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Supplementary Materials: Effects of Fullerenol Nanoparticles on Rat Oocyte Meiosis Resumption

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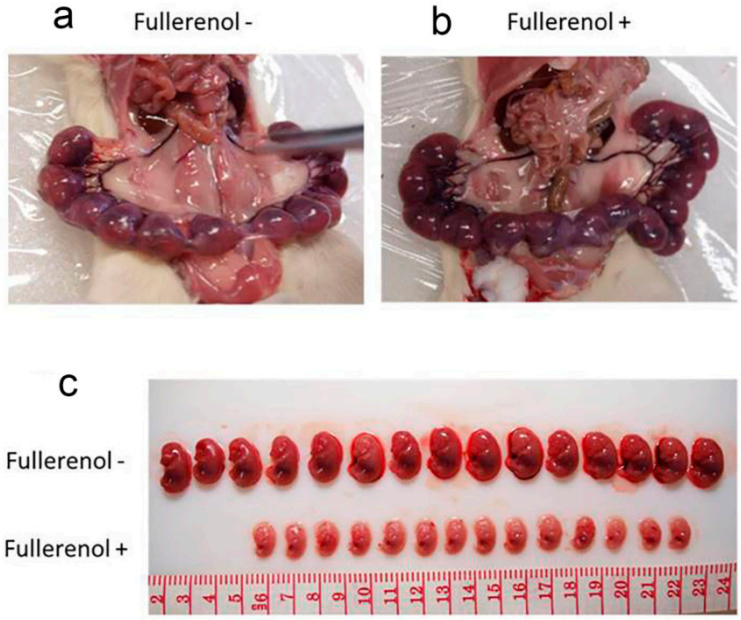

Figure S1. Uteri and fetuses from female rats treated with or without fullerenol. Fullerenol was dissolved in normal saline (25.370 mg/kg body weight) and administered to female rats by tail intravenous injection for 3 consecutive days every week. After a 12-week treatment, copulations between normal males and fullerenol-treated females were initiated. Females were euthanized 18 days after treatment, and the uteri were collected. Fetal development degree was recorded. Uterus of (**a**) control and (**b**) fullerenol-treated rats. Darker color was observed after fullerenol treatment. (**c**) Fetal development in both groups. Smaller fetus was observed after fullerenol treatment (n = 3 in each group).

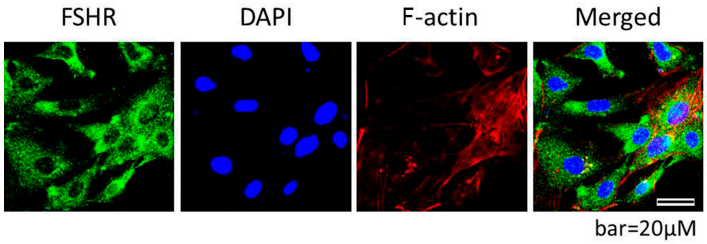

Figure S2. Immunofluorescent staining of follicle-stimulating hormone receptor (FSHR) in granulosa cells. Freshly isolated granulosa cells were routinely cultured for 4 days to attach and then fixed in paraformaldehyde (PFA). Identification with specific cell type marker FSHR indicated the high purity of granulosa cells (green, scale bar: 20 μm). Blue and red color indicate nuclear staining and F-actin skeleton, respectively. Imaging was repeated three times with similar results.

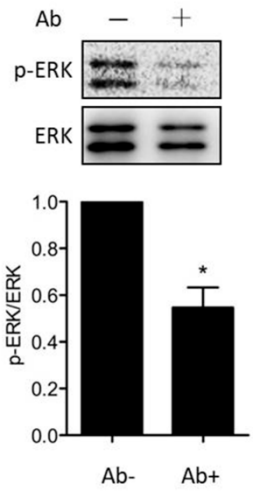

Figure S3. Extracellular domain-targeted anti-epidermal growth factor receptor (EGFR) antibody reduced phosphorylated-extracellular signal-regulated kinase 1 and 2 (p-ERK1/2) activation in granulosa cells. Freshly isolated oocyte-granulosa cell complexes (OGCs) were cultured in M2 medium with or without 2 μg/mL antibody for 2 h, and then granulosa cells were harvested after hyaluronidase treatment for immunoblotting analysis. Ab, extracellular domain-targeted anti-EGFR antibody. Data are shown as mean ± standard deviation (SD) from three replicates. Typical images are presented.

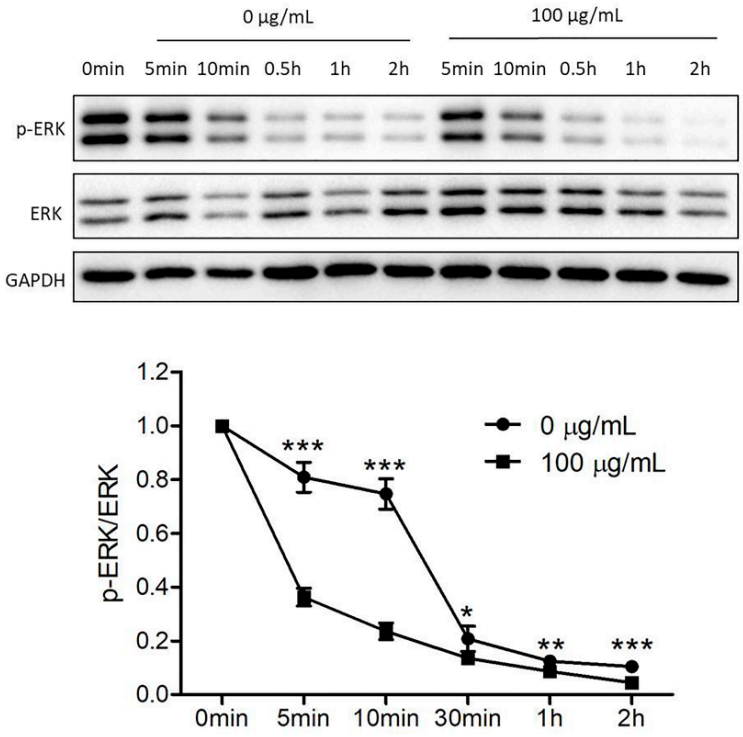

Figure S4. Time-course of extracellular signal-regulated kinase 1 and 2 (ERK1/2) activation in granulosa cells after fullerenol treatment. Freshly isolated oocyte-granulosa cell complexes (OGCs) were cultured in M2 medium with or without 100 μg/mL fullerenol for different times, and then granulosa cells were harvested after removing oocytes using hyaluronidase treatment for immunoblotting analysis. Data are shown as mean ± standard deviation (SD) from three replicates. Typical images are presented.

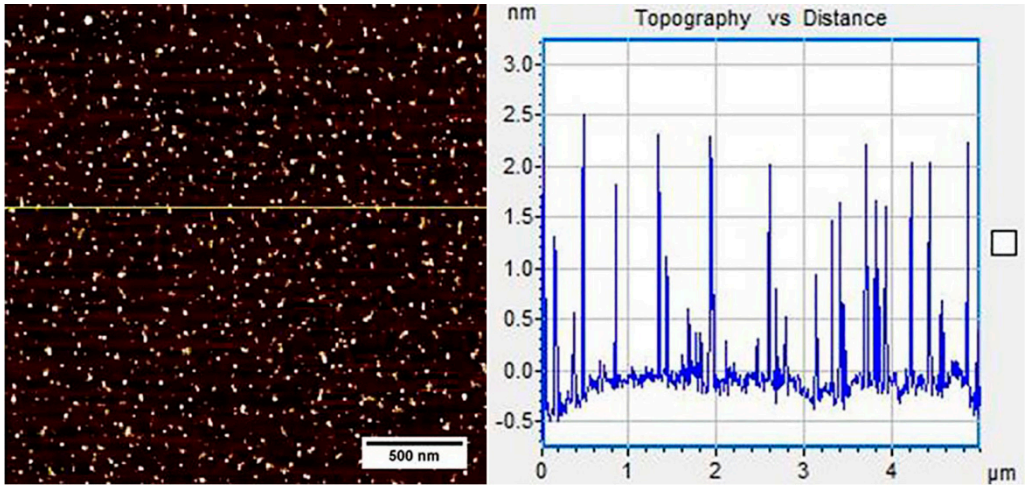

Figure S5. Characterization of fullerenol nanoparticles using atomic force microscopy (AFM). Fullerenol nanoparticles were dissolved in phosphate-buffered saline (PBS) and ultrasonically processed. Newly cleaved mica was pretreated with 0.1 M magnesium chloride ($MgCl_2$), rinsed with deionized water and then dried before nanoparticles deposition. AFM (Agilent 5500, Santa Clara, CA, USA) imaging was performed under air condition and AC mode (tapping mode). High-quality (HQ): NSC15/ALBS probes with nominal spring constant of K = 40 N/m from Mikromasch probes were used. Settings: amplitude, 3–6 V; speed, 0.8 ln/s; scan size, 10 × 10 µm; and range, 1.5 µm. Images were processed off-line using inherent PicoImage software.

Table S1. RT-PCR Primers of *CX43* and *GAPDH*

Gene name	Primers
CX43	Forward: 5′-CTCACGTCCCACGGAGAAAA-3′, Reverse: 5′-CGCGATCCTTAACGCCTTTG-3′
GAPDH	Forward: 5′-TACCCACGGCAAGTTCAACG-3′, Reverse: 5′-CACCAGCATCACCCCATTTG-3′

Table S2. RT-PCR protocol

Reverse Transcription	
1. Prepare the following mixture in each tube:	
Total RNA	3 µg
Olig dT	2 µl
DEPC H_2O	to 14 µl
2. Incubate the samples at 70°C for 5 min and then on ice for at least 1 min.	
3. Prepare reaction master mixture. For each reaction:	
RNase inhibitor	0.7 µl
M-MLV buffer	5 µl
M-MLV	1 µl
dNTP	1 µl
ddH_2O	to 25 µl
4. Incubate the tubes at 37°C for 90 min, heat inactivate at 70°C for 5 min, and then chill on ice.	
5. Store the cDNA at -20°C until use for real-time PCR.	
Real-time PCR	
1. Prepare the following mixture in each tube:	
cDNA	2 µl
Primer (forward and reverse)	1 µl + 1 µl

dNTP	1 μl
Buffer	5 μl
DNA polymerase	0.5 μl
DEPC H_2O	to 25 μl

2. Set up the experiment and the following PCR program as follow.

a. 95°C 3 min, 1 cycle

b. 95 °C 30 s -> 55 °C 30 s -> 72 °C 1 min, 34 cycles

c. 72°C 5 min, 1 cycle

3. After PCR is finished, remove the tubes from the machine. The PCR specificity is examined by 1% agarose gel using 5 μl from each reaction.