“TRPV3 channels mediate strontium-induced mouse egg activation”

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Supplemental Information

Supplemental Experimental Procedures

Immunofluorescence staining

The *zona pellucida* (ZP) was removed, washed several times in 2% goat serum-supplemented Dulbecco’s PBS (PBS–BSA) and then fixed in PBS–BSA containing 2% paraformaldehyde for 45min at room temperature. Eggs were washed and blocked in PBS containing 0.1M glycine, 2% goat serum and 0.01% Tween 20 for at least 1 h. Eggs were then permeabilized with 0.1% Triton X-100 for 15 min at room temperature. Samples were then transferred into PBS–PVA supplemented with 2% goat serum followed by incubation overnight at 4°C with anti-TRPV3 antibody (10 μg/ml, Neuromab). Eggs were washed with PBS–BSA, and incubated with Alexa Fluor 546-conjugated goat anti-mouse IgG (1:500, Life Technologies) for 1 h at room temperature. Eggs were washed and incubated with Hoechst 33342 (Life Technologies) for 15 min at room temperature. WT and *V3-KO* eggs were processed in parallel and mounted on slide glasses, taken at room temperature, and the images acquired the next day with a FluoView-1000 laser scanning confocal microscope (Olympus). The objective used was 60x (UPLSAPO) water immersion NA: 1.20. Images were processing in Image J software.

Sperm isolation and IVF

Sperm cells were obtained from 10-16 week-old male C57BALB6 mice (Jackson labs.). The cauda epididymis of the sacrificed mice was collected and sliced with scissors in 500 μl of M2 medium. The slices were incubated for 10 min at 37° C. The sperm were later transferred to HTF medium equilibrated for 30 min-1 h in 5% CO_2 at 37° C. The released sperm cells were diluted to 2.2-2.5 x 10^6 cells/ml in 500 μl and this stock suspension was incubated either 2 h for standard IVF or 3.5 h for insemination on the microscope stage (**Fig. 5**). Expanded cumulus-oocyte complexes were released from the oviduct and directly transferred to a drop of 100 μl HTF medium and 0.1-0.3 x 10^6 sperms/ml added. Complexes were incubated for 2 h, washed of excess sperm and cultured for an additional 23 h. Eggs were cultured to the 2-cell stage. For insemination on the microscope, populations of 10-25 *zona pellucida*-free eggs loaded with Fura-2 AM were placed in an imaging dish in 90 μl of HCZB covered by mineral oil. The dish was connected to a temperature controller (BIOPTECH Delta T4 culture dish controller) set at 37° C. 10 μl of sperm from a solution of 2.2 x 10^6 sperms/ml. Eggs start to generate their first Ca^{2+} transients in about 1-10 min (Swann, 2013).

Supplemental Results

Supplemental Figure Legends

Figure S1, Related to Figure 1. *TrpV3* deletion does not alter mouse fertility under in-house breeding conditions. **A.** The number of pups from $TrpV3^{-/-}$ females does not differ substantially from the number of pups/litter from $TrpV3^{+/-}$ females. **B**. The number of eggs obtained per superovulation cycle varies widely, but was not significantly different between $TrpV3^{-/-}$, $TrpV3^{+/-}$ and $TrpV3^{+/+}$ animals in this sample size.

Figure S2, Related to Figure 6. Carvacrol terminates calcium oscillations by enabling calcium influx through TRPV3 channels. **A.** Calcium oscillations were induced by injection of 0.01 μg/μl mPLCζ cRNA. *Left panel*: $[Ca^{2+}]_i$ oscillations in control eggs ($TrpV3^{+/+}$, n=9). *Right panel*: $TrpV3^{-/-}$ eggs ($TrpV3^{-/-}$, n=11). 50 μM carvacrol applied to V3-KO eggs yielded the same results as those induced by 100 μM.