Electronic Supplementary Material (ESI) for Analyst.

Supporting Materials

Embryonic development through *in vitro* fertilization using high-quality bovine sperm separated in a biomimetic cervix environment

Cad Designs for SSCs for ICSI and IVF

Figure S1 shows the cad designs for the two sperm sorting chips, (A) ICSI-SSC and (B) IVF-SSC. The PMMA-based substrate of ICSI-SSC has rectangular shape of 16.85 mm×18 mm, and the inlet and outlet are circular shaped. The radius of the inlet is 1.1 mm, and the outlet is 0.5 mm. The width of the channel through which sperm swim is 1 mm, as shown in Fig. S1(A). The IVF-SSC uses PMMA substate of 22.1 mm×25.22 mm, where the inlet radius is 4 mm and the outlet radius is 2 mm. The width of channel is 2.5 mm. Overall, IVF-SSC has larger channel than ICSI-SSC, which facilitates to sort a larger amount of semen.

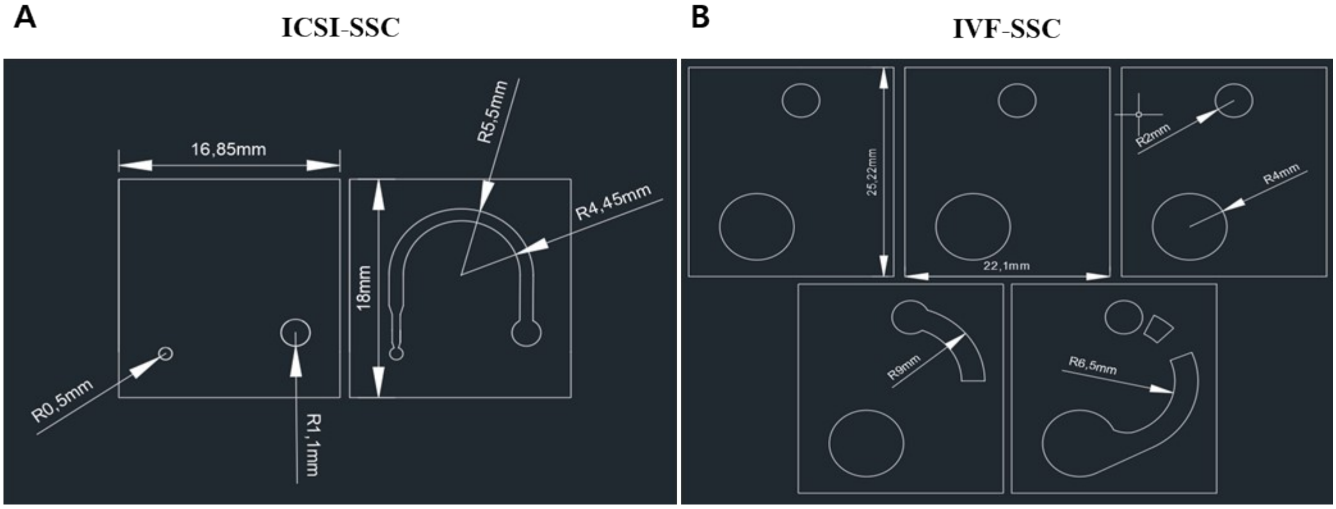

Fig. S1. Cad designs for (A) ICSI-SSC and (B) IVF-SSC.

Viscometry of medium solutions for the SSC

We use quartz tuning fork as a force sensor, and the measured force is associated with fluid viscosity in contact with the tip. First, for the force measurement, we modeled the motion of the quartz tuning fork (QTF) as a driven oscillator:

$$m\ddot{x} + b\dot{x} + kx = F\cos wt + F_{int}, \tag{1}$$

where m is the effective mass, b and k are the damping and elastic constants, F is the driving force, w is the driving angular frequency, and F_{int} is the tip-sample interaction force. The F_{int} (Eq. (1)) is represented as the sum of elastic force F_k and damping force F_b, such that $F_{int} = F_k + F_b$, and they are further characterized by the elastic k_{int} and damping b_{int} coefficients (Ref. [28] in the main text). Here, the viscosity of the sample can be deduced from the inertial flow of the fluid sheared, which induces the elastic interaction k_{int} between the tip and the sample. The k_{int} is measured using the force spectroscopy method of atomic force microscopy (Ref. [31] in the main text),

$$k_{int}/k = \left(\frac{A_0}{AQ}\right)sin\theta + \left(\frac{w}{w_0}\right)^2 - 1, \tag{2}$$

where A_0 is the amplitude of QTF resonator at resonance frequency in free space, A the response amplitude, Q the quality-factor, θ the phase and w_0 the resonance angular frequency. The viscosity of sheared fluid is calculated from the elastic part of interaction, k_{int}, as follows (Ref. [29] in the main text),

$$\eta = 2k_{int}^2/(\sigma^2 w^3 \rho), \tag{3}$$

where σ is the area of shearing tip's bottom and ρ is the density of the fluid. This measurement method allows to probe the fluid's inertial effect k_{int} only, involving the viscosity and the density of fluid, while circumventing interfacial effect such as the solid-liquid line interaction. Thus, fluid's viscosity is accurately measured from the information of k_{int}.

Measurement of mean square displacement of sperm motion

We measured the mean square displacement (*MSD*) of sperm cells by analyzing the position of the sperm over time. This information was collected through tracking the sperm's movement, and the *MSD* at a time *m* is calculated using Eq. (1).

$$MSD(m) = \frac{\sum_{n=0}^{T-m} (x(n+m) - x(n))^2 + (y(n+m) - y(n))^2}{(T-m)/\tau}, \tag{4}$$

where *m* and *n* are integer multiples of the time interval τ between video frames, *T* is the total time, *x* and *y* are the positions of sperm in *x*- and *y*-directions, respectively. The experimentally measured *MSD* (Eq. (1)) yields the diffusion constant D and time t with exponent α, as follows,

$$MSD = Dt^{\alpha}. \tag{5}$$

Here, the parameter α defines sperm motility: α≈1 represents Brownian motion, α≈2 signifies directed motion, and 1<α<2 or irregular MSD curves indicate anomalous motion. In our research, sperm exhibiting directed motion (α≈2) were classified as progressively motile, while those showing Brownian or anomalous motion were classified as non-progressively motile (Ref. [30] in the main text).