## Supporting Information

## JUNO-*Checked* — A live cell electrochemical biosensor for sperm function diagnostics

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**Table 1.** Averaged current values received with chronoamperometry at t = 60 (Fig. 2 B) for the direct enzymatic assay, calculated based on the averaged currents for four different IZUMO1 concentrations:

BiotinIZUMO1	Averaged current response of the JUNO-biosensors
concentrations [ng/ml]	[µA]
0	$-0.16 \pm 0.054$
250	$-2.58 \pm 0.55$
500	$-4.34 \pm 0.64$
1000	$-4.44 \pm 1.04$

**Table 2.** Averaged current values received with chronoamperometry at t = 60 (Fig. 2 D, E) and relative displacement calculated based on the averaged currents for six different IZUMO1 concentrations:

IZUMO1 concentrations [ng/ml]	Averaged current response of the JUNO-biosensors [µA]	Relative displacement [%]
0	$-3.43 \pm 0.71$	100
50	$-2.65 \pm 0.63$	22.88
125	$-2.16 \pm 0.76$	37.04
250	$-2.25 \pm 0.34$	34.47
500	$-1.84 \pm 0.2$	46.28
1000	$-1.15 \pm 0.15$	66.55

**Table 3.** Current responses and relative displacements for the tested semen samples at sperm concentrations of 0, 5, 10, 20 [10<sup>6</sup>]: Representative current response for one JUNO-biosensor with one semen sample at different sperm concentrations, averaged current values received with chronoamperometry at t = 60 and averaged relative displacement for JUNO biosensors (n =9) (Fig. 3 A, B, C).

Sperm cell concentration [10 <sup>6</sup> /ml]	Representative current response for one JUNO-biosensor [µA]	Averaged current response of the JUNO-biosensors [µA]	Relative displacement [%]
0	-1.54	$-1.62 \pm 0.4$	0
5	-1.03	$-1.26 \pm 0.29$	$82.57\pm25.84$
10	-0.78	$-1.09 \pm 0.33$	$67.77 \pm 14.6$
20	-0.4	$-0.93 \pm 0.4$	$57.35 \pm 17.76$

Table 4. Calculated JUNOScore based on the relative displacement of the nine tested semen samples (Fig. 3 D, E, F).

Semen sample	JUNOScore	Progressive motility [%]	Sperm concentration [106]
1	5	46	37
2	3.9	65	101
3	2.6	52	77
4	4.1	58	113
5	2	43	160
6	5.8	32	61
7	0.8	41	43
8	1.6	53	68
9	3.1	27	67

Table 5. Calculated Coefficient of variation (CoV) for Figure 2D

IZUMO (ng/mL)	CoV (%)
0	20,72
50	23,97
125	35,01
250	15,30
500	10,93
1000	13,03

Table 6. Calculated Coeffi	cient of variation	(CoV) for	Figure 2E
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IZUMO (ng/mL)	CoV (%)
0	0,000
50	41,590
125	34,260
250	55,769
500	25,006

1000	29,614
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 Table 7. Calculated Coefficient of variation (CoV) for Figure 3B

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Sperm concentration (ng/mL)	CoV (%)
0	16,9188
5	14,3491
10	11,3005
20	26,3411

## **Table 8**. Calculated Coefficient of variation (CoV) for Figure 3C

Sperm concentration (ng/mL)	CoV (%)
0	0
5	32,984
10	22,703
20	30,551



Figure S1. JUNOSensor's current response to different sperm concentrations in the presence (left) and absence (right) of the JUNO protein.