lopian transfer (GIFT) program. Follicular fluid was obtained from the growing follicle under laparoscopy. Oocytes were inspected and graded. Follicular fluid was centrifuged and the supernatant was used for the experiment. An EGF-like substance, which showed an EGF-antibody binding pattern parallel to that of standard human EGF, was found in the follicular fluid. The mean concentration of EGF-like substance was 0.99 ± 0.28 ng/ml as measured by EGF radioimmunoassay. Membrane filtration study indicated that the substance have a molecular weight less than 10,000. The level of EGF-like substance in the follicle with mature oocyte was higher than that with immature oocyte. The concentration of EGF-like substance also showed positive correlation with the progesterone concentration in the follicular fluid. These results suggest that EGF-like substance is present in human follicular fluid, which may be concerned with oocyte maturation.

250. Partial Purification of Follicle-stimulating Hormone Receptor Binding Inhibitor (FSHRBI) in Porcine Follicular Fluid

K. Furukawa, M. Yamoto and R. Nakano

Dept. Obst. & Gynec., Wakayama Med. College, Wakayama

We have reported that several substances which inhibit the binding of FSH to granulosa cells exist in human and porcine follicular fluid. In the present study, we attempted to purify FSHRBI's from porcine follicular fluid.

Partial purification of FSHRBI was performed by sequential molecular sieving through Sephadex G50 and G15. Consequently, 5 peaks of FSHRBI activity were observed in Sephadex G15 eluate. G15-1 was fractionated by Superose 12-FPLC. A peak of FSHRBI activity was observed at a elution volume of 12 to 14 ml. This fraction was then subjected to anion exchange (MonoQ)-FPLC. FSHRBI activity was observed in the fractions eluted between 0.5 to 0.7 M ammonium acetate. By SDS-PAGE of this fraction, one band was revealed at a position corresponding to Mr 52KD. When G15-2 was applied to MonoQ-FPLC, FSHRBI activity was revealed in the fraction eluted between 0.8 to 0.9 M. MonoQ-FPLC of G15-3 resulted in the emergence of the activities at the unretained and retained (0.2 to 0.3 M) fractions. When the unretained fraction was applied to a RP-HPLC column (TSK-ODS gel), FSHRBI activity was observed in the fractions eluted at 20% methanol-HCOOH (pH 5.0).

The data suggest that high and low molecular weight substances which inhibit the FSH binding to granulosa cells might be present in porcine follicular fluid.

251. Effects of Neuraminidase Pretreatment on the Specific Binding of hLH and hFSH to Porcine Granulosa Cells

K. Nishimori, M. Yamoto and R. Nakano

Dept. Obst. & Gynec., Wakayama Med. College, Wakayama

Previous studies have suggested that specific receptors for gonadotropins are present on granulosa cells and that the binding of gonadotropins to granulosa cells changes during follicular maturation. However, relatively little is known about the mechanism of altering the number of receptors for gonadotropins. It has been proposed that gonadal tissue contains the masked binding sites for gonadotropins. To evaluate the possible existence of masked receptors for gonadotropins in the porcine granulosa cells, we examined the effects of neuraminidase on ^{125}I-hLH and ^{125}I-hFSH binding to porcine granulosa cells. Granulosa cells were obtained by needle aspiration from small (1~2 mm), medium (3~5 mm) and large (6~12 mm) porcine follicles and resuspended in Tris-BSA buffer. Effect of neuraminidase pretreatment (37°C, 30 min) on the specific binding of hLH and hFSH to granulosa cells was investigated and changes in population of receptors were estimated by Scatchard analysis. Specific receptors for hLH and hFSH, with high affinity and low capacity, were demonstrated in porcine granulosa cells. Pretreatment with neuraminidase enhanced specific FSH binding significantly. In contrast, pretreatment with neuraminidase had no effect on specific LH binding. Scatchard analysis revealed that neuraminidase increased the number of binding sites for FSH without altering the affinity for FSH. The results suggest that the porcine granulosa cell contains a population of masked FSH binding sites which can be exposed by in vitro pretreatment with neuraminidase.

252. Serial Cultivation of Human Granurosa Cells: Gonadotropin-like Effect of FGF

T. Hata, N. Makimura, K. Kato, Y. Sawasaki and T. Goto*

Dept. Obst. & Gynec.,