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3 Cases of microsatellite mutation in Somatic Cell Nuclear Transfer cloned cattle

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SCNT cloned animals frequently exhibit developmental abnormalities before or at birth and those that survive to adulthood often display phenotypical variations. Such abnormalities or variations suggest that cloned animals may not share exactly the same genome as a consequence of genetic or epigenetic defects or deregulations associated with the reprogramming of nuclear activities or with *in vitro* culture steps. Somatic cell nuclear transfer (SCNT) animals derived from the same donor genome are generally considered to be genetically identical. However in depth investigation of the genetic identity between clones derived from the same donor genome remains poorly documented. Here, we have applied a modified protocol of RDA (*Representational Difference Analysis*) subtractive technique using Bam HI enzyme in order to reveal possible genetic differences between 10 SCNT Holstein cows of the same genotype including 5 living adult cows and 5 perinatal aborted fetuses, all displaying normal caryotypes. No sequence polymorphism was detected from 129 unique sequences identified after RDA subtractions between these cloned cows. No single nucleotide polymorphism was detected neither after hybridization on a 54K SNP bovine array. However, when using a panel of 16 microsatellites, 2 monoallelic length polymorphisms affecting 2 different markers (ETH 10, INRA 135) were evidenced in one perinatal aborted fetus. When such microsatellite genotyping was extended to 25 other SCNT cloned cows from 4 different genotypes, a third case of microsatellite length polymorphism in one healthy adult cloned cow (INRA 23 marker) was detected. Considering that microsatellite mutation rate is about 10^{-4} in Holstein breed, our data suggest that the observed mutations incidence is related to the SCNT procedure. Microsatellites are known to induce genomic instability through sequence or length modification particularly in centromeres. The present data together with our recent demonstration that adult cloned cows are epigenome variants (de Montera et al. Reproduction, Fertility and Development, 2008, 21(1): 114) provides new insights to the interplay of genomic instability and global DNA methylation variability.

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