(学位第３号様式)

学 位 論 文 要 旨

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題 目	Studies on involvement of the zona pellucida modifications during meiotic maturation of porcine oocytes in the sperm-egg interactions (ブタ卵母細胞の減数分裂時における透明帯変化の精子－卵子間相互作用への関与に関する研究)

It is generally accepted that not only nuclear and cytoplasmic maturation, but also zona pellucida (ZP) modifications during the maturation period are needed for the accomplishment of normal fertilization and developmental competence post-fertilization. However, there is no report regarding whether ZP modifications during meiotic maturation of porcine oocytes are involved in the interactions between the spermatozoa and ZP. Therefore, the present study was conducted to investigate the roles of *N*-glycosylation, sialylation and sulfation of ZP glycoproteins during meiotic maturation in sperm-ZP interactions.

The relative intensities of *N*-acetylglucosamine (GlcNAc) residues in ZP3 detected by lectin-blotting with WGA and S-WGA were increased with the advance of oocyte maturation. These progressive increases of GlcNAc residues elicited by *N*-glycosylation were significantly inhibited in oocytes treated with tunicamycin during IVM, and the incidences of sperm penetration, polyspermy and acrosome reacting (AR) sperm bound to ZP, and the number of sperm binding to ZP were strongly decreased by treatment with tunicamycin. The present study became clear that the increase in terminal GlcNAc residues in ZP glycoproteins through new *N*-glycosylation during the first 24 and 36 h of IVM culture periods in cumulus oocyte complexes (COCs) and denuded oocytes (DOs), respectively, played a critical role in sperm-ZP interactions. Therefore, these results indicate that new *N*-glycosylation of ZP glycoproteins during porcine oocyte maturation was indispensible for sperm-ZP interactions, and suggest that the cumulus cells are partly involved in this *N*-glycosylation, because the longer culture period in the absence of tunicamycin after the onset of IVM culture periods was needed to obtain the sperm penetration at the same levels of untreated oocytes in DOs rather than COCs.

The occurrence of ZP acidification in accordance with sialylation and sulfation of ZP glycoproteins was proved during oocyte maturation, as evidenced by two-dimensional (2D) gel electrophoresis and SSA-lectin blotting. The desialylation and the blocking sulfation by treatment with neuraminidase and $NaClO_3$, respectively, were strongly inhibited ZP acidification, resulting in decreases of the number of sperm bound to ZPs and the percentage of AR in sperm bound to ZPs. The time course of sialylation and sulfation in ZP glycoproteins was correlated with the induction of germinal vesicle breakdown during oocyte maturation, and the cumulus cells during oocyte maturation were not essential in these acidification changes of ZP glycoproteins responsible for sperm-ZP interactions. Interestingly, although the rates of sperm penetration and polyspermy were significantly decreased in desialylated oocytes, the blockage of ZP sulfation by $NaClO_3$ treatment during IVM markedly abolished the incidence of polyspermy with no inhibitory effect on sperm penetration. Moreover, *N*-glycosylation, sialylation and sulfation of ZP glycoproteins during oocyte maturation were not associated with a protective proteolytic modification of the ZP matrix before fertilization.

In conclusion, it is clear from the present study that porcine oocytes during meiotic maturation undergo ZP modifications closely correlated with sperm-ZP interactions, and these findings are the first to demonstrate that the ZP modifications such as *N*-glycosylation, sialylation and sulfation of ZP glycoproteins contribute to ZP acquiring the capacity to accept sperm.