Supporting Information

Experimental Procedures

Acquisition of human oocyte-cumulus-complexes and in vitro maturation

Human ovarian tissue was obtained from subjects following informed consent under Institutional Review Board-approved protocols at Northwestern University. After surgical removal, ovaries were transported to the laboratory in < 2 hours at 4^{o}C in SAGE OTC Holding Media (Copper Surgical, Trumbull, CT). In all cases, the ovaries were processed using a standard technique in ovarian tissue cryopreservation in which the ovarian cortex is separated from the medulla (http://oncofertility.northwestern.edu/media/dissection-human-ovary-preparation-cryopreservation). OCCs released from small antral follicles into the medium as a consequence of tissue processing were collected manually. OCCs were imaged using a Leica DM IRB inverted microscope (Leica Microsystems, Heidelberg, Germany). IVM was performed using the SAGE In Vitro Maturation Kit (Cooper Surgical) according to the manufacturer's instructions. Briefly, OCCs were transferred to 1 ml of SAGE IVM media supplemented with 75 mIU recombinant human FSH (NV Organon, Oss, The Netherlands) and 75 mIU recombinant LH (Ares Serono, Randolph, MA) at 37^{o}C in 5% CO_2 in air for 36-42 hours. Following IVM, cumulus

cells were removed with a brief incubation in 0.3 mg/ml hyaluronidase (Sigma-Aldrich). The meiotic stage of each cell was scored morphologically. Cells with an intact GV remained arrested at prophase I (GV stage) whereas cells that had extruded a polar body had reached MII. Cells without both a GV and a polar body were classified as having undergone germinal vesicle breakdown (GVBD) and were still in meiosis I.

Monastrol treatment and immunocytochemistry

Eggs were incubated in monastrol (Tocris Bioscience, Ellisville, MO) diluted in SAGE IVM media to 100 μM for 1-2 hours to allow for *in situ* chromosome spreading. All treatments were done at 37°C in 5% CO_2 in air. Eggs were fixed in 2% paraformaldehyde (Electron Microscopy Sciences, Hatfield, PA) supplemented with 0.1% Triton X-100 (Sigma-Aldrich, St. Louis, MO) for 1 h at room temperature, permeabilized in PBS containing 0.1% Triton X-100 and 0.3% bovine serum albumin (BSA; MP Biomedicals, Solon, OH) for 15 min, and blocked in PBS containing 0.01% Tween-20 (Sigma-Aldrich) and 0.3% BSA (blocking solution). Eggs were incubated in primary antibodies diluted in blocking solution overnight at 4°C. Kinetochores were immunostained with human CREST autoimmune serum (1:50; Immunovision, Springdale, AZ) and monoclonal anti-α tubulin (1:100; Sigma-Aldrich). The primary antibodies were detected with the appropriate secondary antibodies, including Alexa-Fluor (AF) 488-conjugated donkey anti-mouse IgG, AF 568-conjugated goat anti-mouse IgG, and AF 488-conjugated goat anti-human IgG (1:100; Invitrogen, Carlsbad, CA). F-actin was detected with Rhodamine-phalloidin (1:50; Invitrogen). Eggs were washed in blocking solution in between antibody incubations and then mounted in Vectashield with 4(6-diamidino-2-phenylindole) (DAPI) (Vector Laboratories Inc., Burlingame, CA). Images were taken using a Leica SP5 inverted laser-scanning confocal microscope using either 40x (1.25 NA), 63x (1.4 NA), or 100x (1.4 NA) oil immersion objectives (Leica Microsystems). Images were processed using LAS AF software (Leica Microsystems).

Inter-kinetochore distance analysis

Images were collected at 0.2 μm intervals to span the entire region of the MII spindle, and sister kinetochores were assigned by proximity to a specific sister chromatid pair. Eggs or chromosomes with ambiguous kinetochore staining were excluded from the analysis. The inter-kinetochore distances were measured from the peak pixel intensity of each sister kinetochore using LAS AF software (Leica Microsystems). Most kinetochore pairs were in the same z-plane, but the Pythagorean theorem was used in instances when they were not. Serial confocal

sections were analyzed to obtain a total kinetochore count for each egg. Two individuals, blinded to the subject age, performed these measurements.

Statistical analysis

ANOVA analysis with Bonferroni's Multiple Comparison Test was performed using GraphPad Prism 4.0 software (GraphPad Software, Inc., La Jolla, CA) and the relevant statistical differences are noted. Differences of $P < 0.05$ were considered to be significant.