10.1071/RD15228_AC

Supplementary Material: *Reproduction, Fertility and Development*, 2017, 29(4), 759–767.

Supplementary Material

Age-associated deterioration in follicular fluid induces a decline in bovine oocyte quality

Shun Takeo[A], *Koji Kimura*[B], *Koumei Shirasuna*[A], *Takehito Kuwayama*[A] *and Hisataka Iwata*[A,C]

[A]Tokyo University of Agriculture, Funako 1737, Atsugi Kanagawa 243-0034, Japan.

[B]Laboratory of Reproductive Physiology, Graduate School of Environmental and Life Science, Okayama University, Okayama 700-8530, Japan.

[C]Corresponding author. Email: h1iwata@nodai.ac.jp

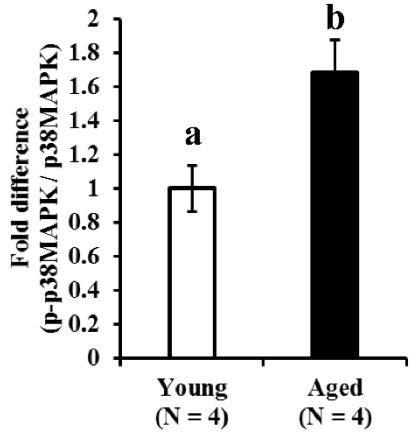

Fig. S1. Expression level of p-p38 MAPK relative to p38 MAPK in granulosa cells by western blot analysis. Granulosa cells were collected from antral follicle (3–6 mm in diameter) of ovaries derived from young and aged cows. Western blot analysis was conducted as described in our previous report (Tanaka *et al.* 2014). Briefly, granulosa cells collected from young and aged cows were washed in 0.2% PVA-PBS and lysed in 40 µL of Laemmli sample buffer (Bio-Rad Laboratories Inc., Hercules, CA, USA). After extraction at 95°C for 5 min, samples were stored at –20°C until use. Extracted proteins were separated by SDS-PAGE on a 10% polyacrylamide gel, and the proteins were transferred to a PVDF membrane (Hybond-LFPPVDF membrane; GE Healthcare, Buckinghamshire, UK). p38 MAPK, rabbit polyclonal p38a antibody (Santa Cruz Biotechnology Inc., Santa Cruz, CA, USA; 1:500) and p-p38 MAPK, rabbit polyclonal phospho-p38MAP kinase (Thr180/Tyr182) antibody (Cell Signaling Technology Inc., Beverly, MA, USA; 1:500) were used as primary antibodies and donkey anti-rabbit IgG HRP-linked antibody (Abcam,

Tokyo, Japan; 1:40,000) was used as a secondary antibody. The band intensity of p-p38MAPK relative to that of p38MAPK was calculated. Data are presented as the mean ± s.e. derived from four different samples. a-b, $P < 0.05$.