Estradiol-17β Increases Bovine Oviductal Tonus via G protein Coupled Estrogen Receptor 1 and RhoA/Rho Kinase Signaling Pathway

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According to the World Health Organization (WHO), human infertility is defined as the failure to achieve a pregnancy after 12 months or more of regular unprotected sexual intercourse. It is estimated that approximately 48 million couples and 186 million individuals live with infertility in the world. Since the transport of gametes and embryos in the oviduct at an appropriate time is important for the establishment of pregnancy, dysfunction of the oviduct is one of the causes of infertility. Early embryo and sperm transport through the oviductal isthmus depends on the contraction and relaxation of the smooth muscle layers. Estradiol-17β (E2) is known to indirectly increase contractility by promoting the secretion of oviductal contractile factors such as endothelin. Previous research has shown that the herbal medicine Tokishakuyakusan directly increases bovine oviductal tonus via G protein coupled estrogen receptor 1 (GPER1). In other reports, E2 regulates contractility via RhoA/Rho kinase (ROCK) signaling pathway and increases the expression of RNDs, which act as ROCK inhibitor in small intestine. Therefore, we investigated the direct effect of E2, the original ligand of GPER1, on the contraction and relaxation of bovine oviductal smooth muscle and its mechanism.

We used bovine oviductal isthmic tissues at four stages of the estrous cycle: stage I (1-4 days after ovulation), stage II (5-10 days), stage III (11-17 days), stage IV (18-20 days). Isthmic tissues cut into 3-4 pieces of 5 mm length were used for the Magnus method to monitor the longitudinal contractility (contraction frequency, contraction force, and tonus). The effect of E2 (1 or 10 nM) and GPER1 agonist (G-1, 1 or 10 μM) on oviductal contractility were examined. Similarly, the effect of E2 (1 nM) with pre-treatment of GPER1 antagonist (G-15, 25 or 250 nM) and ROCK inhibitor (Y-27632, 1 μM) were also examined. The protein expression level of GPER1, RHOA, ROCK II and RND3 in the oviductal smooth muscle tissues of each stage were measured by Western blotting. Furthermore, ROCK II activity in oviductal smooth muscle tissues treated with 1 nM E2 was measured.

E2 had no effects on the frequency and contraction force of oviductal isthmus at all stages. However, the tonus was significantly increased by 1 nM E2 in stage I (P<0.05). The tonus in stages II-IV did not change by E2 treatment. The G-1 treatment also increased oviductal tonus similar to E2 at stage I (P<0.05). The treatment of G-15 and Y-27632 significantly suppressed the increase of oviductal tonus in stage I induced by E2 (P<0.05). There was no significant difference in GPER1 and RHOA protein expression among the estrous stages. On the other hands, the protein expression of ROCK II in stage I was higher than that in stage II (P<0.05). ROCK II activity in stage I was also higher than in stage II and IV (P<0.05). Moreover, the protein expression of RND3 in stage IV was higher than that in stage III (P<0.05). In conclusion, 1 nM E2 directly affects oviductal contractility by increasing tonus via GPER1 and ROCK II activation in stage I. The difference in the effect of E2 on oviductal tonus among each stage may be attributed to the differences in the expression of ROCK II and RND3.