

Review

Genetic and Epigenetic Aspects of Cloning and Potential Effects on Offspring of Cloned Mammals

LAWRENCE C. SMITH and BRUCE D. MURPHY

ABSTRACT

Although the biological mechanisms by which host cytoplasm and donor nuclei interact to produce a developmentally competent reconstructed embryo remain largely unknown, some advances have been made to our understanding of the genetic and epigenetic factors involved in the of reprogramming of the donor nucleus. Genetic alterations, which comprise changes to the genetic information in both the nuclear and cytoplasm compartments, are passed on to subsequent generations at fertilization and are a potential source of variation among cloned animals and their offspring. Apart from the major chromosomal anomalies found in developmentally arrested embryos and fetuses, less detrimental rearrangements and/or mutations are likely to go unnoticed in most donor cell karyotypes, suggesting that such problems could lead to inheritable anomalies among clones and their offspring. Mitochondrial DNA is also relevant to cloning because most animals inherit most or all of their mitochondria from the host oocyte. Epigenetic alterations to the DNA or to the histone packaging proteins are independent of gene sequences. Aberrant epigenetic events may lead to variable gene expression or mitosis and consequent effects on development and phenotype. Although much of the epigenetic marking is reset during embryogenesis and development, the impact of epimutations on progeny remains unexplored.

INTRODUCTION

SINCE THE BIRTH OF DOLLY, animal cloning has been successfully applied to many mammalian species, including cattle, mice, pigs, goats, cats, rabbits, horses, and rats. With a few modifications to allow for species variability, the technique applied has been to introduce nuclei from partially or terminally differentiated cells into the cytoplasm of enucleated eggs, which results in the host cytoplasm "reprogramming" the nucleus

back to a de-differentiated state. In spite of this remarkable achievement, the vast majority of embryos reconstructed by nuclear transfer either die before birth or produce unhealthy offspring, suggesting that a normal developmental outcome is more of an exception than a rule. Elements of the cloning process have been blamed for alterations to the normal developmental pathway of cloned embryos. First, donor somatic cells may be developmentally compromised even before their use for nuclear transfer. Second, the host cyto-

Centre de Recherche en Reproduction Animale, Faculté de Médecine Vétérinaire, Université de Montréal, Saint-Hyacinthe, QC, Canada.

plasm to which nuclei are transferred may not be sufficiently "mature" to undertake the task of reorganizing the developmental pathway of somatic nuclei. Finally, once the nucleus and cytoplasm are united, the *in vitro* handling of reconstructed embryos may be inadequate, leading to immediate and/or long-term consequences to fetal morbidity and mortality. Although the biological mechanisms by which host cytoplasm and donor nuclei interact to enable the production of a developmentally competent reconstructed embryo remain largely unknown, advances have been made to our understanding of factors involved in the genetic and epigenetic reprogramming of the donor nucleus. Many of these have been addressed in recent reviews (Jeanisch et al., 2002; Shi et al., 2003; Vignon et al., 2002). Our intention in this review is to highlight some of the genetic and epigenetic aspects of the technology that have the potential to lead to problems with cloned animals and their offspring.

ALTERATIONS TO THE NUCLEAR DNA SEQUENCE

A standard procedure used to confirm the genetic identity of cloned animals is to perform DNA analysis with a number of known microsatellite markers. To a limited extent, microsatellite analyses also confirms that the DNA content of the cloned animals was not modified in the loci analyzed, as it demonstrates that nuclear transfer does not modify the DNA sequence of donor cells. From information collected to date, it is reasonable to assume that cloned animals are genetically identical among themselves and to the original animal from which donor cells were obtained for nuclear transfer. However, more adequate molecular biology methods could enable a better assessment of whether minor rearrangements or point mutations are present in somatic tissue derived donor cells or result from their inappropriate or prolonged *in vitro* culture in preparation for nuclear transfer. Since the sequencing of large portions of the DNA of clones may be technically unrealistic at this time, other methods such as Amplification Fragment Length Polymorphism (ASLP) analysis could enable a more detailed analysis of larger fractions of the genome of cloned animals (de Montera et al., 2003).

More drastic modification of DNA content in

clones may include alterations caused by chromosome segregation errors or fragmentation resulting from improper nucleo-cytoplasmic interactions due to cell cycle incompatibilities. The importance of synchronizing the cell cycle stage of nuclear donor cells to that of the host cytoplasm has been widely accepted as one of the major causes of failure after nuclear transfer (Campbell et al., 1993; Smith et al., 1988; Szöllösi et al., 1980). It is possible that donor cells may accumulate a wide variety of chromosomal abnormalities due to improper *in vitro* culture environments or to prolonged culture (senescence) prior to their use as donor nuclei. Karyotypic errors are commonly observed during early embryogenesis in traditionally derived and *in vitro*-derived cattle embryos, and have been associated with reduced development and survival (King et al., 1995). Nonetheless, nuclear transfer-derived embryos display higher than usual levels of aneuploidy when compared to embryos derived by *in vitro* fertilization (Bureau et al., 2003). Bureau et al. (2003) demonstrated that cloned embryos derived from granulosa cell nuclei carrying high levels of aneusomy produced significantly more embryos with chromosomal anomalies than those produced by transfer of a male fibroblast primary cell line carrying few chromosomal anomalies, which produced embryos carrying fewer defects. Indeed, all gestations derived from the above granulosa cells were lost during the first trimester, while the fibroblast cells produced several gestations to term, suggesting that donor-derived chromosomal anomalies can substantially affect the outcome of cloning. The contrasting view, that the origin of the alterations in ploidy distribution between clones may be independent of donor cells carrying few chromosome anomalies, has been presented based on an inability to associate donor-derived karyotype errors with embryo development outcome (Booth et al., 2003).

The concept that cloning results in telomere shortening has been highly controversial due to contrasting findings in different species and different laboratories (Shiels and Jardine, 2003). Calves derived from fetal and adult somatic cells have shown longer, equivalent and shorter length telomeres when compared to age-matched controls (Betts et al., 2001; Lanza et al., 2000; Miyashita et al., 2002). Donor cell tissue origin, age of the donor animal and donor cell *in vitro* culture length may be some of the many factors contributing to

the variation in telomere aggregates found in cloned offspring. The decline in telomerase activity in cattle embryonic stem-like cells and the resulting telomeric shortening after long-term culture indicate that culture of donor cells may significantly affect the developmental outcome after nuclear transfer (Betts et al., 2001). Nonetheless, offspring of clones seem to have telomeres of normal length in both somatic and germ cells, indicating that telomere length is reversed to normal levels in the progeny (Miyashita et al., 2003).

ALTERATIONS TO THE MITOCHONDRIAL DNA

Mitochondria are the only cytoplasmic organelles in animals known to carry their own DNA. The mitochondrial genome is composed of double-stranded circular DNA (mtDNA) of approximately 16 kb, which encodes for 13 mRNAs, 22 tRNAs, and two rRNAs. It also contains a highly polymorphic non-coding control region (d-loop) of approximately 1 kb, which is responsible for controlling both mtDNA replication and transcription through interactions with nuclear-encoded transcription factors. Therefore, for mitochondria to perform their task in providing energy for most cell functions they rely heavily on the nucleus to maintain mtDNA copy numbers and transcripts at appropriate levels within the cell.

Since sperm mitochondria are degraded at early stages after fertilization, mtDNA is inherited exclusively through the female germ line and, therefore, retains homogeneity (homoplasmy) within a maternal lineage. Nonetheless, mtDNA is highly mutagenic and polymorphic haplotypes (heteroplasmy) can accumulate in somatic cells during the lifetime of animals, particularly in tissues with high energy requirements, i.e. neurons, muscle and sperm. Contrary to the case in sperm and most somatic tissues, female germ cells have very low metabolic activity and generate few of the reactive oxygen species that cause mtDNA mutations. This favors the transmission of unharmed mitochondrial haplotypes to each generation. Nonetheless, mtDNA heteroplasmy has been shown to occur occasionally in some maternal lineages in cattle usually accompanied by a rapid return to homoplasmy (Ashley et al., 1989; Koehler et al., 1991). The ability to

switch mitochondrial haplotypes within a few or even a single generation has given rise to the mtDNA bottleneck hypothesis, which states that there is stringent segregation of mitochondrial haplotypes during oogenesis. Heteroplasmy can also occasionally involve a mitochondrial haplotype carrying a pathological mutation, as has been reported for many mitochondrial diseases in humans (Wallace and Lott, 2003). In the presence of heteroplasmy, the ratio of wild type (healthy) to mutated (pathological) mtDNA determines the severity of the disease. Therefore, mtDNA heteroplasmy appears to cause phenotypic problems only when (1) one of the mitochondrial haplotypes carries a mutation leading to oxidative phosphorylation failure and (2) the mutant haplotype is present beyond a particular threshold within the affected tissues.

Contrary to the destructive effect of the ooplasm on sperm mitochondria soon after fertilization, analysis of the mitochondrial genetic background of animals derived through the techniques of nuclear and cytoplasm transplantation has indicated that donor-derived mtDNA is not eliminated when injected into oocyte cytoplasm. Indeed, donor-derived mtDNA can be observed at all stages of embryonic and fetal development and in offspring after birth, indicating that mtDNA heteroplasmy commonly occurs in nuclear transfer-derived animals (Meirelles et al., 2001; Steinborn et al., 2000; Takeda et al., 2003). Nonetheless, levels of heteroplasmy vary considerably according to the amount of mtDNA present in the donor cell used for cloning; that is, transfer of somatic cells produces mostly low or undetectable levels of donor mtDNA, whereas blastomere-derived clones produce animals with high levels of mtDNA heteroplasmy. Moreover, ratios of donor:host mitochondria are altered during early embryogenesis, resulting in either morula/blastocyst stage embryo homoplasmy for the oocyte-derived mtDNA, i.e. the loss of all donor mtDNA, or to heteroplasmy, where embryos a large proportions of the mtDNA is donor-derived (Meirelles et al., 2001; Takeda et al., 2003). Increases in the proportion of donor mtDNA are often observed in pronuclear transfers, where large amounts of exogenous mtDNA are fused to the enucleated zygote, indicating that the nucleus preferentially replicates its own mtDNA (Meirelles and Smith, 1998). However, since mtDNA replication does not seem to occur before the blastocyst stage (Piko and Taylor,

1987), it is likely that reconstructed embryos change ratios by the elimination of host mtDNA and simultaneous replacement with donor mtDNA. Interestingly, second generation progeny from heteroplasmic females present stabilized mtDNA ratios, leading to a persistent transmission of constant levels of heteroplasmy (Meirelles and Smith, 1997). It is yet unclear whether persistent heteroplasmy occurs in the offspring of females derived from somatic cell nuclear transfer, as most animals analyzed to date are either homoplasmic for oocyte mitochondria or carry small proportions of donor cell-derived mtDNA. Nonetheless, because somatic cell mtDNA are more prone to mutations, it is possible that mutated haplotypes could become detrimental to the phenotypic outcome of heteroplasmic cloned animals and, in the case of females, also to their germline descendents.

EPIGENETIC ALTERATIONS TO DNA AND CHROMATIN

Epigenetic factors are defined as all processes relating to the expression of genes that are determined by events beyond the level of the genetic information. Several recent review papers have recently addressed the topic of epigenetic reprogramming of cloned offspring (Cezar, 2003; Dean et al., 2003; Vignon et al., 2002). With the exception of some potential nuclear and cytoplasmic genetic alterations, such as those discussed in the sections above, it is assumed that cloned animals are genetically identical. Therefore, it is expected that differences in gene expression profiles among and between clones are the result of epigenetic modifications. Indeed, patterns of gene transcription have been shown to differ substantially among clones, particularly in cloned embryos that fail to develop normal phenotypes. Two major epigenetic programs may be responsible for this transcriptional heterogeneity, variation in the status of covalent modification of the DNA packaging proteins, the histones, and variation in the pattern of methylation of the non-transcribed regions of DNA.

In somatic cells, the DNA is wound about nucleosomes, which are octamers of histone proteins, and the nucleosomes are connected by linker proteins. During spermatogenesis, the histones are replaced by protamines, only to be reinstated on the male pronucleus during early em-

bryogenesis (McLay and Clarke, 2003). The pattern of change in oocyte histones varies from sperm and somatic cells in less understood and more subtle ways. There is at least one oocyte-specific isoform of the linker histone, H1, expressed during oogenesis and early embryogenesis (Tanaka et al., 2003). It is not known to what extent these gamete-specific modifications of DNA packaging proteins occur in cloned embryos. Studies of histone dynamics in cloned animals revealed that removal of the somatic form of histone H1 from donor chromatin occurs shortly after nuclear transfer, within 6–16 h, and that the temporal pattern depends on the cell cycle stage of the donor cell and of the host oocyte (Bordignon et al., 1999). This first step of chromatin remodeling, involving the replacement of somatic by embryonic forms of linker histones, may be required to provide access to other, more subtle modifications to core histones.

Transcriptional activity is closely related to the compactness (structure) of the chromatin, which is largely dependent on components of the nucleosome such as the linker (H1) and core (H2A, H2B, H3, and H4) histones. A variety of covalent modifications to the amino-terminal ends, which extrude from the nucleosome core as a tail, have been shown to be involved in chromatin remodeling, including acetylation, methylation, phosphorylation, polyribosylation and ubiquitination (Fischle et al., 2003). Individual modifications can occur on a number of residues within each histone tail, and some can be subject to more than one modification. Acetylation is a transient state, reversed by deacetylases, and is usually associated with induction of transcriptional events. Phosphorylation can be seen both at interphase, associated with gene expression, and in compacted chromatin during the mitotic phase of the cell cycle (Prigent and Dimitrov, 2003). It is induced by kinases and reversed by phosphatases, in a transitory fashion. Methylation of histone residues is a more persistent state, and has frequently been associated with chromatin compaction and inhibition of transcription (Fischle et al., 2003). Recent evidence suggests that combinatorial patterns of covalent histone tail modification are associated with specific functional states.

Donor cells for nuclear transfer are expected to have patterns of histone modification compatible with their context relative to differentiation (e.g., granulosa cells vs. fibroblasts). It is possible that

the state of histone tail modification of donor cells alters the course of development of the reconstructed embryo, and little is known about histone reprogramming in the embryo. An overview suggests histones in the maternal genome are methylated relative to somatic cells (Reik et al., 2003), while the histones that replace protamines on the paternal gene become rapidly acetylated by mechanisms residing in the oocyte (McLay and Clarke, 2003). As noted above, these modifications have contrasting consequences on gene transcription. Indeed, the methylation of the lysine 9 (K9) residue of histone H3 leads to transcriptional repression, while acetylation is associated with activation. Somatic cell-derived cloned embryos display hypermethylated patterns of H3-K9 compared to fertilized controls (Santos et al., 2003). Moreover, levels of H3-K9 methylation in cloned embryos depend on the donor cell type and correlate closely with the proportion developing to the blastocyst stage. Phosphorylation of H3 at serine 10 is essential for transcription of a subset of genes in the interphase nucleus, and interference with the process at the G2 stage of the cell cycle prevents chromatin compaction and mitosis (Prigent and Dimitrov, 2003). The early transcriptional anomalies observed in cloned embryos demonstrate that the cytoplasm of oocytes is often unable to erase the epigenetic marking on somatic cell chromatin and to initiate a novel epigenetic program to guide the reconstructed zygote through the complex developmental pathways of embryo and fetal differentiation. As a consequence of these epigenetic errors, donor-specific genes may remain transcriptionally active and/or developmentally important genes may show abnormal spatial/temporal patterns of expression. Aberrant histone phosphorylation patterns result in aneuploidy of cultured cells (Ota et al., 2002), and may contribute to the well-known aneuploidic states in cloned embryos. Thus, these abnormalities, particularly those that affect the karyotype, have the potential to be transmitted to offspring. The extent to which this occurs is a fertile field for further investigation.

DNA methylation seems to be substantially altered in embryos and fetuses derived by somatic cell nuclear transfer, both in a genome-wide manner (Cezar et al., 2003; Kang et al., 2001) and in particular regions of the genome (Bourc'his et al., 2001; Ohgane et al., 2001; Shiota and Yanagimachi, 2002). Interclone variability in epigenetic

marks and the expression of developmentally important genes may derive in part from the manner in which donor cells are treated before nuclear transfer. For instance, culture conditions that restrict donor cell proliferation have been shown to alter both the imprinted gene expression pattern and the DNA methylation profile of differentially methylated regions (DMRs) of the insulin-like growth factor-2 (Igf2) and H19 genes in mouse embryonic stem cells (Baqir and Smith, 2003). Preliminary studies of cloned bovine embryos at day 17 after reconstruction demonstrate hypomethylation of the DMRs of the H19 and Snrpn genes, a phenomenon associated with biallelic, and thus, inappropriate, expression of both imprinted genes (unpublished data). Global screening of DNA methylation, as well as examination of DNA methylation in control regions of developmentally important genes indicates consistent abnormalities in the methylation patterns in cloned embryos.

It is yet unclear whether these and other epigenetic anomalies can persist in the germline and be transmitted to the offspring of healthy cloned animals. Following normal fertilization, there is rapid demethylation of the paternal genome on the male pronucleus, while the methylation pattern persists on the female component (McLay and Clarke, 2003; Reik et al., 2003). Wide scale demethylation of single-copy sequences occurs during early embryogenesis (McLay and Clarke, 2003), while imprinted gene methylation appears unaffected (Lane et al. 2003). In the mouse, there is demethylation of imprinted and non-imprinted sequences in primordial germ cells prior to sexual differentiation (Hajkova et al., 2002). This notwithstanding, there is evidence for resistance to epigenetic reprogramming during development, and consequent persistence of methylation of certain classes of DNA sequences (Lane et al., 2003). Thus, there is the potential for the maintenance of an epigenetic mark and consequent germline transmission to the offspring.

CONCLUSION

In conclusion, somatic cell nuclear transfer results in a variety of lethal anomalies and these afflict a high proportion of reconstructed embryos. In contrast, numerous healthy clones from several mammalian species have been born and seem to show no apparent health or reproductive prob-

lems, suggesting that both genetic and epigenetic errors are minimal or absent in these animals. Further, genetic and epigenetic anomalies appear absent in offspring of cloned animals derived by sexual reproduction. This notwithstanding, genetic errors are highly likely to be transmitted via the germline. It is also highly possible that epigenetic alterations and their consequences will appear in offspring. Consequently, further research and fine molecular screening of somatic tissues and gametes is required to determine the long-term effects of cloning on healthy animals and their offspring. This information is required not only to evaluate the economic benefits to the animal breeding industry but also to reassure the public of the social advantages of applying animal cloning to our natural resources.

ACKNOWLEDGMENTS

Financial support was provided by the Canada Research Chair Program (L.C.S.), by the Natural Science and Engineering Research Council and the Canadian Institutes of Health Research (L.C.S. and B.D.M.).

REFERENCES

- Ashley, M.V., Laipis, P.J., and Hauswirth, W.W. (1989). Rapid segregation of heteroplasmic bovine mitochondria. *Nucl. Acid Res.* 17, 7325–7331.
- Baqir, S., and Smith, L.C. (2003). Growth restricted in vitro culture conditions alter the imprinted gene expression patterns of mouse embryonic stem cells. *Cloning Stem Cells* 5, 199–212.
- Betts, D., Bordignon, V., Hill, J., et al. (2001). Reprogramming of telomerase activity and rebuilding of telomere length in cloned cattle. *Proc. Natl. Acad. Sci. USA* 98, 1077–1082.
- Booth, P.J., Viuff, D., Tan, S., et al. (2003). Numerical chromosome errors in day 7 somatic nuclear transfer bovine blastocysts. *Biol. Reprod.* 68, 922–928.
- Bordignon, V., Clarke, H. J., and Smith, L. C. (1999). Developmentally regulated loss and reappearance of immunoreactive somatic histone H1 on chromatin of bovine morula-stage nuclei following transplantation into oocytes. *Biol. Reprod.* 61, 22–30.
- Bourc'his, D., Le Bourhis, D., Patin, D., et al. (2001). Delayed and incomplete reprogramming of chromosome methylation patterns in bovine cloned embryos. *Curr. Biol.* 11, 1542–1546.
- Bureau, W.S., Bordignon, V., Leveillee, C., et al. (2003). Assessment of chromosomal abnormalities in bovine nuclear transfer embryos and in their donor cells. *Cloning Stem Cells* 5, 123–132.
- Campbell, K.H.S., Ritchie, W.A., and Wilmut, I. (1993). Nuclear-cytoplasmic interactions during the first cell cycle of nuclear transfer reconstructed bovine embryos: Implications for deoxyribonucleic acid replication and development. *Biol. Reprod.* 49, 933–942.
- Cezar, G.G. (2003). Epigenetic reprogramming of cloned animals. *Cloning Stem Cells* 5, 165–180.
- Cezar, G.G., Bartolomei, M.S., Forsberg, E.J., et al. (2003). Genome-wide epigenetic alterations in cloned bovine fetuses. *Biol. Reprod.* 68, 1009–1014.
- de Montera, B., Boulanger, L., Renard, J.P., et al. (2003). Genetic identity of clones and methods to explore DNA. Presented at the Workshop on Risk Assessment of Products Obtained from Cloned Livestock.
- Dean, W., Santos, F., and Reik, W. (2003). Epigenetic reprogramming in early mammalian development and following somatic nuclear transfer. *Semin. Cell Dev. Biol.* 14, 93–100.
- Fischle, W., Wang, Y., and Allis, C. D. (2003). Binary switches and modification cassettes in histone biology and beyond. *Nature* 425, 475–479.
- Hajkova, P., Erhardt, S., Lane, N., et al. (2002). Epigenetic reprogramming in mouse primordial germ cells. *Mech. Dev.* 117, 15–23.
- Jeanisch, R., Eggan, K., Humpherys, D., et al. (2002). Nuclear cloning, stem cells, and genomic reprogramming. *Cloning Stem Cells* 4, 389–396.
- Kang, Y.K., Koo, D.B., Park, J.S., et al. (2001). Aberrant methylation of donor genome in cloned bovine embryos. *Nat. Genet.* 28, 173–177.
- King, W.A., Verini Supplizi, A., Diop, H.E.P., et al. (1995). Chromosomal analysis of embryos produced by artificially inseminated superovulated cattle. *Genet. Sel. Evol.* 27, 189–194.
- Koehler, C.M., Lindberg, G.L., Brown, D.R., et al. (1991). Replacement of bovine mitochondrial DNA by sequence variant within one generation. *Genetics* 129, 247–255.
- Lane, N., Dean, W., Erhardt, S., et al. (2003). Resistance of IAPs to methylation reprogramming may provide a mechanism for epigenetic inheritance in the mouse. *Genesis* 35, 88–93.
- Lanza, R.P., Cibelli, J.B., Blackwell, C., et al. (2000). Extension of cell life-span and telomere length in animals cloned from senescent somatic cells. *Science* 288, 665–669.
- McLay, D.W., and Clarke, H.J. (2003). Remodelling the paternal chromatin at fertilization in mammals. *Reproduction* 125, 625–633.
- Meirelles, F.V., Bordignon, V., Watanabe, Y., et al. (2001). Complete replacement of the mitochondrial genotype in a *Bos indicus* calf reconstructed by nuclear transfer to a *Bos taurus* oocyte. *Genetics* 158, 351–356.
- Meirelles, F.V., and Smith, L.C. (1997). Mitochondrial genotype segregation in a mouse heteroplasmic lineage produced by embryonic karyoplast transplantation. *Genetics* 145, 445–451.
- Meirelles, F.V., and Smith, L.C. (1998). Mitochondrial

- genotype segregation during preimplantation development in mouse heteroplasmic embryos. *Genetics* 148, 877–883.
- Miyashita, N., Shiga, K., Fujita, T., et al. (2003). Normal telomere lengths of spermatozoa in somatic cell-cloned bulls. *Theriogenology* 59, 1557–1565.
- Miyashita, N., Shiga, K., Yonai, M., et al. (2002). Remarkable differences in telomere lengths among cloned cattle derived from different cell types. *Biol. Reprod.* 66, 1649–1655.
- Ohgane, J., Wakayama, T., Kogo, Y., et al. (2001). DNA methylation variation in cloned mice. *Genesis* 30, 45–50.
- Ota, T., Suto, S., Katayama, H., et al. (2002). Increased mitotic phosphorylation of histone H3 attributable to AIM-1/Aurora-B overexpression contributes to chromosome number instability. *Cancer Res.* 62, 5168–5177.
- Piko, L., and Taylor, K.D. (1987). Amounts of mitochondrial DNA and abundance of some mitochondrial gene transcripts in early mouse embryos. *Dev. Biol.* 123, 364–374.
- Prigent, C., and Dimitrov, S. (2003). Phosphorylation of serine 10 in histone H3, what for? *J. Cell. Sci.* 116, 3677–3685.
- Reik, W., Santos, F., Mitsuya, K., et al. (2003). Epigenetic asymmetry in the mammalian zygote and early embryo: relationship to lineage commitment? *Philos. Trans. R. Soc. Lond. B. Biol. Sci.* 358, 1403–1409.
- Santos, F., Zakhartchenko, V., Stojkovic, M., et al. (2003). Epigenetic marking correlates with developmental potential in cloned bovine preimplantation embryos. *Curr. Biol.* 13, 1116–1121.
- Shi, W., Zakhartchenko, V., and Wolf, E. (2003). Epigenetic reprogramming in mammalian nuclear transfer. *Differentiation* 71, 91–113.
- Shiels, P.G., and Jardine, A.G. (2003). Dolly, no longer the exception: telomeres and implications for transplantation. *Cloning Stem Cells* 5, 157–160.
- Shiota, K., and Yanagimachi, R. (2002). Epigenetics by DNA methylation for development of normal and cloned animals. *Differentiation* 69, 162–166.
- Smith, L.C., Wilmut, I., and Hunter, R.H.F. (1988). Influence of cell cycle stage at nuclear transplantation on the development *in vitro* of mouse embryos. *J. Reprod. Fertil.* 84, 619–624.
- Steinborn, R., Schinogl, P., Zakhartchenko, V., et al. (2000). Mitochondrial DNA heteroplasmy in cloned cattle produced by fetal and adult cell cloning. *Nat. Genet.* 25, 255–257.
- Szöllösi, D., Balakier, H., Czolowska, R., et al. (1980). Ultrastructure of cell hybrids between mouse oocytes and blastomeres. *J. Exp. Zool.* 213, 315–325.
- Takeda, K., Akagi, S., Kaneyama, K., et al. (2003). Proliferation of donor mitochondrial DNA in nuclear transfer calves (*Bos taurus*) derived from cumulus cells. *Mol. Reprod. Dev.* 64, 429–437.
- Tanaka, M., Kihara, M., Meczekalski, B., et al. (2003). H100: a pre-embryonic H1 linker histone in search of a function. *Mol. Cell. Endocrinol.* 202, 5–9.
- Vignon, X., Zhou, Q., and Renard, J.P. (2002). Chromatin as a regulative architecture of the early developmental functions of mammalian embryos after fertilization or nuclear transfer. *Cloning Stem Cells* 4, 363–377.
- Wallace, D.C., and Lott, M.T. (2003). MITOMAP: A Human Mitochondrial Genome Database. www.mitomap.org.

Address reprint requests to:

Lawrence C. Smith, D.V.M., Ph.D.
 Faculté de Médecine Vétérinaire (CRRRA)
 Université de Montréal
 Saint-Hyacinthe, QC, J2S 7C6 Canada

E-mail: smithl@medvet.umontreal.ca