Electronic Supplementary Material (ESI) for Lab on a Chip.

Supplementary Information

Faster sperm selected by rheotaxis leads to superior early embryonic development in vitro.

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Figure S1

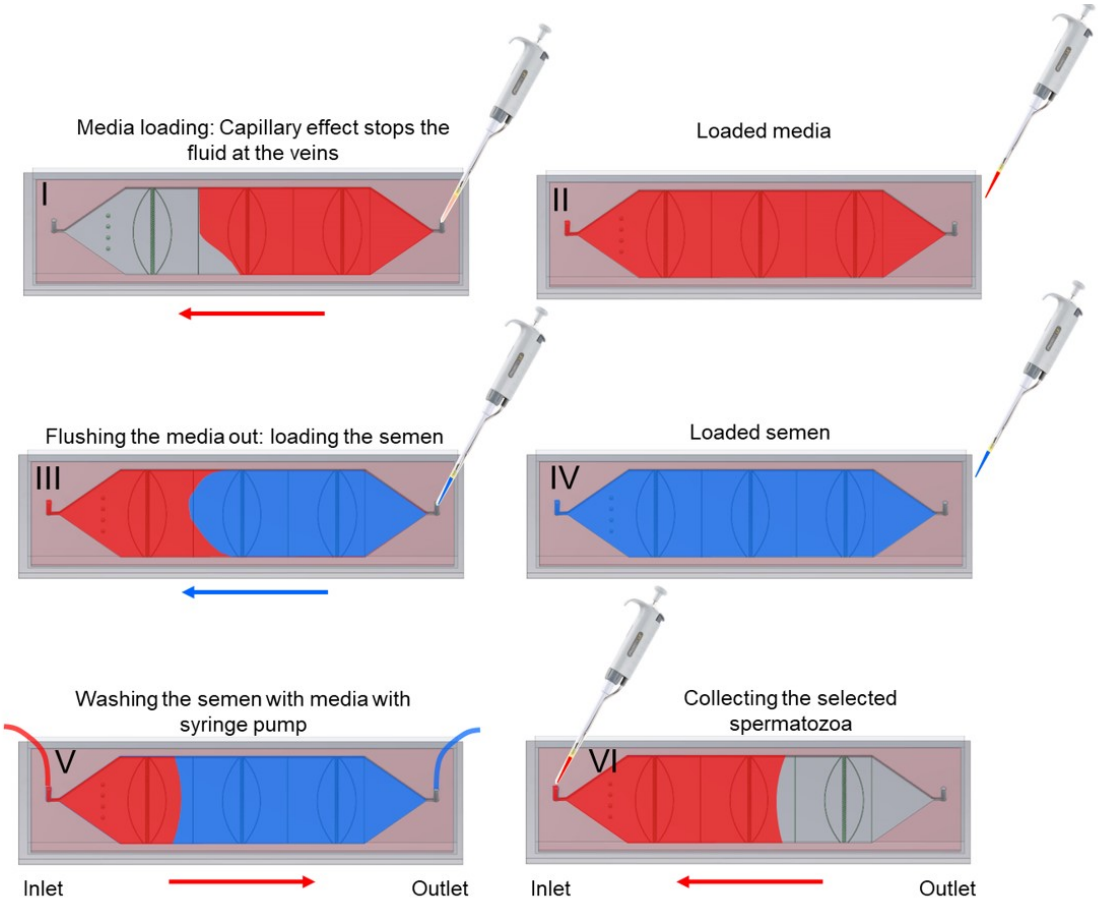


Fig. S1 The procedure of the device operation. First, the empty chip is filled with media (I, and II). The capillary effect of the veins help load the device without air bubbles. Once the device is fully loaded, media is flushed and replaced by semen (III and IV). At last, after the semen is loaded, a syringe pump is used to wash the sperm with media injected from the inlet and the waste is collected in an outlet tube (V). For collecting the selected sperm that remained in the device at step VI, a pipette is used to aspirate the sample from the inlet.

Figure S2

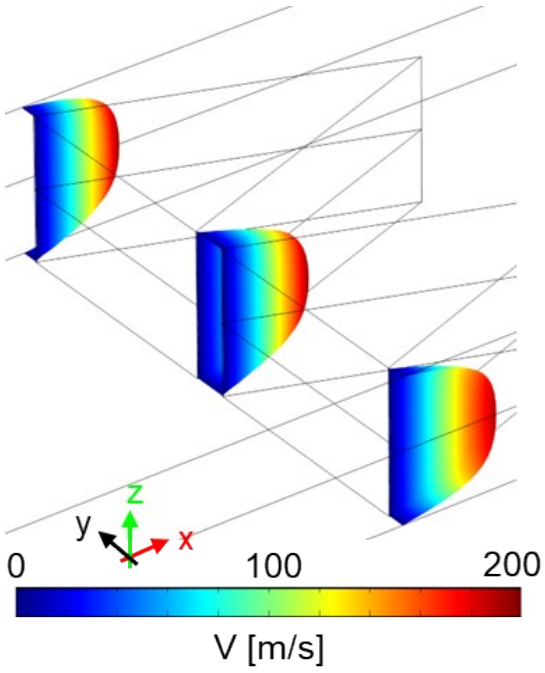


Fig. S2 The velocity profile at the stricture.

Figure S3

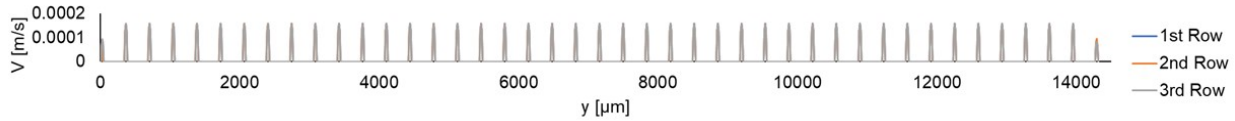


Fig. S3 Velocity profile at the strictures for all the 3 rows of the device using computational fluid dynamics. Except for the strictures at the boundaries all the remaining strictures have the same velocity profile which results in the same shear rate. This, presumably, on its own results in the same accumulation of spermatozoa at the strictures.

Figure S4

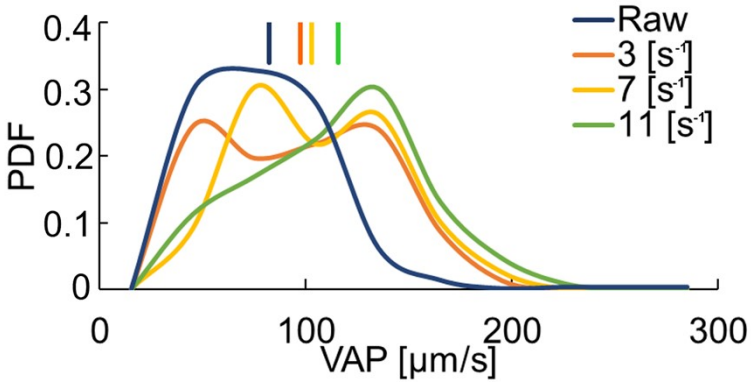
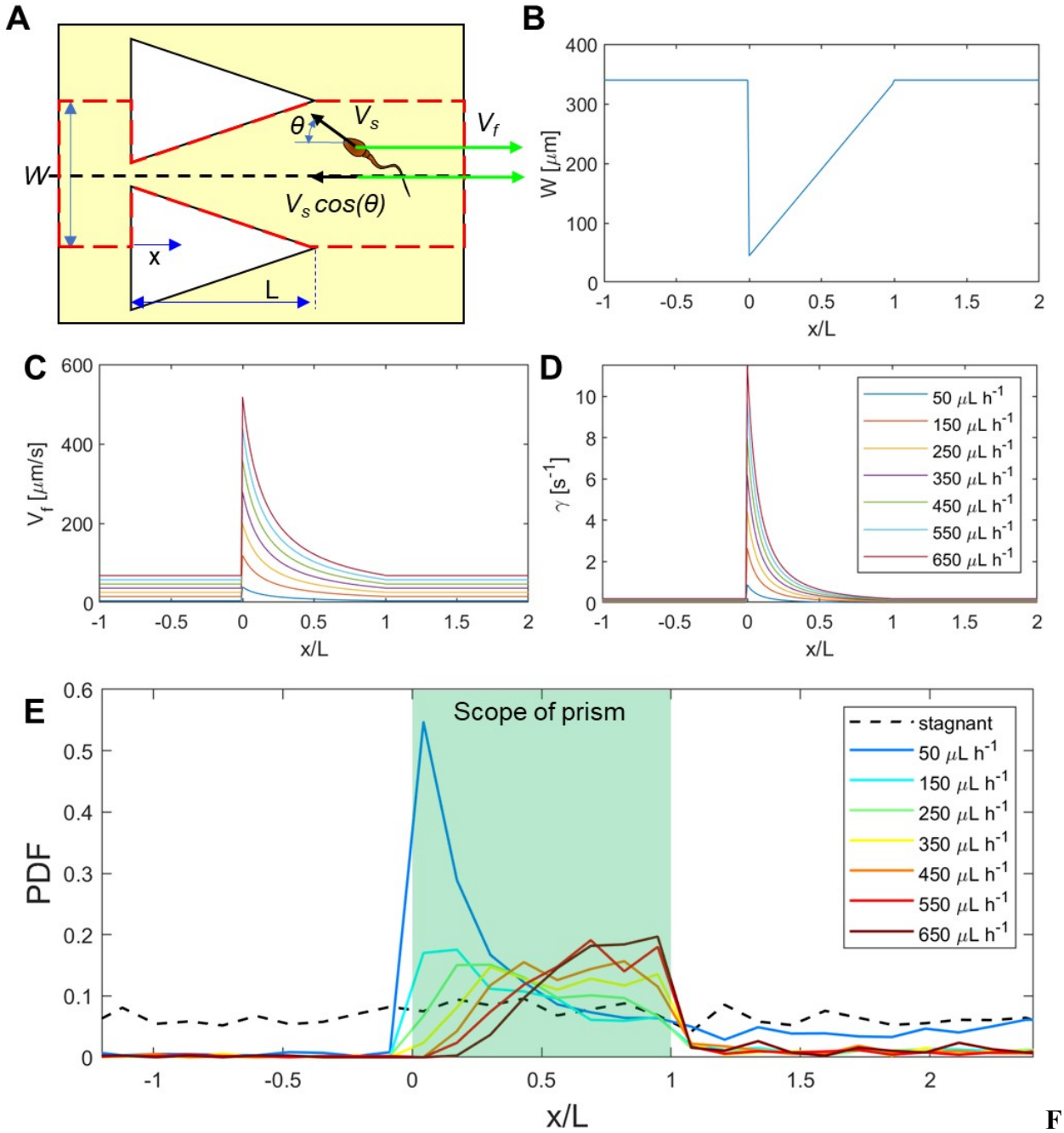


Fig. S4 Filtering effect of the shear rate on the sperm VAP. VAP distribution of raw sample and samples of various shear rates of 3 s^{-1}, 7 s^{-1} and 11 s^{-1}. The lines on the top of the curves indicate the mean of the VAP distributions which are color-coded based on the legend. The vertical axis is mirrored with respect to 0 to avoid overlapping curves.

Figure S5



ig. S5 Minimal model of accumulation of microswimmers behind the barrier in the gate area (scope) of the prism.

The area bound by red-dashed line is projected over the black dashed line to form a one-dimensional convective-diffusive transport ruled by Langevin equation. The width of this area is shown in **Fig. S5B**. Using the equations pointed out in the material and method section the velocity and shear rate on the line are calculated for various flow rates in the channel shown in **Fig. S5C and D**, respectively. The **Fig. S5E** shows the accumulation effect of random distribution of the sperm cells by solving the Langevin equation for various flow rates in the channel.As can be seen, at stagnant conditions the distribution of the sperm is roughly uniform as expected. As the flow rate increases, sperm starts to be accumulated at the gate of the stricture in the shaded area. And as the flow rate increases, the maximum shifts downstream as the experimental results point to the similar observation. The result of these distributions at each flow rate condition is an average of 10 simulations of the same problem with different seeding points of random number generation. The time step for solving the Langevin equation was set to 0.1 s.

Figure S6

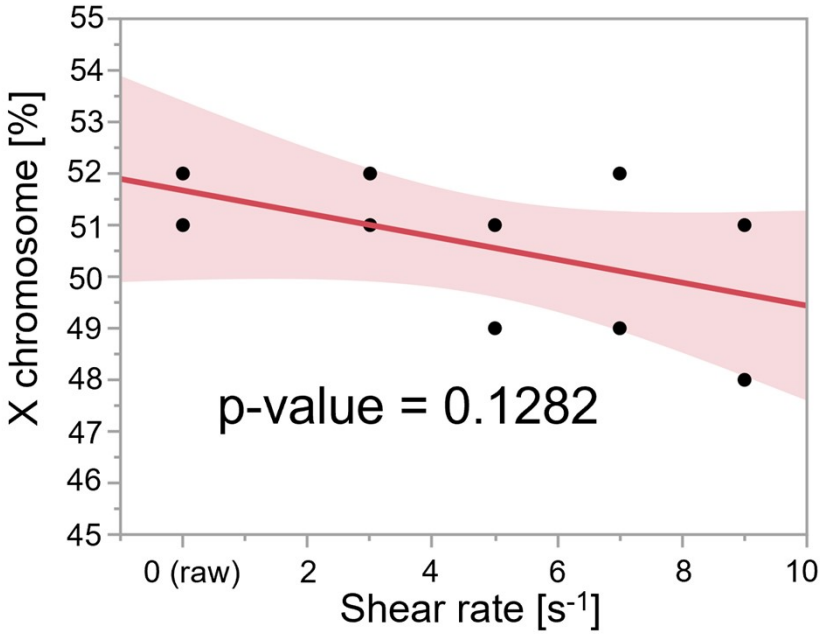


Fig. S6 The variation of X chromosome vs shear rate. The shaded area shows the 95% confidence interval. The results are shown for two patients and the F-test revealed that there is no statistical significance for sex bias however the results are from limited observation and more data is required to evidently conclude the effect of shear rate on sperm paternal content.

Figure S7

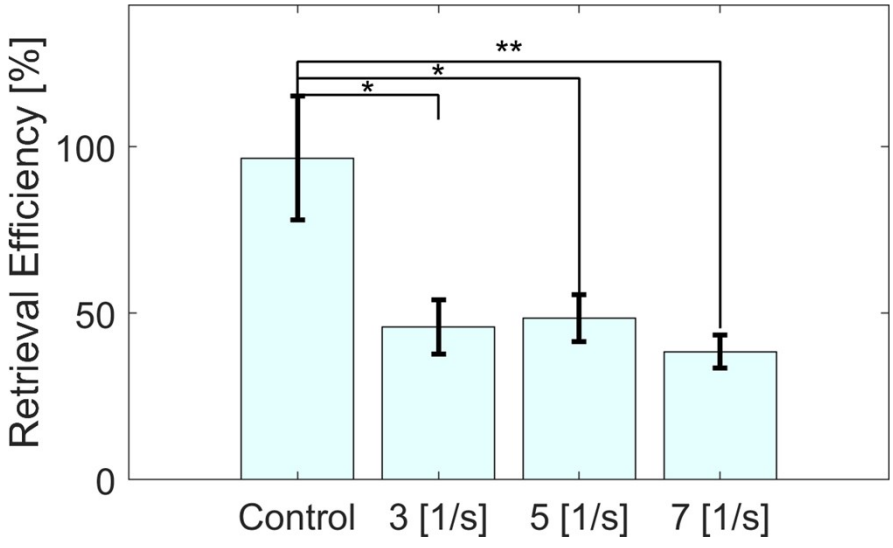


Fig. S7 Retrieval efficiency of the sorted sperm. * $p < 0.05$, ** $p < 0.01$.

Tables

Table S1. Detailed table of human sperm selection experiments via microfluidic device and comparison with DGC processed samples.

	Conditions		Selected Samples			Density Gradient Centrifugation (DGC)					Raw Sample			
Patient #	Dilution factor*	Shear rate [s^{-1}] (Flow rate [$\mu L\ h^{-1}$])	Motility [%]	Morphology [%]	Total Concentration [$M\ mL^{-1}$]	SCF [%]	Motility [%]	Morphology [%]	Total Concentration [$M\ mL^{-1}$]	SCF [%]	Motility [%]	Morphology [%]	Total Concentration [$M\ mL^{-1}$]	SCF [%]
1	1	3 (150)		4		3	93	4	33	3.3	46	4	62	5.9
1	1	3 (150)		4		3	93	4	33	3.3	46	4	62	5.9
2	1	3 (150)		3	1.1		89	3	8	6.1	44	3	13	7.6
2	1	3 (150)		3	0.9		89	3	8	6.1	44	3	13	7.6
2	1	3 (150)		4	1.1		89	3	8	6.1	44	3	13	7.6
2	1	3 (150)			1.2		89	3	8	6.1	44	3	13	7.6
1	1	3 (150)		3	12		93	4	33	3.3	46	4	62	5.9
3	1/3	3 (150)	93	4	2	5.4	91	3	37	7.3	46	3	68	11.1
3	1/3	3 (150)	93	4	2	5.4	91	3	37	7.3	46	3	68	11.1

3	1/3	3 (150)	95	4	2	5.4	91	3	37	7.3	46	3	68	11.1
3	1/3	3 (150)	96	4	2	5.4	91	3	37	7.3	46	3	68	11.1
4	1/3	3 (150)	95	4	5		92	3	79	14.1	46	3	102	9.4
4	1/3	3 (150)	96	4	4		92	3	79	14.1	46	3	102	9.4
5	1	5 (250)	94	5	7		92	4	90	8.8	45	4	93	10.3
5	1	5 (250)	96	5	8	4.1	92	4	90	8.8	45	4	93	10.3
6	1/5	5 (250)	95	4	0.5	3.2	88	3	36	5.6	42	3	38	8.6
7	1/5	5 (250)		4	7	2.5	91	3	78	2.9	45	3	152	5.2
7	1/5	5 (250)	100	4	11	2.5	91	3	78	2.9	45	3	152	5.2
7	1/5	5 (250)	100	5	5	2.5	91	3	78	2.9	45	3	152	5.2
3	1/3	5 (250)	94	5	3		91	3	37	7.3	46	3	68	11.1
3	1/3	5 (250)		3	0.5		91	3	37	7.3	46	3	68	11.1
3	1/3	5 (250)	96	4	2		91	3	37	7.3	46	3	68	11.1
3	1/3	5 (250)	97	4	2		91	3	37	7.3	46	3	68	11.1
4	1/3	5 (250)	99	4	8		92	3	79	14.1	46	3	102	9.4
4	1/3	5 (250)	99	4	13		92	3	79	14.1	46	3	102	9.4
5	1	7 (350)	97	5	8.5	7.4	92	4	90	8.8	45	4	93	10.3
3	1/3	7 (350)	100	5	0.2	2.8	91	3	37	7.3	46	3	68	11.1

3	1/3	7 (350)	99	5	0.3	2.8	91	3	37	7.3	46	3	68	11.1
3	1/3	7 (350)	96	4	3	2.8	91	3	37	7.3	46	3	68	11.1
3	1/3	7 (350)	93	4	4	2.8	91	3	37	7.3	46	3	68	11.1
4	1/3	7 (350)	98	4	5		92	3	79	14.1	46	3	102	9.4
4	1/3	7 (350)		3	0.4		92	3	79	14.1	46	3	102	9.4
3	1/3	9 (450)	94	4	4	3.9	91	3	37	7.3	46	3	68	11.1
3	1/3	9 (450)		3	0.5	3.9	91	3	37	7.3	46	3	68	11.1
3	1/3	9 (450)	99	4	0.1	3.9	91	3	37	7.3	46	3	68	11.1
4	1/3	9 (450)	97	4	5	9.2	92	3	79	14.1	46	3	102	9.4
4	1/3	9 (450)	98	5	6	9.2	92	3	79	14.1	46	3	102	9.4

* Dilution factor: Human semen were diluted in order to reduce viscosity and improve fluid flow distribution. The dilution factor is the ratio of volume of semen to total volume of diluted semen.

Table S2. Total sperm count from the device for bovine experiments, calculated by multiplying concentration with 80 μL of selected samples.

Shear rate [s^{-1}]	3	5	7	9	11
Total sperm count [M]	1.72 ± 0.22	1.25 ± 0.17	1.03 ± 0.16	0.83 ± 0.22	0.31 ± 0.02

Table S3. The fitted curves to the CASA parameters and DFI versus shear rate (γ).

Parameters (y)	Fitted formula
VAP	$VAP = 85.267612 + 9.0720872 \times \log(\gamma)$
Motility	$MOT = 83.051678 - 1.3540038\times\gamma - 1.596778\times(\gamma - 7.03406)^2$
ALH	$ALH = 4.6704762 - 0.0066344 \times \gamma + 0.1084659\times(\gamma - 7.03406)^2$
Concentration	$CONC = 26.983667 - 2.0112154 \times \gamma$
BCF	$BCF = 37.148095 + 0.2753272 \times \gamma - 0.4916333 \times (\gamma - 7.03406)^2$
DFI	$DFI = 7.5244828 - 0.7277971 \times \gamma + 0.3251104 \times (\gamma - 6.21189)^2$

Movies

Legend for Movie S1

Movie S1: Accumulation of sperm near the media inlet over time. The media flows from left to right. Sperm that swim against the flow accumulate at the inlet and are selected.