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Oral Presentation

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Starvation improves sheep fibroblast chromatin remodeling in spermatide-like structure

We have recently demonstrated that the chromatin of somatic cells can be converted into spermatid-like structures, by the transient expression of human protamine 1 gene Prm1. Here we have further advanced our protocol, by mimicking the nuclear remodelling taking place in spermatids. Since nuclear maturation in spermatids occurs in G0, our first aim was to test if protaminization of somatic nucleus increases when G0-stage fibroblasts are trasfected with Prm1 gene. Protamine deposition on DNA is anticipated by a genome-wide histone acethylation. Thus, our second aim was to induce a genome hyper-acethylation of G0 cells, by optimizing timing of exposure/concentration of Histone De-Acethylase Inhibitor (HDAI) Tricostatin A. Results: G0-stage fibroblasts cultured for 24h pre-transfection with medium containing 0.5% FBS showed an higher proportion of spermatid-like cells, compared to the control (CTR: 10% FBS) ($p<0.05$). Furthermore, Bromodeoxyuridine incorporation demonstrated that starved somatic cells were effectively in G0-stage during protaminization ($p<0.0001$). Finally, we have found a greater number of spermatid-like cells with TSA concentration between 25 and 50 nM, comparing to 100 nM ($p<0.05$). To conclude, we have demonstrated that G0 stage, and the open nuclear structure conferred by TSA resulted in a more efficient Prm1-mediated conversion of somatic nuclei into spermatid-like structures.