

# Role of Reactive Oxygen and Nitrogen Species during Meiotic Resumption from Diplotene Arrest in Rat Oocytes

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**Abstract**—Hydrogen peroxide ( $H_2O_2$ ) and Nitric oxide both acts as a signaling molecule and modulates various aspects of cell functions in a wide variety of cells including mammalian germ cells. We examined whether a decreased level of intra-oocyte cyclic<sup>3</sup>, 5'-adenosine monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP), leads to accumulation of  $H_2O_2$  and depletion of NO, if so, whether a moderate increase of  $H_2O_2$  and decrease in NO inactivates maturation promoting factor (MPF) during spontaneous resumption of meiosis in rat oocytes cultured in vitro. Removal of cumulus cells and culture of denuded oocytes in vitro significantly decreased oocyte cAMP and cGMP level led to spontaneous meiotic resumption from diplotene arrest. The reduced oocyte cAMP level was associated with an increased oocyte  $H_2O_2$  level and reduced catalase activity and reduced oocyte. The cGMP level was associated with a decreased oocyte NO level. Exogenous supplementation of  $H_2O_2$  induced meiotic resumption from diplotene arrest. Supplementation of -nitroso-l-acetyl penicillamine (SNAP; an NO donor) inhibited meiotic resumption and aminoguanidine (AG; an inducible NOS [iNOS] inhibitor) induced meiotic resumption in a concentration- and time-dependent manner. The increased intra-oocyte  $H_2O_2$  and decreased NO level induced Thr-14/Tyr-15 phosphorylation of CDK1, while Thr-161 phosphorylated CDK1 and cyclin B1 levels were reduced significantly. These results suggest that a decreased level of intra-oocyte cAMP is associated with an increased level of  $H_2O_2$ . The increased level of  $H_2O_2$  was associated with high phosphorylation of Thr-14/Tyr-15 and dephosphorylation of the Thr-161 residue of CDK1 and reduced the cyclin B1 level. The inhibition of iNOS expression leads to a decrease of NO and cGMP levels thereby decreasing Cdc25B level. The reduced CDC25 B level leads to accumulation of Thr-14/Tyr-15 phosphorylated CDK1 level which eventually inactivated MPF. The inactive MPF finally induced meiotic resumption from diplotene stage in rat oocytes cultured in vitro

**Keywords:** ROS, RNS, meiotic resumption from diplotene arrest, rat oocytes.