

253 Retinoic Acid (RA) stimulates hCG- $\alpha$  subunit secretion by choriocarcinoma cells in Vitro. H.Tanaka, Y.Kato, T.Jimbo, G.D.Braunstein\*, Dept. Perinatology, Kagawa Med. Sch., Dept. Med., Cedars-Sinai Med. Ctr.\*

RA, active metabolite of vitamin A, is an important mediator of cellular differentiation and has been shown to stimulate hCG secretion in trophoblastic cells. In order to evaluate whether RA modulates hCG- $\alpha$  secretion or production, we examine the effect of RA on hCG- $\alpha$  secretion in choriocarcinoma cell line, JEG-3 cells which produce low amount of hCG.

RA stimulated hCG- $\alpha$  secretion in a dose-dependent fashion, starting at  $10^{-10}$ M, with maximal secretion at  $10^{-6}$ M. The time required to give a statistically significant increment of hCG- $\alpha$  over control cells was 48 hours. The addition of RA to cholera toxin or forskolin resulted in synergistic stimulation of hCG- $\alpha$ . Intracellular levels of cAMP were not affected by RA treatment. RA did not alter cell number in the dosages used. These results suggest that RA modulates not only hCG- $\beta$  subunit production but also hCG- $\alpha$  subunit production.

254 Molecular cloning of the cDNA for the human placental oxytocin like substance. A.Sakai, T.Makino, T.Suqi, K.Toyoshima, K.Iwasaki, M.Umeuchi, S.Saito, T.Maruyama, S.Nozawa, Dept. Obst. and Gynec., Keio Univ. Sch. Med., Tokyo.

We have isolated oxytocin (OT) like substance cDNA clone obtained from the human placental cDNA library by using OT antibody. Its total base number of the cDNA clone was approximately 900bp in the position of band appeared in the electrophoresis. Its antigenicity was observed by measuring the concentration of expressed substance transfected into the Chinese hamster ovary cell. It was 200pg/ml in culture medium by the RIA system. Furthermore its bioactivity of uterine muscle contractile effect was observed in the Magnus apparatus. In amino acid sequence on the analogy of nucleotide sequence of OT like substance cDNA in the human placenta, human pituitary OT like structure was not found. Therefore an unknown peptide possessing uterine contractile effect was supposed to exist in the human placenta. Further studies is required to clarify the physiological role of this peptide in the mechanism of pregnancy maintenance and onset of labor pain.

255 Regulation of ovarian oxytocin gene expression in bovine peri-ovulatory phase. K.Furuya, C.Mitsui, I.Nagata, Dep. Obst. and Gynec., National Defense Medical College, Saitama.

Although oxytocin (OT) has been identified in the early luteal cells, little is known about the molecular mechanism of OT production in the peri-ovulatory phase. We investigated a comparative study of gene expression of OT in bovine ovarian cells between the pre- and post-ovulatory phase, evaluating (1) the relationship between follicular fluid estradiol ( $E_2$ ) content and mRNA level of OT in granulosa cells with and without insulin-like growth factor I (IGF-I) treatment, and (2) the effect of IGF-I and  $E_2$  in the early luteal cells. mRNA levels of OT in granulosa cells obtained from the follicles with high  $E_2$  level (pre-ovulatory phase) were markedly increased by IGF-I, however those levels with low  $E_2$  level (early follicular phase) were not increased by IGF-I. In the early luteal cells, IGF-I had a marked stimulatory effect on the mRNA levels of OT, but  $E_2$  had no influence. These results reveal that in the pre-ovulatory phase, OT gene expression is affected by both IGF-I and high level of local  $E_2$ , and that it is regulated by IGF-I alone in the post-ovulatory phase.