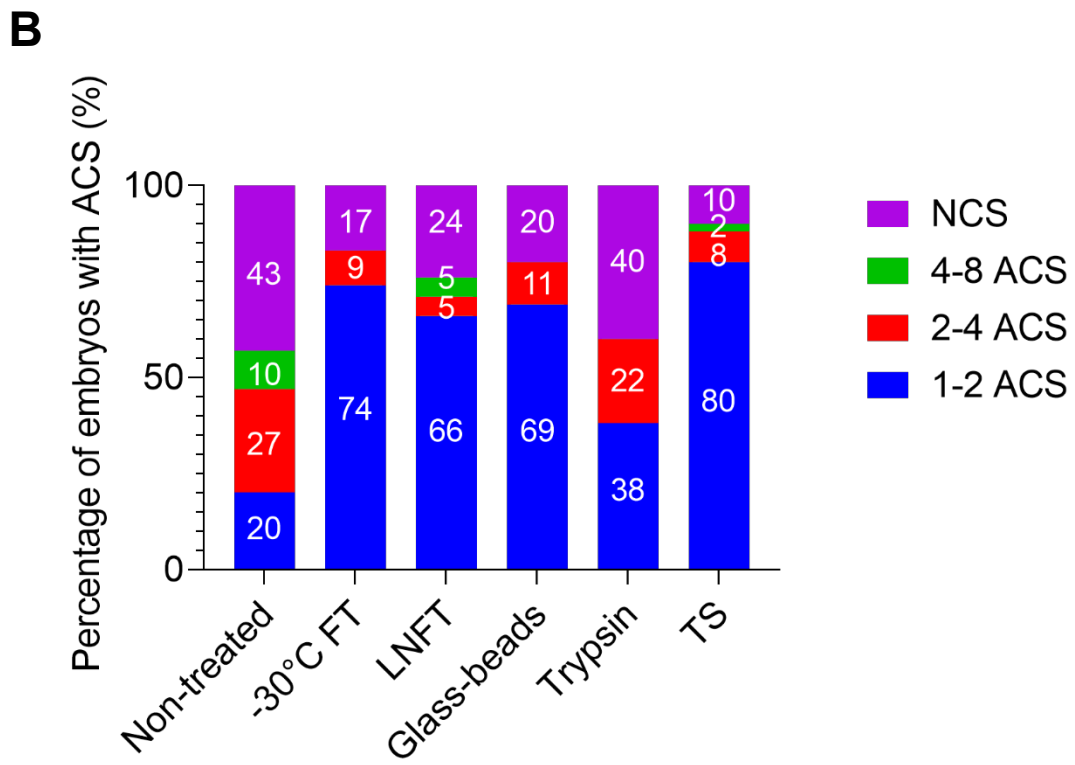
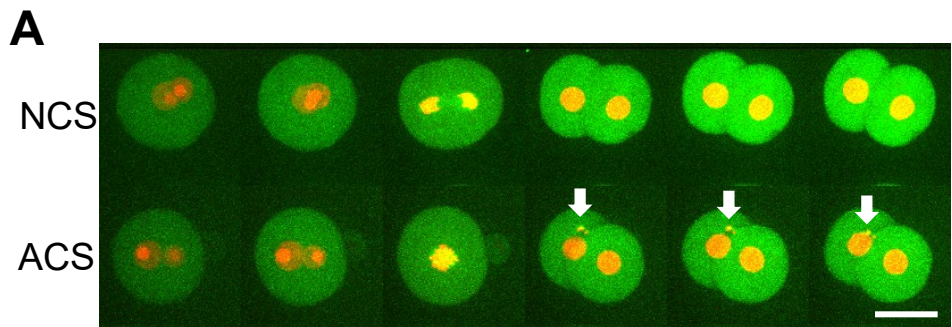
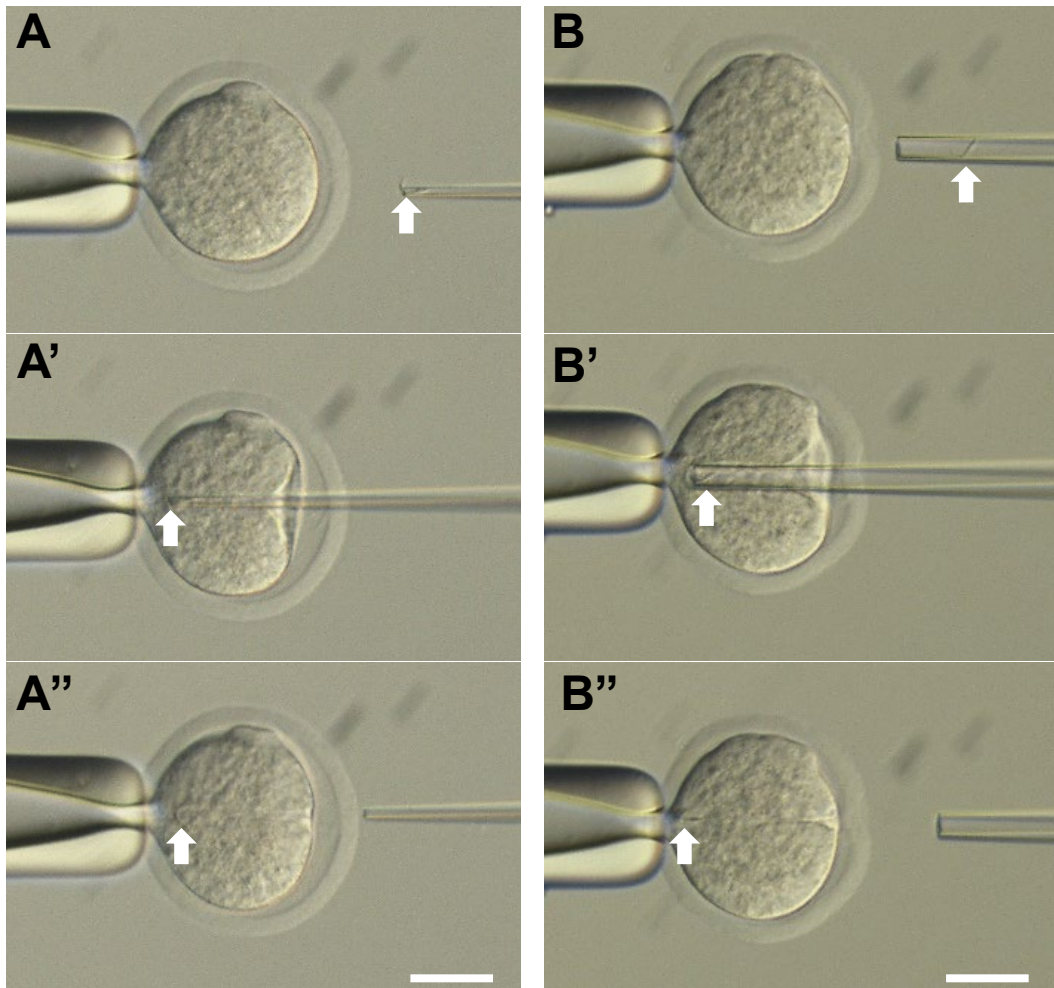


Supplementary Fig. 1. Morphology of mouse and rat sperm after tail removal treatment. (A-F) Bright field images of mouse sperm after tail removal treatment are shown; (A) non-treated control. (B) -30° C freeze-thawed, (C) liquid nitrogen freeze-thawed, (D) glass bead-homogenized, (E) trypsin-treated, and (F) TrypLETM Select-treated. (G-H) Bright field images of rat sperm after treatment. (G) Non-treated, (H) Sonicated, (I) Trypsin-treated. Arrows indicate sperm that have lost their tail. Scale bars in A-F: 20 μ m, in G-I: 15 μ m.



Supplementary Fig. 2

Supplementary Fig. 2. Abnormal chromosome segregation found in ICSI embryos generated with treated sperm. **(A)** Time-lapse images of ICSI embryos with NCS and ACS. Arrows indicate abnormal located chromosomes. Red: H2B-mRFP1, Green: EGFP-tubulin. Scale bar: 40 μm . **(B)** ACS frequency of ICSI embryos using treated sperms. ACS was analyzed at the 1–2, 2–4, and 4–8 cell divisions for each embryo. The percentages of ACS at each division in ICSI embryos from each treated and non-treated control sperm are shown. ACS: abnormal chromosome segregation, NCS: normal chromosome segregation.



Supplementary Fig. 3. Rat ICSI procedure. (**A–A''**) The conventional rat ICSI process using a thin injection needle. (**B–B''**) Rat ICSI using pre-activated oocytes and a thick needle. In **A–A''** and **B–B''**, arrows indicate sperm head. Scale bars in **A–A''** and **B–B''**: 40 μm .

Supplementary Tables**Supplementary Table 1.** *In vitro* development of ICSI embryos using sperm with tails artificially removed

| Treatment | No. of oocytes | No. of embryos developed to | | | | |
|-------------|----------------|-----------------------------|--------------|--------------|--------------|----------------------|
| | | pn (%) | 2-cell (%)** | 4-cell (%)** | Morula (%)** | Blastocyst (%)** |
| Piezo-cut | 40 | 35 (88) | 33 (94) | 31 (88) | 29 (83) | 24 (69) ^a |
| -30°CFT | 95 | 85 (89) | 82 (96) | 75 (88) | 70 (82) | 50 (59) |
| LNFT | 80 | 72 (90) | 69 (96) | 62 (86) | 55 (76) | 43 (60) |
| Glass-beads | 100* | 94 (94) | 87 (93) | 76 (81) | 62 (66) | 39 (41) ^b |
| Trypsin | 87* | 70 (80) | 68 (97) | 67 (96) | 65 (93) | 47 (67) |
| TS | 92* | 85 (92) | 84 (99) | 69 (81) | 63 (74) | 45 (53) |

* These oocytes were activated with SrCl₂. **The percentages relative to no. of embryos that developed into pronucleus (pn). Statistical analysis of blastocyst rate was performed between piezo-cut control and each treatment. Significant χ^2 comparisons a vs. b, P < 0.05.

Supplementary Table 2. Comparison of time burden between conventional ICSI and modified ICSI (pre-activated oocytes injected with trypsin-treated sperm) performed by operators with varying experience

| Operator | Type of sperm | No. of oocytes used | No. of oocytes survived (%) [*] | No. of embryos developed to | | Required time (min) for injection | Required time (min)/ No. of oocyte used | No. of embryos transferred | No. of offspring (%) ^{***} |
|----------|---------------|---------------------|--|-----------------------------|--------------------------|-----------------------------------|---|----------------------------|-------------------------------------|
| | | | | pn (%) ^{**} | 2-cell (%) ^{**} | | | | |
| A | Piezo-cut | 30 | 26 (87) | 26 (100) | 25 (96) | 18.9 | 0.63 | N.D. | N.D. |
| | Trypsin | 30 | 30 (100) | 30 (100) | 29 (97) | 16.1 | 0.53 | N.D. | N.D. |
| B | Piezo-cut | 30 | 23 (77) | 23 (100) | 23 (100) | 19.9 | 0.66 | N.D. | N.D. |
| | Trypsin | 29 | 27 (93) | 27 (100) | 24 (89) | 18.6 | 0.64 | N.D. | N.D. |
| C | Piezo-cut | 30 | 22 (73) | 22 (100) | 22 (100) | 35.2 | 1.17 | N.D. | N.D. |
| | Trypsin | 30 | 27 (90) | 27 (100) | 25 (93) | 30.8 | 1.02 | N.D. | N.D. |
| D | Piezo-cut | 30 | 20 (67) | 19 (95) | 17 (90) | 28.4 | 0.94 | N.D. | N.D. |
| | Trypsin | 30 | 29 (97) | 29 (100) | 29 (100) | 26.8 | 0.89 | N.D. | N.D. |
| E | Piezo-cut | 50 | 45 (90) | 45 (100) | 43 (96) | 32.5 | 0.72 | 43 | 18 (42) |
| | Trypsin | 50 | 50 (100) | 50 (100) | 49 (98) | 23.5 | 0.47 | 24 | 9 (39) |
| F | Piezo-cut | 40 | 36 (90) | 35 (97) | 31(86) | 142.9 | 3.97 | 31 | 8 (26) |
| | Trypsin | 40 | 36 (90) | 33 (92) | 30 (83) | 117.3 | 3.26 | 14 | 5 (36) |
| G | Piezo-cut | 36 | 32 (89) | 24 (75) | 23 (72) | 151.5 | 4.73 | 23 | 2 (9) |
| | Trypsin | 36 | 35 (97) | 29 (83) | 28 (80) | 114.1 | 3.26 | 28 | 2 (7) |
| H | Piezo-cut | 40 | 24 (60) | 23 (96) | 22 (92) | 103.8 | 4.33 | 22 | 6 (27) |
| | Trypsin | 40 | 36 (90) | 36 (100) | 35 (97) | 74.5 | 2.07 | 28 | 7 (25) |

* Percentages relative to the number of oocytes used. **Percentages relative to the number of oocytes survived. *** Percentages relative to the number of embryos transferred.

N.D.: not determined.