Deciphering the contribution of non-genomic estrogen signaling in steroidogenesis in perinatal mouse testis

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Estrogens, and more specifically 17β-estradiol (E_2), play critical roles in male reproduction. E_2 action is in part driven by the estrogen receptor 1 (ESR1) that mediates both a nuclear signaling pathway and a cell membrane-associated non-genomic pathway. ESR1KO mice revealed the critical role of ESR1 for proper development of the male reproductive tract and regulation of perinatal testicular testosterone production. Another mouse model expressing only the nuclear form of ESR1 (Nuclear Only Estrogen Receptor, NOER) highlighted the importance of the non-genomic pathway in the male reproductive tract. However, the contribution of the non-genomic (or membrane) pathway in fetal Leydig cells and perinatal testosterone secretion remained unexplored. Since Leydig cells are hypertrophied in 2-day old ESR1KO testes, we first examined fetal Leydig cell morphology in 2-day old NOER testis. Surface measurement of fetal Leydig cells after 3βHSD immunostaining did not show any difference between NOER and wild-type (WT) testes (n=3), suggesting that the effect previously observed in ESR1KO was due to the genomic pathway. Next, total RNA was extracted from 2-day old testis collected from 3 WT, 3 NOER, and 2 ESR1KO mice, and used for polyA RNA-Seq. Analysis revealed 11 differentially expressed genes (DEG) in NOER (4 down and 7 up; FDR<0.05; FC2) and 192 DEG in ESR1KO (99 down and 93 up) when compared to WT. *Hmga1* and *Cyp17a1* were the only two common DEG between NOER and ERKO. Compared to WT mice, *Hmga1* mRNA levels were significantly increased in ESR1KO while in NOER animals *Hmga1* mRNA levels were decreased below WT levels. This indicates that Hmga1 expression is antagonistically regulated by the genomic pathway but positively by the non-genomic pathway. *Cyp17a1* mRNA levels were increased in both NOER and ESR1KO indicating that expression of this gene is controlled mainly by the non-genomic pathway. Targeted analysis of additional genes important for Leydig cell function revealed a similar increase in *Star*, *Hsb3b1, Cyp11a1*, and *Insl3* in both ESR1KO and NOER suggesting a regulation by the non-genomic pathway. These data provide a better understanding of the regulation of perinatal testicular steroidogenesis by estrogens and reveal new roles for the cell membrane-associated non-genomic ESR1 pathway.