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Collection and culture of mural granulosa cells

The immature rats (postnatal day 21) received an intraperitoneal injection of 10 IU PMSG. Forty-six hours later the animals were killed by decapitation after anaesthesia with pentobarbital sodium. The ovaries were placed in PBS. The mural granulosa cells were collected by repeated puncture of antral follicles with a 5.5-gauge needle (Chinese size, which corresponds to 26-gauge in Western countries). After centrifugation at 2000 rpm for 5 min, the cells were washed three times with PBS. The cells were initially cultured in 35-mm dishes at a density of 1×10^6 cells/dish, and maintained for 48 h in 2 mL Dulbecco's Modified Eagle's Medium supplemented with 10% fetal bovine serum, and 100 IU mL^{-1} penicillin and streptomycin. The medium was then changed to the phenol red-free Dulbecco's Modified Eagle's Medium supplemented with 100 IU mL^{-1} penicillin and streptomycin. Norepinephrine (NE; Sigma, St Louis, MO, USA) was dissolved in ascorbic acid at 10^{-2} M and added to the cultures at final concentration of 10^{-4} M. In addition, ascorbic acid of the same volume was added and performed as the control. They were incubated at 37°C in water-saturated air with 5% CO_2. After 21 h of treatment with NE, BrdU were added to the cultures at final concentration of 20 ug mL^{-1}. After 3 h incubation, the medium was discarded, and the cells were washed three times with PBS and fixed in 4% PFA for 20 min at RT, then immunochemical procedure for BrdU detection was performed as described above.

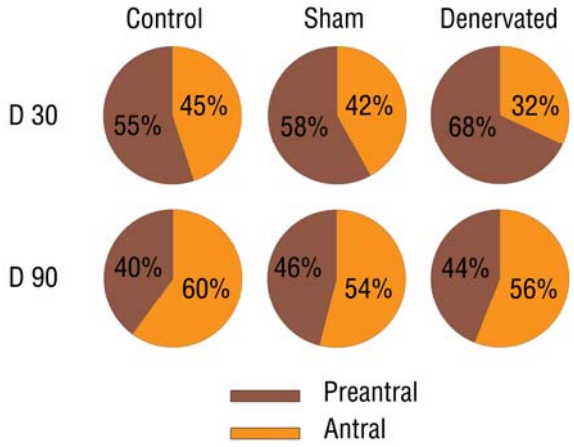

Fig. S1. Neonatal SON transection significantly increases the proportion of preantral follicles, decreases the proportion of antral follicles in denervated ovaries compared with controls on PD 30, but has no obvious effect on the proportion of preantral follicles and antral follicles on PD 90.

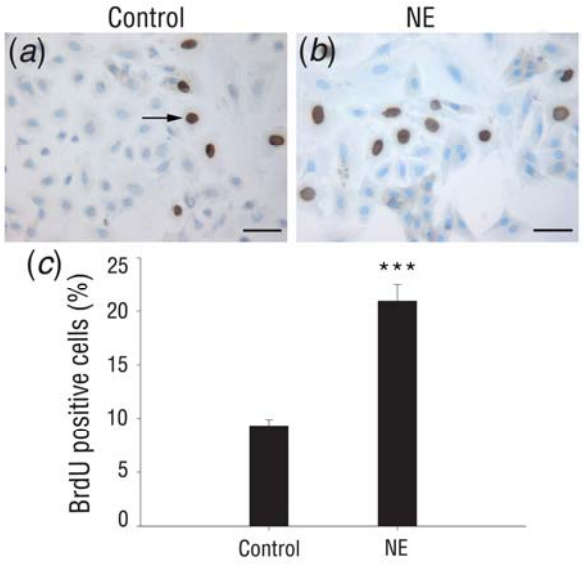

Fig. S2. NE promotes cell proliferation of the cultured ovarian granulosa cells detected by BrdU immunolabeling. (*a*, *b*) Representative micrographs for BrdU expression (arrow) in cultured granulosa cells treated with control (*a*) and treated with NE (10^{-4} M) (*b*) for 24 h. Scale bars = 50 μm. (*c*) NE significantly increases the percentage of BrdU positive cells in cultured ovarian granulosa cells. The values represent mean ± s.e.m. ($n \geq 10$). Asterisks indicate statistical significance compared with the control group (***$P < 0.001$).