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Subject: Is a Clone an Exact Duplicate?
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I appreciate the comments of Dr. Hinrichs pertaining to some points I tried to make in my recent article in the Quarter Horse News. I thought that it might be instructive to provide further commentary about the points she makes. Therefore, I offer the following comments in no particular order as discussed by Dr. Hinrich.

As Dr. Hinrich indicated, I also do not know of any work done on the methylation status of cloned equine embryos but there is some information available in other species and I draw my conclusions based on those published results. In my opinion, there is no reason to believe that methylation in the horse will be dramatically different from that in other mammals including human.

It is important to note that genomic imprinting refers to a special kind of epigenetic variation which involves heritable changes in the expression of genes. Epigenetic modifications themselves are brought about not only by DNA methylation but by chromatin remodeling, RNA interference and DNA acetylation as well. Of these four mechanisms, the most widely studied and best understood is methylation. However, none of the mechanisms have been delineated and what impact impairment of these mechanisms has on embryonic development remains to be realized. Significantly, DNA methylation is only part of the whole picture.

Most imprinted genes studied so far are involved in placental and embryonic development and fetal growth. Imprinted genes are established as sex-specific patterns in the germline during gametogenesis and usually are clustered. Once a gene is methylated and imprinted in the germline, it remains transcriptionally inactive during embryogenesis and development. Due to the existence of only one functional allele, imprinted genes are highly susceptible to lethal mutations and, because these genes are in close proximity, they often are controlled together. As such, a single mutational change can disrupt the functioning of many genes and the consequences can be deleterious. Lethality may be due either to changes in the DNA sequence of the only active allele of an imprinted gene or to an "epimutation" that leads to a heritable change in the gene without altering its DNA sequence. Such epimutations can alter methylation patterns in imprinted genes rendering an inactive allele active or vice versa. Genomic imprinting and its loss can influence the precise functioning of a variety of gene networks, making it an extremely vital epigenetic mechanism in mammalian growth and development.

Disruption or loss of imprinting has been identified as a causative agent in approximately 30 known human diseases or disorders. The impact of genomic imprinting is manifested when an individual has one copy of a normally imprinted allele and the other active allele is either inactivated or deleted. Two striking examples of the loss of imprinting in human are the Prader-

Willi syndrome and the Angelman syndrome.

I do not disagree with Dr. Seidel's point that during gametogenesis, all early imprints are erased and re-established based on the sex of the individual. However, such is not necessarily the case in the original cloned embryo. My only point is that the clone will not be an exact replica of the original. What happens in subsequent generations obviously would follow the basic principles of hereditary genetics. What the outcome will be several generations removed, no one knows. Until there is a sufficient body of knowledge available to answer some important and relevant questions, producing clones for breeding purposes or for performance is playing genetic roulette.

Resetting telomeres in cloned embryos is a critical cellular exercise. I assume that the comment "different donor cell types appear to have different abilities for this" refers to reestablishing the original telomere length. As we know, a telomere is a repeating DNA sequence (for example, TTAGGG) at the end of a cell's chromosomes. Telomeres function by preventing chromosomes from losing base pair sequences at their ends. They also stop chromosomes from fusing to each other. However, each time a cell divides, some of the telomere is lost. When the telomere becomes too short, the chromosome reaches a "critical length" and can no longer replicate. It is well established that critically short telomeres cease to function as protective units and cause the cell to die or to arrest permanently.

The minimal functional telomere length, and whether this length varies among cell types (mammary gland cells and fibroblasts, among others), has not been clearly defined. But even in senescent human cells, telomeric double-stranded repeats are readily detectable, suggesting that several kilobases of TTAGGG repeats are required at all times. Similarly, when the telomere-protection factor TRF2 is over-expressed in telomerase-negative primary human fibroblasts, telomere-shortening rates almost double, and cells enter senescence with considerably shorter telomeres than control populations, indicating that telomere structure, not telomere length, is the main determinant of functional telomeres. Bottom line is that telomeres are highly specialized and regulated complexes in which both length and structure determine integrity and function. A cell not only needs to monitor telomere length but also needs to control the adoption of a functional telomere structure. Whether these activities are healthy normal endeavors in a cloned embryo has not been determined.

Again, I appreciate the comments of Drs. Hinrichs and Seidel and hope that we all will be able to continue a constructive dialog on a matter of such importance to the Quarter Horse industry.

Respectfully,

Lee Bulla