Dynamics of DNA methylation levels in maternal and paternal rabbit genomes after fertilization

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oral n°18 p 14

Program & Abstracts

22nd Wilhelm Bernhard Workshop

August 25 - 29, 2011

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DYNAMICS OF DNA METHYLATION LEVELS IN MATERNAL AND PATERNAL RABBIT GENOMES AFTER FERTILIZATION

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The reprogramming of DNA methylation in early embryos has been considered to be essential for the reprogramming to totipotency, the embryonic genome activation (EGA) and subsequent development. However, its degree appears to differ as a function of species and it may be altered by the in vitro environment. While the rabbit is a pertinent model for species with a delayed EGA because both in vivo and in vitro developed embryos are easily available, the status of DNA methylation levels in both parental genomes after fertilization remains controversial. In order to generate precise data on the DNA methylation status in rabbit zygotes, we first of all defined five pronuclear (PN) stages during the first cell cycle, which we subsequently used to classify in vivo and in vitro developed rabbit zygotes. This allowed us to precisely quantify the methylated DNA during the one cell stage, as well as the total DNA content, which was used for normalization. We thus showed that both pronuclei display distinct patterns of DNA methylation reprogramming. In the maternal pronucleus (MP) the methylation level remains constant throughout the one cell stage, thanks to maintenance methylation during the S-phase. Conversely, in the paternal pronucleus (PP) partial demethylation occurs before replication, probably as a result of active DNA demethylation, while maintenance methylation subsequently takes place during the S-phase. Interestingly, we showed that PP DNA methylation reprogramming is partially altered by the in vitro environment. Taken together, our approach evidences that rabbit is one of the species displaying partial DNA demethylation in the PP, and for the first time demonstrates maintenance methylation activity in both pronuclei during the first S phase.