Is a Clone an Exact Duplicate?

By Lee A. Bulla Jr., PhD

Editor's Note: This is a guest editorial that arrived at Quarter Horse News just as this issue was headed out the door to the printer. Dr. Lee A. Bulla desired to have this published prior to the 2010 AQHA Convention, which is scheduled for March 5-8 in Kissimmee, Fla.

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Is a clone an exact duplicate of the original? The answer is no. Indeed, there are several reasons why a clone is not an exact replica. Before addressing the reasons, let's consider first the natural process of sexual reproduction. Reproduction begins at fertilization, when gametes (sperm and egg) unite to form a single-celled zygote that divides repeatedly and eventually gives rise to an adult body with trillions of cells. The zygote contains genes (DNA) derived from both the mother and the father, and provides all the genetic information necessary to form a new individual. Developmentally, a zygote produces a ball of cells called an embryo, which develops into a fetus and, ultimately, into a newborn individual.

The profound consequences of natural reproduction are the generation of genetic diversity, which is essential to the survival of a species. Genetic diversity means that a population of individuals contains most of the possible variations or alternative forms of genes, called alleles, somewhat evenly distributed throughout the population. The alleles, or changes in the DNA sequence, are the result of mutations.

For certain genes in animals such as horses, parental origin influences the characteristic traits that can be seen in the offspring. These genes are said to be imprinted. In the process of imprinting, certain chemical constituents, i.e., methyl groups, cover a gene or several linked genes and prevent them from being activated or expressed. For a particular imprinted gene, the copy inherited from either the sire or the dam is always covered with these methyl groups. The result of this gene cover-up is that certain features or traits may be modified, sometimes adversely, depending on which parent transmitted the allele. In other words, a particular gene may function if it came from the sire, but not if it came from the dam, or vice versa.

In embryos resulting from natural fertilization, the extent of cover-up (methylation) in the male gamete is reduced (demethylation) within a few hours upon fertilization whereas in the female gamete, the cover-up is reduced more gradually until the embryo reaches the morula (16-cell) stage. At this point, cells of the morula differentiate into blastomeres, some of which become the inner cell mass. Other blastomeres are flattened on the surface and become the trophoblast. The embryo ultimately is implanted in the uterus. Methylation patterns are set in the inner cell mass from this point onward while the trophectoderm (the cell layer from which the trophoblast differentiates) remains hypomethylated and progresses toward formation of the placenta.

Interestingly, embryos resulting from cloning initiate demethylation but, unlike normal embryos, demethylation after the two-cell stage is incomplete if not lacking altogether. Furthermore, cells of cloned embryos are methylated earlier than usual, starting at the four- or eight-cell stages. As a result, the outer cell layer of the trophoblast, which eventually must be hypomethylated, does not have adequate time to undergo complete demethylation, leading to an unusual state of hypomethylation. By the time the embryo has reached the 32- or 64-cell stage, there is little difference between the trophoderm and the inner cell mass with respect to methylation. Hence, disturbance in the normal timing of events results in potentially grave changes in epigenetic reprogramming and in subsequent development of the extra-embryonic lineage, which consequently can be detrimental to the development of the cloned embryo.

So, why is a clone not an exact duplicate? One obvious answer lies in the capacity of certain nuclear genes transferred by somatic cell nuclear transfer (SCNT) to be normally activated or expressed. In normal development, for some genes, one copy is turned off, depending upon which parent transmits it. That is, some genes must be inherited from either the dam or the sire to become active — genomic imprinting. Imprinting, as described above, chemically marks the DNA from the sire and the dam so that only one copy of a gene (either the maternal or paternal gene) is turned on. In SCNT, genes in a donor nucleus skip passing through a sperm or egg, and thus are not imprinted. Loss or disruption of imprinting renders a "cloned" embryo not identical to the animal from which the donor cell was obtained and, oftentimes, leads to abnormalities and disorders resulting from changes in the normal activation and expression of certain embryonic genes. Regulation of gene expression is abnormal at many times during prenatal development. Although the SCNT technology is rather straightforward, the end-product of such manipulation, a cloned cell, has complications.

There are other reasons why a clone is not a precise replica. In some species, the tips of chromosomes, called telomeres, become shorter with aging. Generally, older horses have shorter telomeres and decreased cellular immune responses to help protect them from disease. Premature aging, seen in shortened telomeres, may be why the first cloned mammal, Dolly, died early of a severe respiratory infection.

DNA from a donor cell has had considerable time to accumulate mutations. Such mutations might not be noticeable in one of millions of somatic cells (body cells), but they could be devastating if a nucleus containing such mutations is used to program development of a new individual.

At a certain time in early prenatal development in female mammals, one X chromosome is inactivated. Whether that X chromosome is from the dam or the sire occurs at random in each cell, creating an overall mosaic pattern of expression for genes on the X chromosome. The pattern of X inactivation of a female clone probably would not match that of her donor cell because X inactivation occurs in the embryo, not in the zygote.

Mitochondria (energy centers in a cell) contain genes. A clone’s mitochondria descend from the recipient egg, not from the donor cell because they are in the cytoplasm and not in the nucleus.

The environment is another powerful factor in why a clone is not an identical copy. For example, coat color patterns differ in Smart Little Lena clones. When the animals were embryos, cells destined to produce pigment moved in a unique way in each individual, producing different color patterns.

It is important to understand that for all animals, experience, nutrition, stress, exposure to infectious disease and many other factors join their genes in molding them as individuals. And all individuals, cloned or otherwise, pass on whatever genetic instructions they have to their progeny.