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Sperm Injection into Maturing and Activated Porcine Oocytes

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Chromatin configuration and microtubule assembly were determined in porcine maturing and activated oocytes following intracytoplasmic sperm injection. Microtubule localization was confirmed using a mouse monoclonal antibody to α-tubulin and detected using a fluorescent labeled goat anti-mouse secondary antibody. DNA was stained with propidium iodide. The image of microtubules and chromatin was captured using laser scanning confocal microscope. In germinal vesicle stage oocyte, sperm chromatin remained condensation and sperm derived microtubules were not observed at 8 to 12 h after sperm injection. At 24 h after injection, the sperm nucleus developed to the metaphase chromatin along the metaphase structure of female nucleus. In some metaphase I stage oocytes, sperm chromatin decondensed at 8 h to 12 h after injection, sperm aster was seen soon after sperm injection. At 24 h after sperm injection into metaphase I stage oocyte, male chromatin developed to the metaphase chromatin while female chromatin extruded first polar body and formed the metaphase chromatin. At 12 to 15 h after sperm injection into preactivated oocytes, condensed sperm nucleus was located in close proximity of female pronucleus. However, the condensed nucleus did not fuse with female pronucleus. In preactivated ocytes, injected sperm remained condensation, a few sperm organized small microtubular aster. Instead, maternal derived microtubules were organized near the female chromatin, which seem to move condensed male chromatin near to the female pronucleus. These results suggest that sperm nuclear decondensing activity and nucleation activity of centrosome during fertilization are cell cycle dependent. In absence of male functional centrosome, female origin centrosome takes over the role of microtubule nucleation for nuclear movement.

(Key words) ***Sperm injection, Microtubules, Centrosome***