Animal Reproduction

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**Folliculogenesis, oogenesis and superovulation**

# Heat stress during mouse oocyte growth uncouples DNA methylation reprogramming from developmental competence

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## Resumo

Oocytes are susceptible to heat stress (HS) but its impact on epigenetic reprogramming remains elusive. Oocyte-specific DNA methylation occurs synchronously during oocyte growth in newborn mice, which allows exploring the effect of environmental challenges. We determined the impact of HS during oocyte growth on DNA methylation and developmental competence. Swiss mice with F0 litters were weighted on postnatal day 9 (P9) and randomly allocated to control (CTL) (21°C/24h) or HS (35°C/12h/light and 21°C/12h/dark) from P10 to weaning on P21. F0 females remained under 21ºC until puberty on P35. F0 females received 10 IU eCG to collect fully-grown (FG) oocytes 46h later for whole genome bisulfite sequencing (WGBS) or were subject to natural mating with fertile males. Superovulation of F0 females was with 10 IU eCG and 10 IU hCG 44-48h apart to collected mature oocytes 12-14h post-hCG for parthenogenetic activation (PA) or mated for *in vivo* embryo production and their collection was 94h post-hCG. PA was in calcium-free M16 medium with 10 mM $SrCl_2$, 5.0 µg/mL cytochalasin B, and 0.1 mg/mL PVA under 5% $CO_2$ at 37 ºC for 5h. Activated oocytes underwent *in vitro* culture in KSOMaa and embryonic development recorded at 24h (cleavage) and 96h (morulae + blastocysts) post-activation. Data was subject to ANOVA using general linear model of SAS. FG oocytes were subjected to next-generation sequencing of DNA converted by sodium bisulfite and differently methylated regions (DMRs) located by the DSS method. Functional analysis relied on g:Profiler. Exposure to HS did not affect survival of lactating females (P = 0.95) or offspring (P = 0.95). However, HS reduced body weight at P21 and weight gain for both lactating females (P = 0.0002) and offspring (P < 0.0001). Groups did not differ for mean number of ovulated oocytes (P = 0.86) and oocyte viability (P = 0.85). Exposure of F0 females to HS during oocyte growth did not affect cleavage rates (P = 0.12) after PA. However, HS tended to reduce (P= 0.06) embryonic development. F0 females has similar litter sizes after natural mating (P = 0.94) and *in vivo* embryo production (P = 0.35), which had similar percentage of viable embryos (P = 0.77). WGBS indicated more hypo-methylation in differentially methylated regions (DMRs) from HS oocyte than in the CTL counterparts. Enrichment analysis of DMRs found binding sites of transcription factors linked to HS response (Hsf4), genome activation (Klf4/6), trophectoderm development (Tfap2a/c), Wnt signaling/histone deacetylation (Kaiso), TGF-β signaling (Smad3/Rreb1), nuclear receptor signaling (Vdr), and uncharacterized factors (Pax4/5, Sall1). Nitric oxide and calcium signaling (Cacna1d-Kcnn4 complex) were novel processes affected by HS. In conclusion, HS during oocyte growth compromises genome-wide DNA methylation without affecting developmental competence. These results uncouple these biological processes and suggests that oogenesis tolerates substantial epigenetic noise.