517 The Role of active oxygen in the ovulatory process of rat. H.Koyama, N.Tanaka, H.Okamura, and M.Inoue*, Dept.OB/GYN ,*Dept. Biochemistry, Kumamoto Univ. Med. Sch. Kumamoto, Japan.

To investigate the role of active oxygen in the ovulatory process, we synthetized long-acting superoxide dismutase derivative (SM-SOD) which scavenges superoxide anion. [Methods] On 22 days old immature Wistar rats, 10iu of Pregnant Mare Serum Gonadotropin(PMSG) were injected subcutaneously, followed by 5iu of human chorionic gonadotropin(hCG) in 48 hours, which started the ovulatory process. We analyzed that (1) the change of glutathione concentration in ovary and the activity of gamma glutamyltranspeptidase(γ-GTP) after hCG injection, (2)the turnover rate of glutathione in ovary after hCG injection, (3) the effect of SM-SOD and buthionine sulfoximine (BSO;specific inhibitor for glutathione synthetase) on ovulation rate, (4) vascular permeability in ovary by the Evans-blue method. [Results] SM-SOD significantly decreased ovulation rate by intravenous injection, and glutathione metabolic rate was significantly much higher in the ovary treated by hCG injection than control. The γ-GTP activity significantly increased 8 hours after hCG injection. BSO inhibited the increase of ovarian glutathione level, and ovulation rate significantly. Ovarian vascular permeability increased in ovulatory process, however, this enhancement of vascular permeability was inhibited by SM-SOD injection. [Conclusion] These data suggested that the active oxygen plays a significant role in follicle rupture by mediating ovarian vascular permeability, and enhancement of glutathione turnover rate is also mandatory for ovulation.

518 Effect of macrophages on granulosa cell proliferation in rat. Y. Fukumatsu, H. Katabuchi, M. Naito*, K. Takahashi*, H. Okamura, Dept. Obst. and Gynec., Kumamoto Univ. Med. Sch., Kumamoto, *2nd Dept. Pathology, Kumamoto Univ. Med. Sch., Kumamoto.

The effect of macrophages(Mϕ) on granulosa cell(GC) proliferation was examined in gonadotropin-primed immature rats. By immunohistochemistry using anti-rat Mϕ monoclonal antibody TRPM-3, Mϕ were observed in granulosa cell layer and antrum of follicles and corpus luteum. Electronmicroscopy also demonstrated Mϕ with some vacuoles of various sizes in the growing follicles. On the 8 μm serial sections which contain the maximal diameter of various follicles, the average ratios of GC and TRPM-3 positive cells(TR3/GC) were 0.008, 0.007, 0.002 in the preantral, antral and mature follicles, respectively. Preantral follicle was most rich in Mϕ population. ^{3}H-thymidine labeling index of GC cultured with peritoneal Mϕ(PMϕ) was significantly increased, and peaked when the ratio of GC and PMϕ(PMϕ/GC) was 0.01 (25.0%) in comparison with GC alone (14.2%). This ratio was almost coincident with TR3/GC in the preantral and antral follicles in vivo. These results suggested that Mϕ may participate in promoting granulosa cell proliferation.

519 Distribution of Ca^{2+}/calmodulin-dependent protein kinase II in the rat ovary. T.Ohba,H.Katabuchi,K.Miyazaki,K.Fukunaga*,H.Okamura,E.Miyamoto*, Dept.Obst.and Gynec.,*Dept.of Pharmacology, Kumamoto Univ.Med.Sch., Kumamoto.

We previously reported the presence of Ca^{2+}/calmodulin-dependent protein kinase II(CaM kinase II) in rat granulosa cells and the possible involvement of CaM kinase II in granulosa cell functions. In this study, we examined the distribution of CaM kinase II in the rat ovary during the ovulatory process. Indirect immunofluorescence was used to determine the distribution of CaM kinase II in cultured rat granulosa cells. CaM kinase II was localized diffusely in the cytoplasm in which the enzyme was concentrated on punctate structures. The immunohistochemical observation demonstrated that the oocyte, granulosa cell layer, theca cell layer, and vessel were immunostained with the anti-CaM kinase II antibodies. The stroma surrounding the primary follicle did not react with the antibodies. Immunoreactivity of the theca cell layer was weakly observed in the early antral follicle and apparently in the mature follicle. These results suggest that the expression of the enzyme is correlated with the differentiation of the theca cell layer during the follicle maturation.