Animal Reproduction

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Physiology of reproduction in male and semen technology

The fate of porcine sperm CRISP2 from the perinuclear theca before and after in vitro fertilization.

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In a previous study (Zhang et al., Biol Reprod 105:1160-1170; 2021) we reported that porcine sperm cysteine rich secretory protein 2 (CRISP2) is localized in the post-acrosomal sheath (PAS)-perinuclear theca (PT) as reduction-sensitive oligomers. In the current study, the decondensation and removal of CRISP2 was investigated during *in vitro* sperm capacitation, both after induction of the acrosome reaction and after *in vitro* fertilization. Confocal immunofluorescent imaging revealed that additional CRISP2 fluorescence appeared on the apical ridge and on the equatorial segment (EqS) of the sperm head following capacitation, likely a result of the local de-oligomerization of CRISP2. After an ionophore A23187 induced acrosome reaction, CRISP2 immunofluorescence disappeared from the apical ridge and the EqS area partly due to the removal of the acrosomal shroud vesicles but also partly due to its presence in a subdomain of EqS (EqSS). The fate of sperm head CRISP2 was further examined post-fertilization. *In vitro* matured porcine oocytes were co-incubated with boar sperm cells for 6-8 h and the zygotes were processed for CRISP2 immunofluorescent staining. Notably, decondensation of CRISP2, and thus of the sperm PT, occurred while the sperm nucleus was still fully condensed. CRISP2 was no longer detectable in fertilized oocytes in which sperm nuclear decondensation and paternal pronucleus formation was apparent. These data indicate that PT decondensation and degradation may be executed in advance of sperm DNA decondensation post-fertilization. This rapid dispersal of CRISP2 in the PT is likely regulated by redox reactions for which its cysteine rich domain is sensitive. Reduction of disulfide bridges within CRISP2 oligomers may be instrumental for PT dispersal and PT elimination. These results raise important questions such as whether the dispersed PT proteins in the oocyte cytoplasm may be involved in oocyte activation, as well as in male nuclear chromatin decondensation in order to form the male pronucleus.

Keywords: CRISP2; porcine; sperm; perinuclear theca; decondensation; fertilization; oocyte.