sperm, which would in turn vary the population DFI. For instance, more motile sperm with non-fragmented DNA may reach the outlet due to the additional time, leading to further decreases in DFI. The FertDish nevertheless demonstrated effective selection of sperm with non-fragmented DNA and high B/A values, which was consistent for both 1 h and 2 h testing times, from raw patient samples of various qualities (8-17% DFI). The testing of the FertDish in-clinic and in real-time shows that the platform is applicable and adaptable to the clinical workflow, and outperforms clinical DG+SU in terms of performance.

Fig. 3 Sperm selection outcomes with human patient semen samples. a) DFI of sperm selected by the FertDish at 1 h and 2 h testing times as compared to sperm from the initial raw semen and DG+SU selection. b) Distribution of sperm B/A levels from raw, DG+SU selection, and FertDish at 1 h and 2 h. The dashed line shows the threshold value of 0.34 separating fragmented and non-fragmented DNA. c) Peak sperm B/A levels from raw, DG+SU selection, and FertDish at 1 h and 2 h. Given three patient samples, for each test sample, $N = 1$ replicate for raw and DG+SU, and $N = 2$ replicates for FertDish at 1 h and 2 h, with $n = 1000-2000$ sperm analyzed for each replicate. * $p < 0.05$ for DG+SU and FertDish 1 h and 2 h when compared to raw. • $p < 0.05$ for FertDish 1 h and 2 h when compared to DG+SU. Error bars represent standard error of the mean in a, b, c.

Evaluating concentration of selected sperm from donor and patient samples

To ensure that the FertDish provides sufficient throughput and recovery of selected sperm for ICSI, the concentrations of selected sperm at 1 h and 2 h testing times, from human donor and patient semen samples, were evaluated and compared alongside DG+SU selection.

Donor raw semen had an average of 6.6 million sperm (6.6×10^7 sperm/mL). The FertDish recovered more than 2.3×10^4 sperm (2.3×10^5 sperm/mL) at 1 h and 3.3×10^4 sperm (3.3×10^5 sperm/mL) at 2 h, while DG+SU selection recovered ~6,000 sperm (6.0×10^4 sperm/mL) (Fig. 4a). Given an average of 8.9 million sperm (8.9×10^7 sperm/mL) in patient raw semen, the FertDish recovered more than 1.2×10^4 sperm (1.2×10^5 sperm/mL) at 1 h and 1.5×10^4 sperm (1.5×10^5 sperm/mL) at 2 h. DG+SU selection recovered $\sim 1.4 \times 10^4$ sperm (1.4×10^5 sperm/mL) (Fig. 4b).

Compared to DG+SU selection, the selected sperm concentrations from FertDish at 1 h and 2 h were considerably higher (4-5 fold) in donor sample tests, and comparable in patient sample tests (no significant difference, $p > 0.05$). For both donor and patient sample evaluations, the concentrations of selected sperm displayed a positive trend between 1 h and 2 h testing times, indicating that a higher number of motile sperm can be selected (*e.g.* reach the outlet) given the additional time.

ICSI ultimately requires one viable sperm for injection (from a minimum standard of ~1000 sperm), however, distinguishing and locating the individual sperm is often challenging. The FertDish enabled a high recovery rate of sperm with high DNA quality (Figs. 2 and 3) at concentrations that are more than sufficient for ICSI. In a clinical context, the incubation time and thus concentration can be adapted based on the numbers required for the particular workflow given

the quality of the original semen sample. For instance, normozoospermic samples may require shorter incubation times, whereas oligozoospermic or asthenozoospermic samples may require longer incubation times, to recover an adequate amount of viable sperm.

Fig. 4 Sperm concentrations from donor and patient sample testing. a) Sperm concentrations from donor samples, including raw, DG+SU, and FertDish at 1 h and 2 h. b) Sperm concentrations from patient samples, including raw, DG+SU, and FertDish at 1 h and 2 h. $N = 3$ replicates for DG+SU and FertDish 1 h and 2 h. Error bars represent standard error of the mean in a and b.

Conclusion

The FertDish provides a clinically applicable and single stage procedure for the selection of sperm with high DNA quality for ICSI. By incorporating the traditional ICSI petri dish with an array of confined microchannels *via* a patterned film, the FertDish setup fits within the existing clinical workflow yet leverages microfluidic technology to select a high quality sperm sub-population based on boundary-following behavior. Testing with human donor semen samples showed that sperm selected by the FertDish at 1 h and 2 h were of high DNA quality – improvements of 91% in DFI, 46% in distribution of non-fragmented sperm, and 75% in peak sperm B/A when compared to the initial raw semen. In addition, quantifying with in-clinic human patient semen samples demonstrated the ability of the FertDish to select sperm with high DNA quality while effectively operating in clinical settings. Selected sperm, at 1 h and 2 h, from patient samples displayed improvements of 74-79% in DFI, 10% in distribution of non-fragmented sperm, and 11% in peak sperm B/A over raw semen. Notably, the FertDish outperformed the current clinical standard, DG+SU. The technician preparation time for sperm selection was considerably reduced, going from the standard 2 h multi-stage process for DG+SU to a 10 min single stage setup for the

FertDish. When compared to DG+SU, the DNA quality of sperm selected by the FertDish (at 1 h and 2 h) displayed improvements of > 50% in DFI in donor sample tests, and > 63% in DFI, > 5% in distribution of non-fragmented sperm, and > 10% in peak sperm B/A in patient sample tests.

The FertDish demonstrated high sperm recovery, with selected sperm concentrations surpassing the minimum criteria for ICSI, which provides flexibility and higher probability in determining the top sperm candidates for injection. A positive correlation was observed between the selected sperm concentration and testing time, additionally indicating that testing time can be adapted for the desired clinical workflow to enable testing that is more patient-oriented. For instance, accommodating for factors such as different semen qualities, and the coinciding of sperm-oocyte preparation schedules such that both sperm and oocyte are simultaneously ready for ICSI.

The straightforward assembly approach, low-cost and widely accessible fabrication method, and use of only pre-existing clinical materials enable the FertDish to be readily available for large scale, rapid, and inexpensive manufacturing. The reductions in cost, equipment needs, and personnel time offered by FertDish are particularly welcome in the context of the COVID-19 pandemic. There are now increased financial and operational pressures on clinical fertility practices, and clinical procedures and workflows require increased sanitation time and social distancing of staff within clinical settings.^{33,34} The FertDish approach streamlines the process of microfluidic sperm selection and performing ICSI in a clinically applicable manner, and has the potential to improve ICSI outcomes.

Experimental

Platform fabrication

The FertDish features a two-layer biocompatible film that is incorporated within a standard ICSI petri dish. The two-layer film follows a semi-circle shape and consists of a patterned film, containing an array of 60 open microchannels, and a cover film, which overlays a section of the patterned film to establish open inlet and outlet regions. Inkscape was used to design the films, and a cutting plotter (Silhouette Studio 3; Silhouette Inc., Canada) was used to cut the films out of opto-adhesive PCR film (Sigma Aldrich, Canada). The etch function of the cutting plotter was used to pattern the array of microchannels, whereby etching the surface of the film with the plotter blade tip (triangular shaped) created channels with 10 μm (bottom plane) to 50 μm (top plane) width, 10 mm length, and 100 μm depth. The length of a complete channel (patterned film along with cover film) consisted of 6 mm open and 4 mm closed geometry, with the open portion of the channels defining the inlet ($\sim 230 \text{ mm}^2$). The channels, past the closed portions, connected to an open outlet region ($\sim 40 \text{ mm}^2$). After cutting, the patterned film was wiped with 70% ethanol, rinsed with distilled water, and dried in air. The patterned and cover films were treated with plasma (BD-20AC; Electro-Technic, Canada) to render hydrophilic, combined together, and applied onto a 60 mm diameter petri dish (MedStore, Canada).

Semen sample preparation

Donor semen samples, which were cryogenically frozen, were obtained from ReproMed Ltd (Toronto, Canada) and stored in a freezer at -80°C . Samples were thawed at 37°C for 20 min prior to each experiment. Patient semen samples were obtained from the Hannam Fertility Centre (HFC) (Toronto, Canada) from men undergoing fertility assessments. Fresh samples were incubated at 37°C for 30 min to allow liquefaction, and experiments were performed immediately after at HFC. Donors and patients provided informed consent for research purposes in accordance with the regulations of the Assisted Human Reproduction Act (approval number: 37629). The University of Toronto Biosafety Office approved the experimental use of frozen donor samples and fresh patient samples.

Media preparation

10% polyvinylpyrrolidone (PVP) was prepared by dissolving PVP powder (1 g) (Sigma Aldrich, Canada) in PureSperm wash (10 mL) (Nidacon, Canada). Media (70 μ L) used for each test consisted of a mixture with PureSperm wash (30 μ L) and 10% PVP (40 μ L).

FertDish sperm selection procedure

The full testing procedure includes media and semen loading, sperm selection, and sperm retrieval. Media (70 μ L) was first loaded in the outlet region. After $\sim$ 2 min waiting time, all channels were filled with media *via* capillary action. Semen (100 μ L) was then loaded in the inlet region. The platform was then placed inside an incubator at 37°C for the duration of the experiment. Experiments were performed for 1 h and 2 h, and the effect of time on selected sperm concentration and DNA integrity was examined. Selected sperm were retrieved from the outlet by repeated aspiration and re-suspension (with PureSperm wash ($\sim$ 40 μ L)) of the outlet media with a pipette until a final retrieved volume of $\sim$ 200 μ L was achieved.

DG and SU sperm selection procedure

To isolate viable sperm from semen, two-layer density gradient centrifugation was performed using PureSperm 80 and PureSperm 40 (Nidacon, Canada) at 500 g for 15 min. The supernatant was aspirated, leaving a pellet containing viable sperm. The pellet was re-suspended in PureSperm wash (Nidacon, Canada) and centrifuged at 300 g for 5 min. The aspiration, pellet re-suspension, and centrifugation process was repeated twice. The sperm swim-up assay was then performed, in which the final pellet was laid below a layer of PureSperm wash in a culture tube and placed in an incubator (at 37°C) for 1 h to isolate motile sperm. 200 μ L at the top of the swim-up layer was aspirated for sperm quality assessments. Donor samples used 100 μ L each for PureSperm 80 and 40, and PureSperm wash (during centrifugation steps), and 500 μ L for swim-up PureSperm wash. Patient samples used 1 mL each for PureSperm 80 and 40, and PureSperm wash (during centrifugation steps), and 2 mL for swim-up PureSperm wash.

Sperm DNA quality assessment

Sperm were assessed for DNA quality directly following selection. The DNA quality of sperm was examined using the sperm chromatin dispersion (SCD) or “Halo” assay, and conveyed as percent DNA fragmentation index (DFI), and distribution of, along with peak, sperm B/A values. The SCD kit was obtained from Fertitech Canada Inc. (Montreal, Canada), and the assay was performed

according to the provided protocol. Briefly, 1% low melting point agarose gel (~80 μ L) was first dissolved (incubated at 80°C for 20 min). Sperm (60 μ L) were then dispensed into the dissolved gel. The sperm-agarose suspension (30 μ L) was dispensed onto an agarose-coated (0.65%) glass slide, covered with a slide cover, and placed in a refrigerator (2-8°C) for 5 min for gel solidification. The slide cover was then removed, and the slide was introduced to an acid denaturing solution (2 mL; 0.08 N HCl) for 7 min followed by a neutralizing and lysing solution (2mL; 0.4 M Tris, 0.8 M DTT, 1% SDS, and 50 mM EDTA, pH 7.5) for 25 min at room temperature. The slide was washed with distilled water for 5 min, placed in various ethanol baths (70%, 90%, 100%) for 2 min each sequentially, and dried in nature. For sperm staining, Wright's stain (300 μ L) and Wright's buffer (750 μ L) were dispensed onto the slide. After 15 min, the slide was rinsed with water and air-dried. The slide was analyzed using the EVOS FL Auto Imaging Microscope (ThermoFisher Scientific, Canada). Stained sperm, with various halo sizes, were automatically located and imaged (20 $\times$ magnification) using the EVOS scan program. The images were analyzed using in-house software, and sperm head sizes and halos were automatically measured and B/A values were computed.

Sperm concentration assessment

Selected sperm were stored in a refrigerator (at 2-8°C) for three days prior to assessing concentration. Propidium iodide (1 μ L) (ThermoFisher Scientific, Canada) was introduced to the sperm (100 μ L) for labeling. The suspension (10 μ L) was loaded into a hemocytometer (Sigma Aldrich, Canada) and analyzed under the EVOS microscope using the fluorescence mode, and sperm concentration was measured.

Author contributions

S.X., J.R. and D.S. planned the research; S.X. developed and tested the platform; S.X. and M.S. established the SCD sperm DNA assay; S.X., J.R. and R.N. interpreted and validated the data; S.X., J.R., R.N. and D.S. wrote the manuscript; S.X., J.R., M.S., A.L., T.H., K.J., R.N. and D.S. helped with the interpretation of data and provided insightful feedback.

Acknowledgements

This work was supported by the Natural Sciences and Engineering Council of Canada (NSERC) through the Collaborative Health Research Projects (CHRP) program, the Canadian Institutes of Health Research (CIHR), the NSERC Discovery Grants and Discovery Accelerator programs, an NSERC E.W.R. Steacie Memorial Fellowship, and the Canada Research Chairs program. The authors also acknowledge support from the Australian Research Council Discovery Program (R.N. and D.S.), and the Monash Interdisciplinary Research Grant (R.N.).

Conflicts of interest

The authors declare no conflict of interest.

Notes and references

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