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Reprogramming of genomic imprints by *in vitro* culture and cloning procedures in cattle

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ABSTRACT

***Background*:** Procedures for cloning cattle by somatic cell nuclear transfer (SCNT) have been widely used in cattle due to commercial interests in traditional animal breeding and to produce transgenic animals for the pharmaceutical industry. Even though cloning has proven to be more efficient in cattle than other species studied, certain problems are common, such as low efficiency, pregnancy failure and gross placental structural and functional abnormalities. Most of the developmental abnormalities observed in cloned animals are related to the proliferation of the fetus and placenta, a phenomenon known as "large offspring syndrome (LOS) in ruminants. It has been hypothesized that the epigenetic control of imprinted genes, *i.e.* genes that are expressed in a parental-specific manner, is at the root of LOS.

***Review*:** Our recent research has focused on understanding the epigenetic alterations associated with different technologies used for assisted reproduction. We compared samples from naturally derived individuals produced by artificial insemination (AI) to samples of individuals obtained from *in vitro* embryo culture (IVC) and SCNT. In order to perform parental-specific analysis of genetic imprinting we identified single nucleotide polymorphisms in DNA of *Bos indicus* that were used for analysis of parental alleles and their respective transcripts in the tissues of hybrid individuals obtained by crossing *Bos indicus* and *Bos taurus*. To investigate the epigenetic abnormalities, we performed bisulfate sequencing to analyze the differentially methylated domains (DMD) of a set of genes that are subjected to genomic imprinting in cattle, that is to say, SNRPN, H19 and IGF2R. Since the expression of imprinted genes varies significantly in different tissues during development, we examined embryonic and extra-embryonic membranes at different periods, i.e. preimplantation (day 17), post-implantation (day 40- 60), and after birth. For SNRPN, day 40 fetuses in the IVF group showed significantly less methylation when compared to the AI group and SNRPN expression was mostly paternal in all fetal tissues studied, except in placenta. However, the SCNT group presented severe loss of DMR methylation in both day 17 embryos and 40 fetuses and biallelic expression was observed in all stages and tissues analyzed. For H19, biallelic expression was tightly associated with a severe demethylation of the paternal *H19* DMD in SCNT embryos, suggesting that these epigenetic anomalies to the *H19* locus could be directly responsible for the reduced size and low implantation rates of cloned. Preliminary analysis of the paternally imprinted IGF2R gene, indicates that, although the methylation patterns of the DMD are reduced in the SCNT day-17 vesicles, expression is consistently bi-allelic, regardless of whether the embryos were derived *in vivo*, *in vitro* or by SCNT.

***Conclusion*:** Therefore, these results indicate a generalized hypomethylation of DMD in all three imprinted genes analyzed which, with the exception of IGF2R, seems to lead to the bi-allelic expression of individuals produced by SCNT. Together, these results suggest that epigenetic marks of imprinted genes are erased during the reprogramming of somatic cell nuclei during development, indicating that such epigenetic defects may play a key role in mortality and morbidity of cloned animals.

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