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Transgenerational analysis of embryonic heat exposure in Japanese quail

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Changes in gene activity induced by perinatal environmental challenges are known to impact the phenotype, health and disease risk of animals. The epigenome is an essential contributor to phenotypic plasticity, and learning how environmental exposures translate into persisting epigenetic changes may open new doors to improve the robustness and resilience of developing animals. In that respect, birds are species of choice to directly manipulate the embryonic environment with limited direct maternal influence. It was previously reported that the heat tolerance of male commercial chickens was improved by cyclically elevating the egg incubation temperature. This procedure named embryonic thermal manipulation (TM) was associated an enhanced gene response when animals were heat challenged at slaughter age, 35 days post-hatch (D35). Unpublished work of our team shows that TM is associated with two epigenetic marks changes in the hypothalamus of D35 chickens that may contribute to the molecular basis of TM-induced programming of gene expression.

To further explore the molecular mechanisms of heat acclimation, we took advantage of an inbred line of Japanese quails (*Coturnix japonica*) to investigate the transgenerational impact of TM on bird epigenome. Among other advantages, the quail short generation cycle is 3-4 time faster than the one of chicken and the use of an inbred genotype should help reducing phenotypic variations due to genetic variability. TM was transposed to quail by elevating the incubation temperature from 37.8°C to 39.5°C during 12 hours per day from the 12th hour of incubation until E13 (E0-13). TM affected the hatching rate and the survival during the first four weeks of life, the growth until 25 days of age and the surface temperature of the shank at D35. We also found that TM impacted some blood metabolites in interaction with sex at D35. The thermal response of TM animals was assessed by a heat challenge at D35 that had no impact on survival. Nevertheless, according to beak surface temperature and blood sodium levels, TM animals differentially responded to the heat challenge, in a sex dependant manner. To explore the molecular impacts of TM, a genome-wide study of gene expression by RNA-seq and of DNA methylation by whole genome bisulfite sequencing (WGBS) is currently ongoing on D35 hypothalamic tissues of TM and control animals.

TM was repeated on the progeny of TM animals to a total of 4 consecutive generations in order to evaluate the multigenerational impact of the treatment, in parallel to 4 generation of untreated animals crossed in a mirror manner as controls. In addition, we derived 2 generations of

control treatments from two consecutive generations of TM to assess the transgenerational impact of TM. A phenotypic characterisation including physiological, reproductive and behavioural measurements is currently underway at all generations and the first results will be presented.

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Key words: embryonic environment; thermal manipulation; Japanese quail; transgenerational; epigenetics