**Epimutations – Where Do They Come From And Where Do They Go?**

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Epimutations are heritable defects in epigenetic programming that do not involve changes in the underlying DNA sequence and may or may not impact gene expression. Epimutations can occur naturally, but are more likely to be induced by environmental factors that disrupt the normal epigenome in a particular cell type. Previous studies have shown that the use of assisted reproductive technologies (ART) can induce epimutations in offspring produced by these methods. Our study was designed to chronicle the occurrence of epimtuations in mice produced by somatic cell nuclear transfer (SCNT, = cloning), or by intracytoplasmic sperm injection (ICSI = a type of ART), and to determine if epimutations incurred in one generation of mice are transmitted to subsequent generations. We analyzed allele-specific DNA methylation and expression at three imprinted genes, *H19*, *Snrpn* and *Peg3*, in somatic cells from juvenile and adult mice generated by SCNT, ICSI or natural reproduction. Surprisingly, we detected a greater incidence of epimutations in mice produced by ICSI than in those produced by SCNT. No epimutations were detected in naturally conceived mice. We then allowed the ICSI mice carrying epimutations to reproduce naturally and found no epimutations in the offspring, indicating that epimutations induced in ICSI mice are typically not transmitted transgenerationally. We examined germ cells from the ICSI mice and found that the epimutations present in the somatic cells had been corrected by germline-specific epigenetic reprogramming, although the reprogramming process is delayed in mice produced by ICSI.

The greater incidence of epimutations in ICSI mice than in SCNT mice provided a unique insight into one potential contributing effect of the ICSI process, because oocyte genomes that had been exposed to gonadotropin stimulation (superovulation) were retained during the ICSI process whereas they were removed during the SCNT process and replaced with somatic cell nuclei that had not been exposed to gonadotropin stimulation. To test the hypothesis that gonadotropin stimulation contributes to the occurrence of epimutations, we produced mice by natural reproduction using females that had been subjected to gonadotropin stimulation and found that many of the offspring carried epimutations in somatic tissues and also showed delayed reprogramming in the germ line.

Taken together, our results indicate that aspects of the ART process, including gonadotropin stimulation, can induce or predispose the occurrence of epimutations in offspring, but that while such epimutations are likely to persist in somatic tissues of these offspring, they are typically corrected by epigenetic reprogramming in the germ line and therefore not transmitted to subsequent generations.