

241 Changes of the plasma membrane fluidity of granulosa cells during the process of luteinization. H.Negishi, T.Okuda, S.Makinoda, T.Tanaka, S.Fujimoto, M.Kinjou*, T.Koyama*, Dept. of Obst. and Gynec., Hokkaido Univ. Sch. Med., Sapporo, *Dept. of Div. of Physiology., Res. Insti. App. Elect., Sapporo.

To elucidate the membrane fluidity of granulosa cells (GCs) in luteinization process, porcine GCs were cultured in the serum free medium under the stimulation of hCG and their plasma membrane anisotropy (rs), lipid composition and progesterone (P) synthesis were measured. rs, which is reverse to membrane fluidity (MF), was measured with the spectrofluorometer using diphenyl-hexatriene. The results were as follows. 1) The value of the rs (MF) stimulated by hCG decreased (increased) significantly ($P<0.05$) as compared to that without hCG. 2) The concentration of cholesterol (C) decreased significantly ($P<0.05$) by hCG. In contrast, the concentration and composition of phospholipids (PL) showed no significant changes. 3) P in culture medium significantly ($P<0.01$) increased by hCG at 30, 300mIU/ml. These results indicate the increase of MF is due to a decrease of C/PL ratio. This study suggests C of plasma membranes was utilized for P synthesis at least in the serum free medium during the process of luteinization.

242 Effect of Activin A on human granulosa luteal cell culture K.Ishimoto, Y.Hasagawa, M.Seki, Y.Ibuki, M.Igarashi. Dept.Obst. and Gyne. Gunma Univ. Sch. Med. Gunma.

Activin A plays a important role in ovarian regulation. We examined the effect upon human granulosa luteal cells that were obtained by follicular aspiration from women undergoing ovum retrieval for IVF. Granulosa luteal cells were cultured at 37°C in 95%O₂-5%CO₂ for 24h and activin A, hFSH, hCG added in various manner. After 72h the supernatant of the medium was aspirated and frozen for radioimmunoassay of estradiol, progesterone, and inhibin. Addition of lng/ml of activin A suppressed estradiol and progesterone production significantly, ($p<0.05$) but the higher dose of activin A does not have a significant effect in this culture. Human FSH and hCG stimulated inhibin secretion in this culture dose dependent manner, but activin A does not have a significant effect in inhibin secretion. These result suggest that activin A may have a dual function in steroidogenesis.

243 Evidence for tight coupling of gonadotropin-releasing hormone receptor occupancy to stimulated phosphoinositide turnover and anti-gonadotropin action in granulosa cells. K.Iida, A.Imai, T.Tamaya, Dept. Obstet. and Gynecol., Gifu Univ. Sch. of Med., Gifu.

Gonadotropin-releasing hormone (Gn-RH) stimulates phosphoinositide turnover by binding to its specific receptor, and suppresses Gn-dependent maturation and steroidogenesis in granulosa cells. This study was undertaken to determine whether persistent receptor occupancy was necessary for Gn-RH to exert such actions on rat granulosa cells, using competitive antagonist, antide. Gn-RH stimulated phosphorylation of phosphatidylinositol (PtdIns), which could be terminated by displacement of previously bound Gn-RH from its receptor by antide and restarted by reoccupying the receptors with Gn-RH. The antide could prevent the Gn-RH-stimulated PtdIns phosphorylation at whenever it was added to incubations. An identical effect of antide was observed also in anti-FSH action of Gn-RH. FSH enhanced the aromatase activity, which was quenched by Gn-RH and restarted at a time when Gn-RH was removed from its receptor by antide. These two responses associated with occupancy of Gn-RH receptor suggest a tight coupling of stimulated PtdIns turnover to suppression of aromatase activation.