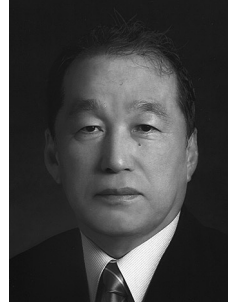


Japan Academy Prize to:

Eimei SATO
President, National Livestock Breeding Center
Professor Emeritus, Tohoku University

for “Studies on the Regulatory Mechanism of
Oogenesis in Mammals”

***Outline of the work:***

In the 1970s, the study of in vitro fertilization in various mammals was extensively conducted worldwide for the elucidation of information on the early stages in the fertilization process, efficient production of high-quality domestic animals, and medical treatment of infertile women. The preparation of capacitated spermatozoa and mature oocytes are essential for fertilization. However, at that time, studies on the in vivo maturation of ovarian oocytes and in vitro maturation of immature oocytes isolated from ovaries had just begun. To enhance the understanding in this area, Dr. Eimei Sato elucidated the regulatory mechanisms of oogenesis, particularly selective oogenesis and oocyte maturation, and developed methods for the induction of follicular development and in vitro maturation of ovarian oocytes.

I Regulatory mechanisms of follicular development

Mammalian oocytes grow, differentiate, and mature within ovarian follicles in a process termed as oogenesis. Although a large number of oocytes are present in the ovaries in various mammalian species, a small number is recruited for oogenesis. Follicular growth, induced simultaneously with oogenesis, is stimulated by the follicle-stimulating hormone (FSH). Dr. Sato identified 3 components involved in selective follicular growth: an FSH inhibitor that is produced and secreted in the follicles, a substance that promotes viability of oocytes, and angiogenic factors involved in the formation of a capillary network that influences the transport of FSH into the follicles. Identification of the FSH inhibitor initiated the study of inhibin. These 3 components were originally identified in porcine and bovine ovaries and were later functionally demonstrated in mice.

The presence of angiogenic factors in the ovaries was evidenced by the morphologically well-developed capillary network extending to the developing follicles. Dr. Sato first provided evidence of developing follicles with a high angiogenesis-inducing ability in mice, and later in pigs and cows. In addition, a gene-vector that is functional within the follicles was identified.

Dr. Sato developed a novel method for the proliferation of the capillary networks that surround the follicles by expressing an angiogenic factor in the follicles. This resulted in 5 times the number of fertilizable oocytes than that generated during spontaneous ovulation. Dr. Sato was highly lauded internationally because this was the first report on the direct effect of capillary networks on follicular development.

II Regulatory mechanisms of oocyte maturation

Oocyte maturation is induced in the final stage of oogenesis. An outline of the regulatory mechanisms involved in oocyte maturation was first reported by Dr. Sato. Oocytes isolated from ovarian follicles were shown to undergo meiotic resumption when cultured in vitro.

Dr. Sato discovered that the induction of meiotic resumption is suppressed when isolated oocytes are cultured in adhesion with a layer of granulosa cells isolated from ovarian follicles. Further investigation on the factor influencing meiotic arrest revealed that adenosine and cyclic adenosine monophosphate (cAMP) function as inhibitors that influence the cAMP level and phosphorylation of mitogen-activated protein (MAP) kinase cascade as well as the movement of phosphorylated MAP kinase into the oocyte nucleus. In addition, fertilization and reprogramming of the somatic cells incorporated into the oocytes were found to be enhanced when the oocytes were cultured with these substances for a defined period at the start of culture.

Dr. Sato then demonstrated the relationship between the induction of oocyte maturation and cumulus expansion in pigs. Hyaluronan, with its CD44 receptor located in the cumulus cells, was determined to be the main component of glycosaminoglycans that accumulate in the intercellular space of cumulus cells. The binding of hyaluronan with the CD44 receptor induces the phosphorylation of tyrosine residues on connexin 43, the gap junction protein present between cumulus cells and the oocyte, resulting in the inhibition of gap junction communication. Consequently, the oocyte is released from the inhibitory influence of the granulosa cells and oocyte maturation is induced. Dr. Sato was the first to report the above-mentioned mechanism for the induction of oocyte maturation.

As previously mentioned, Dr. Sato has conducted research on the regulatory mechanisms of follicular growth in mammalian ovaries, particularly on selective oogenesis and oocyte maturation in laboratory and domestic animals including pigs and cattle, using morphological, biochemical, and molecular biology techniques. The findings obtained from these studies have been shown to be standard in all mammals. Dr. Sato's contribution to the development of an in vitro culture system for immature oocytes and elucidation of the molecular mechanisms of oocyte maturation has greatly influenced animal reproduction, reproductive biology, and reproductive medicine, and earned him several accolades including international awards.

Reference:

- 1) Sato, E., Miyamoto, H., Ishibashi, T., Iritani, A.: Identification, purification and immunohistochemical detection of the inhibitor from porcine ovarian follicular fluid to compensatory ovarian hypertrophy in mice. *J. Reprod. Fertil.*, 54: 263-267, 1978
- 2) Sato, E., Iritani, A., Ishibashi, T.: Purification and action sites of a follicle-stimulating hormone inhibitor from bovine follicular fluid. *J. Anim. Sci.*, 55: 873-877, 1982
- 3) Sato, E., Ishibashi, T., Iritani, A.: Meiotic arresting substance separated from porcine ovarian granulosa cells and hypothetical arresting mechanism of meiosis. In "Intra-ovarian Control Mechanisms", Edited by C.P. Channing and S.J. Segal, pp.161-173, Plenum Press, New York and London, 1982
- 4) Sato, E., Ishibashi, T., Koide, S.S.: Inducement of blood vessel formation by ovarian extracts from mice injected with gonadotropins. *Experientia*, 38: 1248-1249, 1982
- 5) Sato, E., Koide, S.S.: A factor from bovine granulosa cells preventing oocyte maturation. *Differentiation*, 26: 59-62, 1984
- 6) Sato, E., Wood, H.N., Lynn, D.G., Koide, S.S.: Modulation of oocyte maturation by cyclic adenosine 3, 5-pyrophosphate. *Cell Differ.*, 17: 169-174, 1985
- 7) Sato, E., Koide, S.S.: Biochemical transmitters regulating the arrest and resumption of meiosis in oocytes. *Int. Rev.Cytol.*, 106: 1-33, 1987
- 8) Sato, E., Ishibashi, T., Koide, S.S.: Prevention of spontaneous degeneration of mouse oocytes in culture by ovarian glycosaminoglycans. *Biol. Reprod.*, 37: 371-376, 1987
- 9) Sato, E., Kawamura, N., Ishibashi, T.: Chemicals influencing maturation, activation and degeneration of bovine oocytes in culture. *J. Dairy Sci.*, 71: 3482-3488, 1988

- 10) Sato, E., Matsuo, M., Miyamoto, H.: Meiotic maturation of bovine oocytes in vitro: Improvement of meiotic competence by dibutyryl cyclic adenosine 3, 5-monophosphate. *J. Anim. Sci.*, 68: 1182-1187, 1990
- 11) Sato, E., Miyamoto, H., Koide, S.S.: Hyaluronic acid-like substances from mouse ovaries with angiogenic activity. *Z. Naturforsch.*, 45c: 873-880, 1990
- 12) Sato, E., Miyamoto, H., Koide, S.S.: Glycosaminoglycans in porcine follicular fluid promoting viability of oocytes in culture. *Mol. Reprod. Dev.*, 26: 391-397, 1990
- 13) Miyamoto, Y., Nakayama, T., Haraguchi, S., Miyamoto, H., Sato, E.: Morphological evaluation of microvascular networks and angiogenic factors in the selective growth of oocytes and follicles in the ovaries of mouse fetuses and newborns. *Develop. Growth Differ.*, 38: 291-298, 1996
- 14) Sato, E., Tanaka, T., Takeya, Y., Miyamoto, H., Koide, S.S.: Ovarian glycosaminoglycans potentiate angiogenic activity of epidermal growth factor in mice. *Endocrinology*, 128: 2402-2406, 1991
- 15) Sato, E., Inoue, M., Toyoda, Y.: Morphological profiles of mouse ovarian follicles: extensive accumulation of a strongly negative-charged substance at specific foci in follicular tissue during oocyte maturation. *Arch. Histol. Cytol.*, 56: 293-302, 1993
- 16) Nakayama, T., Inoue, M., Sato, E.: Effect of oocytectomy on glycosaminoglycan composition during cumulus expansion of porcine cumulus-oocyte complexes cultured in vitro. *Biol. Reprod.*, 55: 1299-1304, 1996
- 17) Inoue, M., Naito, K., Nakayama, T., Sato, E.: Mitogen-activated protein kinase translocates into the germinal vesicle and induces germinal vesicle breakdown in porcine oocytes. *Biol. Reprod.*, 58: 130-136, 1998
- 18) Jiang, J.Y., Miyoshi, K., Umezu, M., Sato, E.: Superovulation of immature hypothyroid rdw rats by thyroxine therapy and the development of eggs after in vitro fertilization. *J. Reprod. Fertil.*, 116: 19-24, 1999
- 19) Jiang, J.Y., Macchiarelli, G., Miyabayashi, K., Sato, E.: Follicular microvasculature in the porcine ovary. *Cell Tissue Res.*, 310: 93-101, 2002
- 20) Kimura, N., Konno, Y., Miyoshi, K., Matsumoto, H., Sato, E.: Expression of hyaluronan synthases and CD44 messenger RNAs in porcine cumulus-oocyte complexes during in vitro maturation. *Biol. Reprod.*, 66: 707-717, 2002
- 21) Yokoo, M., Miyahayashi, Y., Naganuma, T., Kimura, N., Sasada, H., Sato, E.: Identification of hyaluronic acid-binding proteins and their expressions in porcine cumulus-oocyte complexes during in vitro maturation. *Biol. Reprod.*, 67: 1165-1171, 2002
- 22) Jiang, J.Y., Macchiarelli, G., Tsang, B.K., Sato, E.: Capillary angiogenesis and degeneration in bovine ovarian antral follicles. *Reproduction*, 125: 211-223, 2003
- 23) Shimizu, T., Jiang, J.Y., Iijima, K., Miyabayashi, K., Ogawa, Y., Sasada, H., Sato, E.: Induction of follicular development by direct single injection of vascular endothelial growth factor gene fragments into the ovary of miniature gilts. *Biol. Reprod.*, 69: 1388-1393, 2003
- 24) Shimizu, T., Miyahayashi, Y., Yokoo, M., Hoshino, Y., Sasada, H., Sato, E.: Molecular cloning of porcine growth differentiation factor 9 (GDF-9) cDNA and its role in early folliculogenesis: direct ovarian injection of GDF-9 gene fragments promotes early folliculogenesis. *Reproduction*, 128: 537-543, 2004
- 25) Yokoo, M., Sato, E.: Cumulus-oocyte complex interactions during oocyte maturation. *Int. Rev. Cytol.*, 235: 251-291, 2004
- 26) Hoshino, Y., Sato, E.: Protein kinase B (PKB/Akt) is required for the completion of meiosis in mouse oocytes. *Dev. Biol.*, 314: 215-223, 2008
- 27) Tunjung, W.A.S., Yokoo, M., Miyake, Y., Hoshino, Y., Sato, E.: Effect of hyaluronan to inhibit caspase activation in porcine granulosa cells. *Biochem. Biophys. Res. Commun.*, 382: 160-164, 2009