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Follicular Growth and Oocyte Maturation : A 2003 Perspective

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The birth of the clone animals is influencing the frontier of research of animal biotechnology. It has effects on research of animal biotechnology itself by necessitating setting of new research subjects, modifications of the strategy of ongoing research projects, and challenges to schemes formerly considered impossible. In my talk, such topics including mass production of fertile ova and oocyte maturation will be discussed. (1) Oocytes are needed for the production of a clone by nuclear transplantation. Mitochondrial DNA inherited via the oocyte are involved also in the morphogenesis. Therefore, oocytes from the same animal must be used as recipients to produce genuine clones by nuclear transplantation. Experimenting on the assumption that selective oogenesis can be avoided, and apoptosis of oocytes can be prevented, by using ovarian angiogenic factos will be introduced. (2) It is important to clarify the factors of oocytes involving in reprogramming of somatic cells. Such factors are thought to be expressed in oocytes during oogenesis and oocyte maturation.Therefore, molecular mechanisms of oogenesis and oocyte maturation must be clarified extensively. Topics in this field including our recent advances will be discussed.

VEGF gene fragment stimulates follicular growth and prevents follicular atresia:

Perifollicular angiogenesis is closely associated with ovarian follicular development. To investigate whether additional induction of perifollicular angiogenesis would support subsequent follicular development,

we directly injected vascular endothelial growth factor (VEGF) gene fragments into the ovaries of miniature gilts, followed by gonadotrophin treatment to stimulate follicle growth. In addition, to confirm extraexpression of the VEGF gene after injection, we assessed the expression of two isoforms of VEGF (VEGF 120 and VEGF164) in granulosa cells, and expression of fms-like tyrosine kinase (Flt-1), fetal liver kinase (Flk-1) and density of capillary networks intheca cells. Direct injection of VEGF gene fragments into the ovaries was performed 7 days before equine chorionic gonadotropin (eCG) treatment. The ovaries in miniature gilts were removed 72 h after eCG treatment for histological examination. Granulosa cells and thecal tissues in the antral follicles (diameter, > 4mm) were collected to detect the mRNA expression of VEGF isoforms in the granulosa cells, and Flt-1 and Flk-1 in the thecal tissues by semiquantitative reverse transcription-polymerase chain reaction. VEGF levels were measured the in follicular fluid by enzyme immunoassay. Injection of VEGF gene fragment increased the level of mRNA expression of VEGF 120 and 164 isoforms in the granulosa cells and VEGF protein contents in the follicular fluid. The number of preovulatory follicles and the capillary density in the theca interna increased significantly in the ovaries injected with VEGF gene fragment compared with those treated with eCG alone. The Flt-1, but not Flk-1, mRNA expression show a tendency toward increasing in the thecal tissues of antral follicles in the ovaries injected with VEGF gene fragment. These results demonstrate that Flt-1 may be predominantly involved in the regulation of the capillary network in the theca interna during follicular development. Our data suggested that the regulation of perifollicular angiogenesis during follicular development is a very important factor in the development of ovulatory follicles. Our findings may offer an innovative technique for enhanced induction of follicular development in the ovary through gene and hormonal treatment, which may lead to prevention of infertility caused by ovarian dysfunction.

Hyaluronan-CD44 system involving in oocyte maturation

The process of cumulus expansion is a current topic of interest for *in vitro* production of matured oocytes with the ability for reprogramming of

somatic cells. In the present study, we examined the components of cumulus expansion, molecular mechanisms of cumulus expansion, and role of cumulus expansion for porcine oocyte maturation. The degree of cumulus expansion in the porcine cumulus-oocyte complexes (COCs) increased gradually until 48 h in culture in TCM-199. On the other hand, when the COCs were cultured in TCM-199 with a hyaluronan synthesis inhibitor and hyaluronidase, they showed no evidence of cumulus expansion during the culture period. Furthermore, the expression of hyaluronan synthase 2 (has2) in cumulus cells is accompanied by cumulus expansion. Hyaluronan receptor CD44 mRNA expressed in the cumulus cell, but not in the oocyte extracts. CD44 protein also expressed in/on the membrane of cumulus cells and its expression increased in a manner dependent on the degree of cumulus expansion. Moreover, we found that hyaluronan-CD44 system during cumulus expansion induces the activation of maturation promoting factor, resulting ingerminal vesicle breakdown of the oocytes, and the tyrosine-phosphorylation of Cx43 in the COCs. The present results showed that the main component of cumulus expansion in theCOCs is hyaluronan, the hyaluronan-CD44 system during cumulus expansion regulates the disruption of gap junctions in the COCs, and concurrently control the incidence of meiotic resumption in the porcine oocytes.

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