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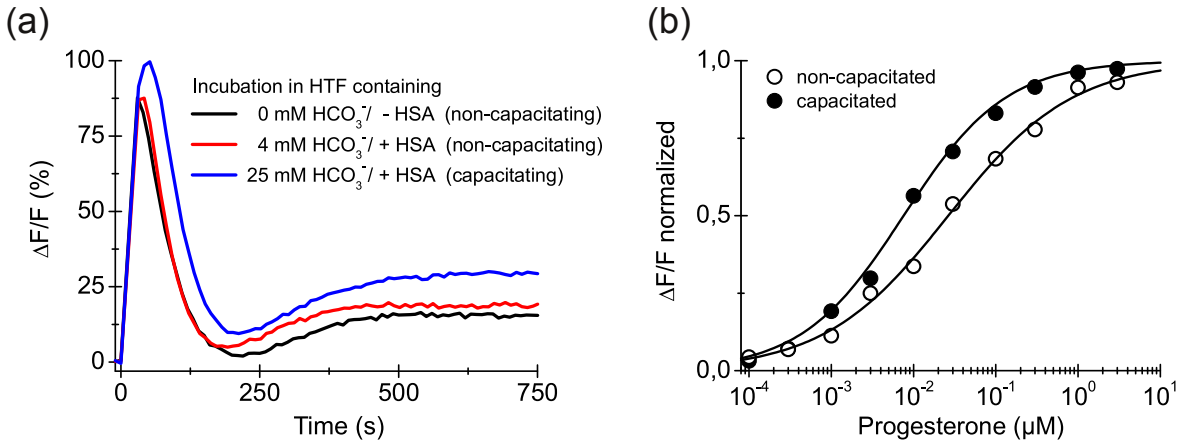
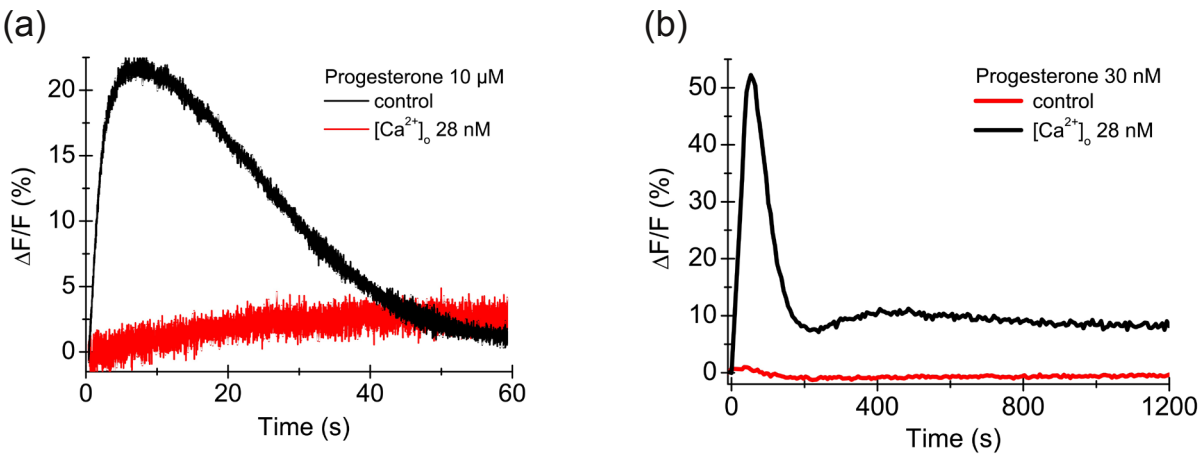
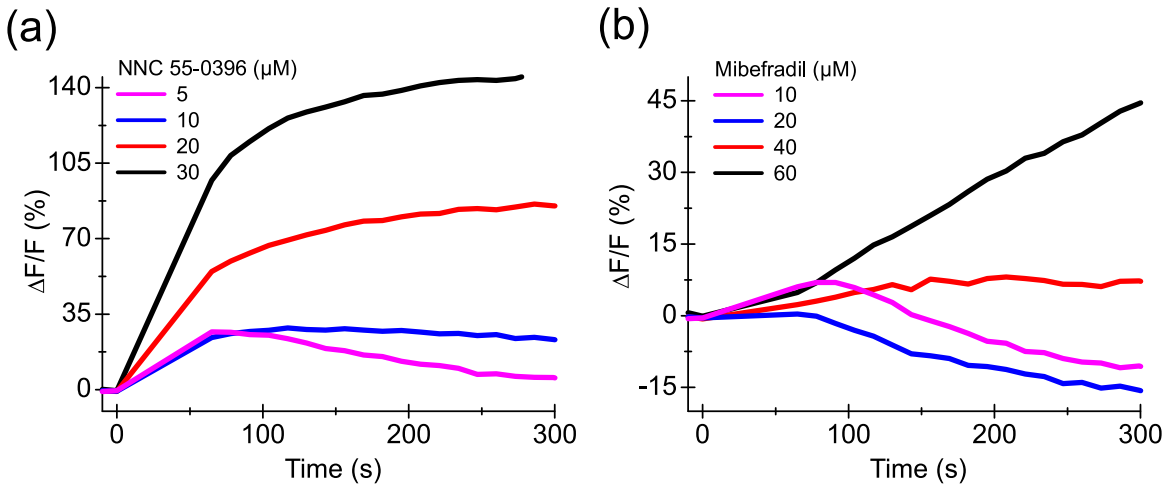


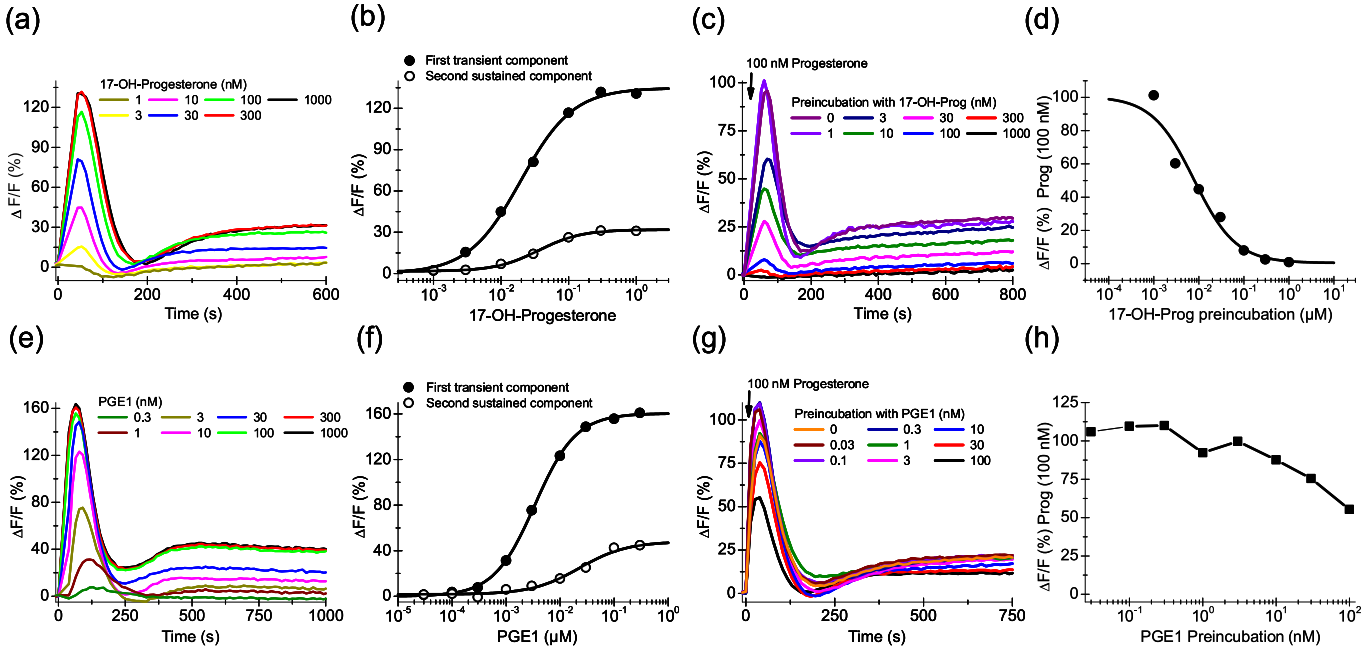
Supplementary Figure 1: Progesterone-induced Ca^{2+} signals in sperm incubated under capacitating and non-capacitating conditions. **a**, Ca^{2+} signals evoked by 3 μM progesterone. Sperm were incubated (> 2 h) in buffer with different concentrations of HCO_3^- and human serum albumin (HSA). Only incubation in 25 mM HCO_3^- + HSA led to capacitation. **b**, Dose-response relationship of the first kinetic component of progesterone-induced Ca^{2+} signals in non-capacitated and capacitated sperm. The continuous line represents a fit of the Hill equation to the data. Fluorescence values were normalized to the bottom (0) and top (1) values obtained by fitting the Hill equation to the raw data. The $K_{1/2}$ values of the first component were 27 nM and 8 nM in non-capacitated (incubated in HTF containing 4 mM HCO_3^- + HSA) and capacitated sperm (25 mM HCO_3^- + HSA). The signals were measured in a fluorescence plate reader. Capacitation was assessed with a FITC-CD45 assay.



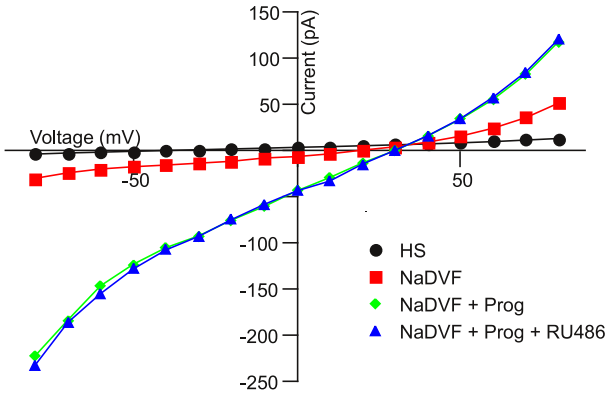
Supplementary Figure 2: Progesterone-induced Ca^{2+} signals in human sperm evoked at 28 nM external Ca^{2+} concentration $[Ca^{2+}]_o$. Final $[Ca^{2+}]_o$ was rapidly established by mixing of sperm suspension (in 2.04 mM $[Ca^{2+}]_o$) 1:1 (v/v) with a Ca^{2+}-free solution containing 12 mM of BAPTA and 20 μM (a) or 60 nM progesterone (b). Experiments were done in a stopped-flow apparatus (a) and in a fluorescence plate reader (b). $[Ca^{2+}]_o$ was calculated and verified using the Ca^{2+} indicators fura-2 and mag-fura-2.



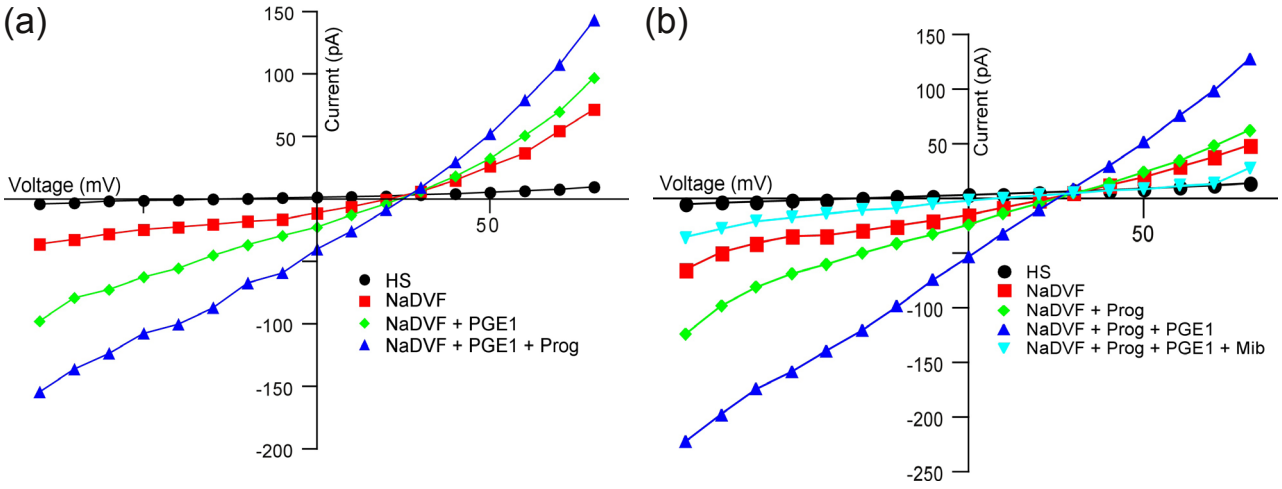
Supplementary Figure 3: Ca^{2+} signals in human sperm evoked by NNC 55-0396 and mibefradil itself. **a**, Ca^{2+} signals produced by 5 to 30 µM NNC 55-0396. **b**, Ca^{2+} signals produced by 10 to 60 µM mibefradil. The signals were measured in a fluorescence plate reader.



Supplementary Figure 4: Ca^{2+} signals evoked by 17-OH-progesterone and PGE1. **a**, Ca^{2+} signals produced by 1 nM to 1 µM 17-OH progesterone **b**, Dose-response relationship of the first and second kinetic component of the Ca^{2+} signals evoked by 17-OH-progesterone. The continuous line represents a fit of the Hill equation to the data. The $K_{1/2}$ values of the first and second component were 19.6 nM and 36 nM, respectively. **c**, Progesterone-induced Ca^{2+} signals (100 nM progesterone) of sperm that had been preincubated with 1 nM to 1 µM 17-OH-progesterone. **d**, Dose-response relationship of Ca^{2+} signals evoked by 100 nM progesterone after preincubation of sperm with various 17-OH-progesterone concentrations, demonstrating a dose-dependent decrease of the signal amplitude. The continuous line represents a fit of the Hill equation to the data. The apparent K_i value was 7.7 nM. **e**, Ca^{2+} signals produced by 0.3 nM to 1 µM PGE1. **f**, Dose-response relationship of the first and second kinetic component of the Ca^{2+} signals evoked by PGE1. The continuous line represents a fit of the Hill equation to the data. The $K_{1/2}$ values of the first and second component were 3.4 nM and 22.9 nM, respectively. **g**, Progesterone-induced Ca^{2+} signals (100 nM progesterone) of sperm that had been preincubated with 0.3 nM to 100 nM PGE1. **h**, Dose-response relationship of Ca^{2+} signals evoked by 100 nM progesterone after preincubation of sperm with various PGE1 concentrations, indicating only a slight linear reduction of the signal amplitudes at saturating PGE1 concentrations. The continuous line connects data points; a dose-response curve could not be fitted to the data. The signals were measured in a fluorescence plate reader.



Supplementary Figure 5: Control currents in HS solution and monovalent currents in divalent-free solutions (NaDVF), in the absence of intracellular divalent ions. Currents were recorded at pH_i 7.3, stepping the voltage from -80 mV to +80 mV in steps of 10 mV. RU486 (1 μM) did not inhibit the currents evoked by 1 μM progesterone.



Supplementary Figure 6: Control currents in HS solution and monovalent currents in divalent-free solutions (NaDVF), in the absence of intracellular divalent ions. Currents were recorded at pHi 7.3, stepping the voltage from -80 mV to +80 mV in steps of 10 mV. **a**, Extracellular application of 100 nM PGE1 or 100 nM PGE1 and 100 nM progesterone enhanced these currents. **b**, currents evoked by application of 1 μM PGE1 and 1 μM progesterone were completely blocked by 30 μM mibefradil.