

DMRT1 is a Sex Determining Gene in Rabbits

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The gonad-specific transcription factor DMRT1 is considered as the Testis Determining Factor in many vertebrates. In mammals, *SRY* is the testis-determining gene, and according to the mouse species, DMRT1 has no longer function in testis determination since *Dmrt1* disruptions only affect post-natal testis differentiation and fertility. Nevertheless, human patients with *DMRT1* mutations exhibit 46, XY DSD with testicular dysgenesis, suggesting an earlier role for DMRT1 in non-rodent mammals. Thanks to CRISPR/Cas9 technology, our laboratory generated *DMRT1* mutant rabbits. We showed that the absence of DMRT1 leads to early male-to-female sex reversal in XY homozygous mutants that developed ovaries. Indeed, when observed a few days after sex determination, XY *DMRT1*^{-/-} gonads were devoid of testis cords structures and presented a typical ovarian organization with a thicker epithelium and ovigerous cords. Molecular studies confirmed the ovarian signature with an up-regulation of ovary-driving genes (*RSPO1*, *WNT4*, *FOXL2*, and *CYP19A1*) and downregulation of testis-specific genes (*SOX9*, *SOX10*, and *DHH*) in the developing XY *DMRT1*^{-/-} gonads (bulk RNA-sequencing and RT-qPCR data). Interestingly, although some SOX9 protein remained detectable in a few somatic cells, it was not sufficient to trigger its target gene expression (*AMH* and *DHH*, for example, were down-regulated, while *SRY* expression was highly maintained). Our data highlight the role of DMRT1 as a key player in sex determination in rabbits. DMRT1 is required for SRY action on *SOX9* gene expression and may also be necessary for SOX9 protein function on its target genes acting as a pioneer factor. Our investigations are currently focused on characterizing DMRT1 direct targets in the rabbit testis by ChIP-seq.