Supplementary Methods

Immunofluorescence imaging of meiotic spindles in rescued zinc-insufficient eggs and 5-methylcytidine in fertilized zinc-insufficient eggs

Zinc supplemented oocytes were fixed in a microtubule stabilizing buffer$^1$. Following a blocking step, they were stained with monoclonal alpha-tubulin antibody (1:100), followed by a cocktail of Alexa Fluor 633-conjugated goat anti-mouse IgG (1:500) and Alexa Fluor 488-phalloidin (1:100). Ethidium homodimer-1 (Invitrogen) was used as a nuclear stain. Visualization of 5-methylcytidine in fertilized oocytes was accomplished following the protocol of Santos and Dean$^2$. Briefly, oocytes were fixed 7 hours post-fertilization with 4% paraformaldehyde at room temperature. After a blocking step, they were probed with a monoclonal antibody for 5-methylcytidine (1:500, Eurogentec, San Diego, CA). Alexa Fluor 633-conjugated goat anti-mouse IgG was used as a secondary antibody (1:500, Invitrogen) and DNA was visualized with TOTO-3 (1 µM, Invitrogen). All images were acquired on a TCS SP5 confocal microscope (Leica Microsystems) equipped with a 40x oil-immersion objective and Ar (488 nm) and HeNe (543 and 633 nm) laser lines. Images were processed using the LAS AF software (Leica Microsystems).

In vitro fertilization

Sperm were isolated from the cauda epididymides of a proven breeder CD-1 male using Percoll gradient centrifugation (PGC) as described previously$^3$. The selected sperm population was capacitated in potassium simplex optimized medium (KSOM) supplemented with 3 mg/ml bovine serum albumin (BSA, MP Biomedicals, Solon, OH) and 5.36 mM D-glucose for up to 1 hr. Capacitated sperm were added to cumulus-free, in vitro matured oocytes to a final concentration of $1.0 \times 10^6$ sperm/ml. Newly fertilized oocytes were liberated of sperm by gentle aspiration through a narrow-bore pipette 7-8 hours post-fertilization (hpf) and transferred into KSOM supplemented with amino acids (Millipore, Billerica, MA) for extended culture. Samples were imaged at 8 hpf, 24 hpf, and every 24 hrs thereafter up to 120 hpf.

Time-lapse live cell imaging of calcium oscillations

Oocytes were loaded with Calcium Green-1 AM (5 µM, Invitrogen) and Pluronic F-127 (0.02% v/v, Invitrogen) for 30 min at 37 °C. These were washed in calcium-free KSOM (Millipore) and transferred to a 35 mm glass-bottom dish (Bioptechs Inc., Butler, PA) containing 50 µl of 10 mM strontium chloride (Sigma-Aldrich) in calcium-free KSOM overlaid with oil (Irvine Scientific). Images were acquired every 4 sec for a total duration of 3 hours. All images were acquired on a TCS SP5 confocal microscope (Leica) equipped with a stage top incubator (Tokai Hit, Shizuoka, Japan), 40x oil-immersion objective and an Ar (488 nm) laser line.
Elemental analysis of *in vitro* maturation medium

The complete maturation medium containing 200 mM L-glutamine, 10% FBS, 1.5 IU/ml human chorionic gonadotropin (hCG, Sigma-Aldrich), and 5 ng/ml epidermal growth factor (EGF, Sigma-Aldrich) was treated with Chelex treatment overnight following established protocols\(^4\). Elemental analysis of the media was conducted by inductively coupled plasma mass spectrometry (ICP-MS, PQ ExCell, TJA Solutions, Franklin, MA).

Supplementary References

S1. Ibanez, E., Sanfins, A., Combelles, C.M., Overstrom, E.W. & Albertini, D.F. Genetic strain variations in the metaphase-II phenotype of mouse oocytes matured in vivo or in vitro. *Reproduction* **130**, 845-55 (2005).

S2. Santos, F. & Dean, W. Using immunofluorescence to observe methylation changes in mammalian preimplantation embryos. *Methods Mol Biol* **325**, 129-37 (2006).

S3. Xu, M., West, E., Shea, L.D. & Woodruff, T.K. Identification of a stage-specific permissive in vitro culture environment for follicle growth and oocyte development. *Biol Reprod* **75**, 916-23 (2006).

S4. Suhy, D. A., Simon, K. D., Linzer, D. I., & O’Halloran, T. V. Metallothionein is part of a zinc-scavenging mechanism for cell survival under conditions of extreme zinc deprivation. *J. Biol. Chem.* **274**, 9183-92 (1999).

Supplementary Figures

**Supplementary Scheme 1.** Chemical structures of membrane permeable small molecule chelators used in the manuscript. TPEN was utilized as a zinc-selective chelator, and neocuproine and tetrathiomolybdate were used as copper-selective chelators.

**Supplementary Figure 1.** Dose-dependent response of maturing oocytes to the heavy metal chelator TPEN. Oocytes mature normally in the presence of 1 µM (a) or 5 µM (b) TPEN, as illustrated by their ability to divide asymmetrically. Arrowheads denote polar bodies. Only 10 µM TPEN clearly perturbed asymmetric division without cytotoxic effects (c, replicated from Fig. 2). Concentrations 20 µM (d) or higher (50 µM, e) block polar body formation and result in oocytes that appear unhealthy, with granular cytoplasm and shrinkage away from the zona pellucida.

**Supplementary Figure 2.** The reduction of zinc in the maturation medium by Chelex treatment is insufficient to cause the same phenotypes as TPEN. Control *in vitro* matured oocytes (a) divide asymmetrically, yielding a large egg and a small polar body. This is recapitulated by oocytes matured in a low-zinc media (b), suggesting that even reduced zinc levels are sufficient to permit normal maturation. The media was analyzed by ICP-MS to confirm there was a reduction in the total zinc concentration (c).

**Supplementary Figure 3.** TPEN specifically disrupts the accumulation of zinc in the maturing oocyte. There is no difference between total copper (a) in control *in vitro* matured (IVM, N = 9) oocytes versus those that had been matured in the presence of TPEN (IVM
+TPEN, N = 10). However, the total content of zinc was significantly lower in IVM +TPEN oocytes compared to the control (b). Dotted lines indicate the concentration in GV oocytes, as shown in Fig. 1c. Note that there was no significant difference in copper or zinc content between in vivo ovulated MII eggs (as shown in Fig. 1c) and IVM eggs. Data represent mean values ± s.e. Asterisks denote significance (p<0.05); ns = not significant.

Supplementary Figure 4. Zinc insufficiency can be rescued prior to polar body extrusion. At the end of a 16-hour in vitro maturation period, normal oocytes display a metaphase II spindle and the first polar body (a, PB). Zinc insufficiency will cause arrest in a telophase I-like state after the same duration. A metaphase II spindle can be restored in zinc-insufficient oocytes as late as 9 hrs post-maturation and representative images of supplementation are shown after 7 (b), 8 (c), and 9 (d) hrs of exposure to TPEN. The spindle enlarges in both length (e) and width (f) with delayed rescue. Polar body diameter is not completely restored by zinc supplementation (g), although asymmetric division is fully rescued. Magenta = alpha-tubulin, yellow = DNA, green = F-actin. Carats (^) denote cumulus cells. Scale bar = 25 µm.
TPEN  neocuproine  tetrathiomolybdate
**Supplementary Table 1.** Summary of numerical values for total iron, copper, and zinc content in the maturing oocyte and two-cell embryo.

|             | GV oocyte                  | MII egg                   | two-cell embryo             |
|-------------|----------------------------|----------------------------|-----------------------------|
|             | Fe (× 10^9) | Cu (× 10^9) | Zn (× 10^9) | Fe (× 10^9) | Cu (× 10^9) | Zn (× 10^9) | Fe (µg/cm²) | Cu (µg/cm²) | Zn (µg/cm²) |
| atoms       | 4.65 ± 0.45 | 2.23 ± 0.22 | 37.46 ± 2.97 | 5.92 ± 0.47 | 3.32 ± 0.18 | 57.79 ± 4.99 | 5.52 ± 0.17 | 2.18 ± 0.09 | 45.97 ± 1.38 |
| µg/cm²      | 0.00588 ±   | 0.00319 ±   | 0.05546 ±    | 0.00638 ±   | 0.00398 ±   | 0.07211 ±    | 0.00871 ±   | 0.00391 ±   | 0.08500 ±    |
| mean ROI area| 7325 um²      | 9034 um²      | 6098 um²      |                   |             |               |             |             |             |

*µg/cm² × ROI area (cm²) = total amount in µg. Converted to atoms using atomic weight for each element and Avogadro’s number.
Supplementary Table 2. Summary of phenotypes\(^a\) following \textit{in vitro} oocyte maturation.

|                  | N\(^b\) | GVBD   | PB     | DG     |
|------------------|---------|--------|--------|--------|
| control          | 121     | 18.2%  | 81.8%  | 0.0%   |
| 10 \textmu M TPEN| 116     | 5.2%   | 75.8%  | 19.0%  |

\(^a\) GVBD = germinal vesicle breakdown, PB = polar body, DG = degenerate

\(^b\) N = total number of oocytes cultured \textit{in vitro}
Supplementary Table 3. Total number of atoms of iron, copper, and zinc in oocytes following *in vitro* maturation (IVM) in the absence (control) or presence of 10 µM TPEN.

|                  | Fe     | Cu     | Zn     |
|------------------|--------|--------|--------|
| IVM control      | 6.72 ± 0.21 | 3.55 ± 0.16 | 52.31 ± 2.40 |
| IVM + 10 µM TPEN | 8.72 ± 0.21* | 3.28 ± 0.06 | 40.86 ± 1.80  |

*The iron content increases in a small but statistically significant manner in oocytes treated with 10 µM TPEN. The concentrations of iron and zinc in the total maturation medium are 8 and 4 µM, respectively. Assuming a stoichiometric chelation of available zinc in the medium, some of the iron in the medium may remain available for uptake by the oocyte’s zinc transporters.*
Supplementary Figure 2

(a) Control

(b) Chelex-treated

(c) Molarity (mol/L)

Control: 3.99 ± 0.494 μM
Chelex-treated: 0.0779 ± 0.0121 μM
