CRISP (Cysteine Rich Secretory Proteins) as novel regulators of epididymal epithelium differentiation

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Epididymal CRISP1 and CRISP4 associate with the sperm surface during maturation and are key mediators of fertilization. Whereas single knockout (KO) males for these molecules showed *in vitro* sperm fertilizing defects but normal fertility, all double KO (DKO) animals for these proteins exhibited impaired *in vivo* fertilization and fertility. In addition, one third of DKO showed bigger testes and epididymides not observed in single KO. Based on this, in the present work we investigated the mechanisms underlining this DKO phenotype. Histological studies of DKO testes and epididymides showed that whereas mice with normal tissues (Group 1) were not different from controls, those with bigger organs (Group 2) had clear histological defects as well as an abnormal presence of immune cells in the interstitium and lumen. RT-qPCR for different immunomodulator molecules revealed higher levels of *Il-6* and *Il-10* and a downregulation of *Tgf*-β in DKO from Group 2 not observed in Group 1. Interestingly, immunofluorescence experiments using specific markers for each of the different epididymal epithelial cells revealed fewer and shorter basal cell projections in the initial segment known as axiopodia, defects in principal cells and clear cells with an immature phenotype in both groups. Accompanying these epithelial changes, males from both groups also exhibited an increase in intraluminal pH. Altogether, these observations support the relevance of CRISP proteins for male fertility through their involvement in epididymal epithelium differentiation and luminal acidification which are critical for sperm maturation and storage.