

Review Article

Chromatin Modifying Agents in the *In Vitro* Production of Bovine Embryos

**Fabio Morato Monteiro,^{1,2} Clara Slade Oliveira,¹ Letícia Zoccolaro Oliveira,¹
Naiara Zoccal Saraiva,¹ Maria Eugênia Zerlotti Mercadante,² Flavia Lombardi Lopes,¹
Daniel Robert Arnold,¹ and Joaquim Mansano Garcia¹**

¹Departamento de Reprodução Animal, FCAV-UNESP, 14884-000-Jaboticabal, SP, Brazil

²Instituto de Zootecnia, Estação Experimental de Zootecnia de Sertãozinho, Caixa Postal 63,
14.160-970-Sertãozinho, SP, Brazil

Correspondence should be addressed to Fabio Morato Monteiro, monteiro@iz.sp.gov.br

Received 31 May 2010; Revised 28 July 2010; Accepted 10 September 2010

Academic Editor: Kyle Orwig

Copyright © 2011 Fabio Morato Monteiro et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

The low efficiency observed in cloning by nuclear transfer is related to an aberrant gene expression following errors in epigenetic reprogramming. Recent studies have focused on further understanding of the modifications that take place in the chromatin of embryos during the preimplantation period, through the use of chromatin modifying agents. The goal of these studies is to identify the factors involved in nuclear reprogramming and to adjust *in vitro* manipulations in order to better mimic *in vivo* conditions. Therefore, proper knowledge of epigenetic reprogramming is necessary to prevent possible epigenetic errors and to improve efficiency and the use of *in vitro* fertilization and cloning technologies in cattle and other species.

1. Introduction

Despite being utilized for nearly three decades [1], the production of embryos *in vitro* still has limitations, such as, lower efficiency when compared to *in vivo* production. In bovine oocytes, matured and fertilized *in vitro*, high cleavage rates are currently obtained. However, only 25%–40% of these zygotes are capable of progressing to the blastocyst stage [2]. Recent studies suggest that epigenetic alterations and consequent changes to chromatin conformation may take place during *in vitro* culture [3]. Enright et al. [4] reported elevated levels of histone H3 and H4 acetylation following long-term culture of embryos. Furthermore, *in vitro* produced bovine embryos showed altered gene expression patterns when compared to *in vivo* controls [5].

In somatic cell nuclear transfer (SCNT), perturbations in epigenetic patterning are also thought to play a role in the low efficacy seen following the use of this technology. In SCNT, the nucleus of a differentiated adult somatic cell is reprogrammed by factors in the oocyte cytoplasm in order to regain the pluripotent patterns of an embryonic cell [6–9].

However, failure in this nuclear reprogramming [10, 11], resulting in abnormal epigenetic patterns in cloned embryos, has been reported [12]. Furthermore, development to the blastocyst stage and survival to birth are significantly lower in clones in comparison to embryos produced by *in vitro* fertilization [11–14]. In cloned mice, animals do not appear to pass an abnormal trait on to their offspring produced *in vivo*. This may suggest that a perturbation to proper epigenetic patterning, rather than to genetic sequences, is responsible of the aberrant phenotypes and also of the low efficiency seen following the use of this technology [15].

In view of these concerns following the use of *in vitro* technologies for the production of embryos, recent studies aim at understanding the chromatin structure modifications during preimplantation and the improvement of *in vitro* approaches to most closely mimic the *in vivo* changes that allow the growth and survival of embryos. In the present paper, we will discuss some of the epigenetic mechanisms involved in embryo development and the use of treatments to manipulate epigenetic patterning *in vitro*.

2. Mechanisms of Epigenetic Regulation

Epigenetic refers to the control of gene function and expression without changes to the gene sequence. Such control allows for different expression patterns, from an identical genome sequence, to take place in separate cells or tissues, establishing the basis for tissue-specific gene expression [16].

Epigenetic modifications control gene expression by a variety of processes which include DNA methylation, post-translational histone modifications, noncoding RNAs [17]. The processes of DNA methylation and histone modification have gained great interest in the fields of mammalian development [16]. We will further discuss these studies and how they may offer insight into the production of embryos *in vitro*.

2.1. DNA Methylation. DNA methylation is one of the most well-described epigenetic mechanisms and plays a key role in several biological processes such as, transcriptional regulation, chromosomal organization, X-inactivation, and genomic imprinting [18–22]. A family of DNA (cytosine-5)-methyltransferases (DNMT) is responsible for the addition of a methyl group to the 5th position of the cytosine ring, and five DNMTs have been already characterized in mammals: DNMT1, 2, 3A, 3B, and 3L [23]. DNMT1 is responsible for the maintenance of methylation through the remethylation of new strands during replication if the mother strand was also methylated [22]. The DNMT3 enzymes (DNMT3a, 3b, and 3L) are involved in the acquisition of *de novo* methylation on DNA previously devoid of methylation [24]. Loss of methylation patterns through genetic ablation of these enzymes is lethal at early embryonic or postnatal stages in mice [24, 25].

Amongst all the biological processes involving DNA methylation, genomic imprinting is one of the most intriguing, as it involves the formation of an epigenetic “mark” at certain loci in a parent-of-origin-specific manner such that genes are expressed monoallelically [26]. Biallelic expression or silencing of imprinted genes is detrimental to fetal growth, phenotype, and survival, considering that the accurate maintenance of inherited methylation patterns on imprinted genes in preimplantation is critically important for the success of both *in vivo* and *in vitro* embryonic developments.

Patterns of DNA methylation are erased in the primordial germ cells during embryonic life and re-established in a sex-specific manner in the gametes during germ cell development. However, for the purpose of the review, we will discuss another important wave of demethylation that occurs postfertilization. Genome-wide methylation is erased, except for methylation of imprint genes and certain repeat sequences, with reacquisition of methylation in mice taking place during the peri-implantation stage in the expanded blastocyst (review in [26]).

2.2. Histone Modifications. Another important epigenetic process is histone modification. Histones are proteins bound to DNA forming the highly conserved structural polymer

known as chromatin. Nucleosomes are the fundamental repeating units of chromatin. A nucleosome consists of 146-base pairs of DNA wrapped around a core of histone proteins. The histone proteins that make up this core are 2 copies each of H2A, H2B, H3, and H4. A linker histone, H1, is bound to the DNA between nucleosomes, allowing for the solenoid helical fiber structure of DNA in the nucleus. These core histones are highly conserved across species. Histone proteins have a globular carboxy-terminal domain that binds the DNA and a flexible amino-terminal tail that extends out of the nucleosome structure. Modifications of histones occur on amino residues, primarily on the amino-terminal tail (reviewed in [27]). These covalent modifications have fundamental functions on chromatin condensation, DNA replication, DNA repair, and gene regulation. Depending on the type of modification that occurs, the nucleosome will either open up to allow for transcriptional factors to bind or remain tightly wound.

Histone acetylation is commonly associated with activated transcription, whereas deacetylation is associated with transcriptional repression [27–29]. Research has shown that acetylation of histone H4 is reduced on the inactive X chromosome in female mammals, suggesting that absence of acetyl groups is a prerequisite for a more condensed and inactive chromatin stage [30]. Histone amino groups can also be methylated, phosphorylated, and ubiquitinated [27, 31].

Examples of some modifications that are commonly associated with euchromatin, regulating gene expression are acetylation of histone H3 and H4, as well as di- and trimethylation of lysine 4 on histone H3 [31]. The region of DNA on the chromosome that is constitutively silenced (telomere, centromeres, and heterochromatin) is hypoacetylated [31–33]. In addition, these regions are highly methylated on particular amino acid residues (lysine 9 and lysine 27 of histone H3) [31, 34]. Among the histone modifications, acetylation and methylation have been the most studied. Unlike histone acetylation, histone methylation is more complex, with gene expression being affected differently depending on which residue is modified [35]. Loci-specific modifications of histones, and the combination of these modifications, have been described as a “histone code” that defines the state of a cell’s transcriptional potential [36].

Several enzymes have been reported to control histone modification. Two proteins involved with controlling the acetyl groups are histone acetyltransferase (HAT; adds acetyl groups) and histone deacetylase (HDAC; removes acetyl groups) [37]. Several of these enzymes (HDACs 1, 2, 3, 7, and HAT1) have been detected in bovine embryos [38]. As more research is conducted, it becomes apparent that crosstalk between enzymes is a common feature. For example in *Schizosaccharomyces pombe*, HDAC is required for deacetylating the histone 3 lysine 9 residue in order for histone methyltransferase to act on that particular residue [34]. The HAT enzymes not only affect chromatin, but also act as coactivators on certain transcription factors [39]. Interactions also occur between HDACs and DNMTs [40–42].

3. Epigenetic Modifications in Mammalian Embryos

Embryonic development involves a wide array of epigenetic modifications, including DNA methylation and histone modifications, which are fundamental for genomic imprinting and X-chromosome inactivation in female embryos [43]. In mammals, levels of histone methylation are higher and histone acetylation levels are lower in male gametes compared to female gametes [12, 44–47], resulting in minimal gene expression at this stage.

During normal bovine embryo development, histone demethylation occurs soon after fertilization [12]. Histone methylation is reduced in 2 to 4 cell embryos, and starts to increase at the 8- to 16- cell stage, concurrently with zygotic genome activation [48]. Histone acetylation levels peak at the time of zygotic genome activation, corresponding to the time of increased gene expression, then diminish during the morula stage [49]. The first cellular differentiation of the developing embryo occurs at the blastocyst stage giving rise to the inner cell mass (ICM) and trophectoderm [48]. At the blastocyst stage, DNA and histone methylation is elevated in the ICM, whereas DNA and histones are hypomethylated in the trophectoderm. Thus, blastocyst formation establishes the beginning of epigenetic differences between the two cell lineages [50].

Another important event in normal mammalian embryo development is X-chromosome inactivation (XCI). Gene expression from one X chromosome is sufficient to allow for normal embryonic development, as seen in male embryos [51]. Therefore, in female embryos, the extra X chromosome must be silenced [12]. Establishment and maintenance of XCI are regulated by epigenetic mechanisms. Research has shown XCI imprinting in the bovine [52] and murine [53, 54] placentas. In mice, imprinting of XCI occurs during preimplantation development, with the paternal chromosome preferentially silenced [52–54]. The paternal X chromosome is silenced at the 4-cell stage, shortly after zygotic genome activation [55]. This pattern persists in the trophectoderm lineage, where the active X chromosome in the placenta is of maternal origin. However, in the ICM of developing embryo, the paternal X chromosome is reactivated and forthwith both maternal and paternal X chromosomes are randomly selected for inactivation [55, 56].

Regards to DNA methylation, during the early postfertilization stages there is an intense wave of DNA demethylation in both pronuclei, albeit at different speeds, followed by a global remethylation that takes place at peri-implantation stages in the mouse. Further, not all sequences have their methylation patterns removed, as this is the case for imprinted sequences, which maintain their methylation marks throughout life except in the germ cell line [43].

3.1. Epigenetic Modifications in Cloned Embryos. In cloning by somatic cell nuclear transfer, the epigenetic patterns of the adult somatic cell must be erased and reprogrammed to those of a totipotent embryonic cell. During the development of embryos *in vivo*, patterns carried by both gametes are each reprogrammed in a very specific and timely manner and

embryonic patterns of the totipotent cell must be established to support the embryonic genome activation that ensues [57–59]. Failure is the re-establishment of correct epigenetic marks that can affect totipotency and proper differentiation and development of embryos [60, 61]. Initial epigenetic patterns of the adult somatic cell used for cloning can affect reprogramming following nuclear transfer [62]. In fact, differences in embryo development have been described following the use of cells of different tissue origins for SCNT [63–65].

Several studies have demonstrated abnormal DNA and histone methylation patterns in SCNT bovine embryos compared with *in vivo* and *in vitro* produced embryos [43, 48, 66–68]. Also, global transcriptome profile experiments revealed down regulation of genes involved in chromatin remodeling in cloned embryos compared with *in vitro* fertilized embryos [69].

As mentioned above, early embryos present an intense and very well-orchestrated reprogramming of DNA methylation, and questions arise as to whether the nucleus of the donor cell in SCNT embryos, carrying a somatic cell pattern of methylation, can be manipulated in order to mimic this intense reprogramming taking place during early embryonic stages [43]. Unlike normal embryos, SCNT embryos have incomplete demethylation after the one cell stage, regardless of species [43]. In addition, these SCNT embryos started to undergo *de novo* methylation prematurely (4-cell stage versus 8- to 16- cell stage for *in vivo* embryos), so by the morula stage, methylation in the blastomeres resembled that of the donor cells [43]. The methylation status of ICM in SCNT bovine blastocysts is similar to *in vivo* blastocyst embryos. However, the trophectoderm of SCNT blastocysts is abnormally hypermethylated [12, 43]. These changes in the normal timing of methylation result in potentially serious consequences in epigenetic reprogramming and further development of the trophectoderm, turning mammalian cloning to an impractical reproductive technology until the timing of events taking place during *in vitro* production can be controlled by scientists [70].

In addition to abnormal DNA methylation, histone modifications are also altered in SCNT embryos. Santos and coauthors [48] demonstrated that SCNT bovine embryos had hypermethylation histone H3-K9 associated with genome-wide hypermethylation. A study also reported that acetylation of lysine 5 on histone H4 (H4-K5ac) appears to change dramatically during early embryo development of IVF produced embryos, but remains consistently elevated in SCNT produced bovine embryos [71]. Compared to *in vitro* fertilized embryos, SCNT bovine embryos have elevated heterochromatic histone methylation (H3K9me2) and H3K9-acetylation in the trophectoderm layer [48]. These and other modifications could explain the altered expression of vital developmental genes later in development.

Another complication with nuclear transfer is the reprogramming of imprinted genes. Abnormalities generally associated with cloned animals resemble those observed in mice with imprinted gene mutations [72]. Analysis of bovine H19 demonstrated that SCNT animals that died shortly after birth had biallelic expression [73]. The placenta of SCNT animals

appears to be especially vulnerable to abnormal expression of imprinted genes [12]. Inoue et al. [74] observed abnormally low levels of expression of both imprinted and nonimprinted genes in the placentas of SCNT mice, whereas expression in the embryo was not altered. Similarly, Yang et al. [75] observed abnormal expression of the imprinted gene IGF2R in the placenta but not in the organs of SCNT calves. Smith et al. [76] found reduced expression of the placenta-specific gene *Cd81* between SCNT and *in vivo* bovine blastocysts. In early pregnancy, expression of the imprinted placental genes *Ascl2* has been shown to be altered in SCNT bovine embryos and placental tissues [77]. In SCNT embryos, *Ascl2* was overexpressed.

Nuclear reprogramming also affects X chromosome inactivation, particularly in the placenta. Reports have shown that the ICM of SCNT embryos had normal XCI (i.e., random paternal/maternal inactivation), whereas the placenta had altered gene expression from X-linked genes [12]. However, in day 8 blastocysts, Smith et al. [76] found no evidence of abnormal expression of X-linked genes from SCNT bovine embryos. It must be noted that complete XCI in bovine embryos has been reported to occur at day 14–15 [78]. At later stages, reports of bi-allelic expression of X-linked genes in the placenta of deceased clones indicate the absence of the paternal XCI that is observed in non-SCNT calves [52]. Live SCNT calves had one active X-chromosome, similar to non-SCNT calves, suggesting that abnormal XCI could contribute to fetal loss [52].

These results provide further evidence towards the incomplete epigenetic reprogramming of SCNT embryos and the trophoctoderm cell lineages appear to be highly vulnerable to these defects.

4. The Use of Histone Deacetylase Inhibitors for *In Vitro* Production of Bovine Embryos

The first reports of utilizing HDAC inhibitors were in cancer therapies. Inhibitors of HDAC promote global chromatin acetylation, which leads to excessive gene transcription. This increase in protein production in tumor cells would induce cellular differentiation, modifying their characteristics of excessive cell growth [79]. Inhibitors of HDAC have been reported to have antiproliferative and apoptotic effects, which are beneficial in cancer therapies. Mutation of the HDAC1 gene in mice caused reduced cell proliferation which led to the embryonic death at midgestation [80]. These effects were associated with increased gene expression of cell cycle inhibitors [80]. Similar antiproliferative effects were seen in embryonic stem cells [81, 82]. Crystallography studies indicated that these inhibitors act by binding the catalytic site of the enzymes, blocking therefore the access to the substrates [83]. Among the known inhibitors, some of the most used are (I) trichostatin A, (II) valproic acid, clinically used in the treatment of epilepsy, and (III) sodium butyrate [84]. These substances cause reversible inhibition of the majority of class I and II HDACs [85].

To improve SCNT efficiency, researchers have turned to stimulating donor cell reprogramming by chemical treat-

ments. Donor cells treated with trichostatin A (TSA) have been shown to have a slight improvement on the development of cloned embryos in cows [10, 62, 86, 87], mice [49, 88–90], pigs [91–95], and rabbits [96, 97]. In pigs, live births were reported following TSA treatment [92, 95]; in rabbits, however, there was no survival of offspring to adulthood [97]. In the bovine, both treatments of donor cells [4, 10, 86] or of the reconstructed embryos [10, 87] were beneficial to the development of clones. The type of donor cell also appears to have a role on the benefits of TSA treatment. In mice, blastocyst formation was 5-fold higher in embryos treated with TSA that were produced from cumulus cells [88]. However, TSA had no effect on blastocyst development from embryos produced by embryonic stem cells. These results suggest that inhibition of HDAC is only beneficial in donor cells that are more differentiated [88]. Similar results also suggest that hypoacetylation may be a limiting factor in the development of cloned embryos [96, 98].

It is thought that TSA aids nuclear reprogramming and subsequently improves expression of embryonic genes such as, *Nanog* [87], *SOX2*, and *cMyc* [90], and regulates expression of genes related to chromatin structure and DNA methylation [90]. Furthermore, the use of TSA improves derivation of embryonic stem cell lines from cloned embryos [88].

The use of general HDAC inhibitors affects all chromatin, which may have negative effects when utilized at elevated concentrations for extended periods of time. Tsuji et al. [89] demonstrated that the beneficial effects of TSA treatment on cloned embryos declined after 12 hours, leading to reduced blastocyst formation and fetal loss. Li et al. [92] reported that in pigs, cloned embryos treated with TSA had a 15-hour delay to reach the stage of compacted morula. Furthermore, embryos fertilized *in vitro* had elevated apoptotic levels following TSA treatment [99]. Apoptosis, or programmed cell death, is a physiological event that has been associated with reduced viability and death of bovine embryos [100, 101]. TSA has been shown to induce expression of proapoptotic genes [102] and, by facilitating histone hyperacetylation, allows the DNA to be available for endonucleases [103].

The addition of TSA, during fertilization, promoted increased levels of histone acetylation on the sperm. Cleavage rate and blastocyst formation were not altered. However, there were more cells in the ICM of embryos that received TSA during fertilization [104]. These results suggest that alteration of histone acetylation during fertilization affects subsequent cell proliferation and differentiation [104]. Embryos cultured with TSA after fertilization, in an effort to aid the activation of embryo genome, had increased levels of histone acetylation, but blastocyst development was not affected [99]. Higher levels of apoptosis were seen in high quality embryos but not in the lower quality ones, suggesting that the use of TSA can benefit this group of embryos. Further, they reported gender differences in the response to histone hyperacetylation following TSA treatment, with female embryos being more sensitive than males [99]. Future studies are warranted to investigate gene expression patterns in embryos following TSA treatment in order to further evaluate potential effects of this agent in early embryo development.

Other HDAC inhibitors are currently being investigated for the use in nuclear transfer in pigs: valproic acid, which resulted in higher levels of Oct4 expression [105] and *scriptaid* [94] and sodium butyrate [106], which resulted in cloned embryos with acetylation levels similar to fertilized *in vitro*. However, special attention must be paid to the fact that the use of valproic acid as an antiepileptic drug (AED) in the first trimester of pregnancy was associated with significantly increased risks of several congenital malformations [107]. Antiepileptic drugs in general are related to malformations, and it is probable that they affect development through multiple mechanisms [108]. Valproic acid has been shown to disrupt the Wnt signalling pathway. However, valproic acid analogues that do not alter Wnt signalling, do not produce teratogenic effects. Other main mechanisms have been connected to valproic acid teratogenic effects, such as, inhibition of folate metabolism and neural apoptosis induction [108], and recent studies reveal HDAC inhibition as one of the possible mechanisms for valproic acid teratogenic effects [109].

In this respect, experiments involving HDAC inhibitors supplementation must be aimed to use the minimum working concentration, in order to inhibit minimum HDAC and obtain the desired effect. Even though the dosages applied for embryo culture are lower for trichostatin A, which is supplemented at nanomolar concentrations, further studies accessing teratogenic effects of this and others HDAC inhibitors are needed.

5. Use of DNA Methylation Inhibitors

As mentioned earlier, several studies indicated that aberrant epigenetic reprogramming occurs in cloned embryos [60, 66, 68]. Partial demethylation and early methylation, at the four- to eight-cell stage in cloned embryos [110], indicate the presence of inadequate nuclear reprogramming in regards to DNA methylation [43].

Researchers aimed at preventing DNA hypermethylation in animal cloning through the use of methylation inhibitors. Pretreatment of donor cells with an inhibitor of DNA methylation, 5-aza-2'-deoxycytidine (5-aza-Dc), before nuclear transfer does not appear to improve development of cloned embryos [111, 112]. These results may indicate that inhibition of excess methylation alone fails to aid reprogramming in the donor cell nucleus, as erasure and reacquisition of methylation during the preimplantation period in embryos is a very complex procedure and may require the use of a combination of agents to try and mimic this process *in vitro*.

6. Conclusions and Future Directions

As discussed here and in the work of others, somatic patterns of histone modifications, as well as DNA methylation, present in differentiated adult cells, must be erased and reprogrammed in a highly organized and timely manner. The acquisition of unique embryonic epigenetic marks must take place in cloned embryos in a similar manner as seen following erasure and acquisition of epigenetic marks in the female and male pronuclei. Failure during erasure and/or

acquisition of these marks will affect subsequent embryonic development [60, 61].

Current studies aim at (1) understanding the factors involved in nuclear reprogramming, (2) identifying possible failures in reprogramming induced by cell manipulation and/or culture conditions, and (3) utilize inhibitors and other agents that could assist in the establishment of proper epigenetic patterning during early embryo development. All these efforts to elucidate the complex steps of epigenetic reprogramming during embryogenesis, as well as how to manipulate these steps, are necessary to improve the efficiency and applicability of *in vitro* production of embryos and cloning.

References

- [1] B. G. Brackett, D. Bousquet, and M. L. Boice, "Normal development following in vitro fertilization in the cow," *Biology of Reproduction*, vol. 27, no. 1, pp. 147–158, 1982.
- [2] P. Lonergan, "Growth of preimplantation bovine embryos," *Acta Veterinaria Scandinavica*, vol. 35, no. 4, pp. 307–320, 1994.
- [3] V. L. Wilson and P. A. Jones, "DNA methylation decreases in aging but not in immortal cells," *Science*, vol. 220, no. 4601, pp. 1055–1057, 1983.
- [4] B. P. Enright, B. S. Jeong, X. Yang, and X. C. Tian, "Epigenetic characteristics of bovine donor cells for nuclear transfer: levels of histone acetylation," *Biology of Reproduction*, vol. 69, no. 5, pp. 1525–1530, 2003.
- [5] C. Wrenzycki, D. Herrmann, A. Lucas-Hahn, E. Lemme, K. Korsawe, and H. Niemann, "Gene expression patterns in in vitro-produced and somatic nuclear transfer-derived preimplantation bovine embryos: relationship to the large offspring syndrome?" *Animal Reproduction Science*, vol. 82–83, pp. 593–603, 2004.
- [6] I. Wilmut, A. E. Schnieke, J. McWhir, A. J. Kind, and K. H. S. Campbell, "Viable offspring derived from fetal and adult mammalian cells," *Nature*, vol. 385, no. 6619, pp. 810–813, 1997.
- [7] T. Wakayama, A. C. F. Perry, M. Zuccotti, K. R. Johnson, and R. Yanagimachi, "Full-term development of mice from enucleated oocytes injected with cumulus cell nuclei," *Nature*, vol. 394, no. 6691, pp. 369–374, 1998.
- [8] A. Baguisi, E. Behboodi, D. T. Melican et al., "Production of goats by somatic cell nuclear transfer," *Nature Biotechnology*, vol. 17, no. 5, pp. 456–461, 1999.
- [9] T. Wakayama, V. Tabar, I. Rodriguez, A. C. F. Perry, L. Studer, and P. Mombaerts, "Differentiation of embryonic stem cell lines generated from adult somatic cells by nuclear transfer," *Science*, vol. 292, no. 5517, pp. 740–743, 2001.
- [10] X. Ding, Y. Wang, D. Zhang, Y. Wang, Z. Guo, and Y. Zhang, "Increased pre-implantation development of cloned bovine embryos treated with 5-aza-2'-deoxycytidine and trichostatin A," *Theriogenology*, vol. 70, no. 4, pp. 622–630, 2008.
- [11] J. B. Cibelli, R. P. Lanza, K. H. Campbell, and M. D. West, *Principles of Cloning*, Academic Press, San Diego, Calif, USA, 2002.
- [12] X. Yang, S. L. Smith, X. C. Tian, H. A. Lewin, J.-P. Renard, and T. Wakayama, "Nuclear reprogramming of cloned embryos and its implications for therapeutic cloning," *Nature Genetics*, vol. 39, no. 3, pp. 295–302, 2007.

- [13] T. A. M. Kruip and J. H. G. den Daas, "In vitro produced and cloned embryos: effects on pregnancy, parturition and offspring," *Theriogenology*, vol. 47, no. 1, pp. 43–52, 1997.
- [14] J. Zhu, E. E. Telfer, J. Fletcher et al., "Improvement of an electrical activation protocol for porcine oocytes," *Biology of Reproduction*, vol. 66, no. 3, pp. 635–641, 2002.
- [15] K. L. K. Tamashiro, T. Wakayama, H. Akutsu et al., "Cloned mice have an obese phenotype not transmitted to their offspring," *Nature Medicine*, vol. 8, no. 3, pp. 262–267, 2002.
- [16] E. Li, "Chromatin modification and epigenetic reprogramming in mammalian development," *Nature Reviews Genetics*, vol. 3, no. 9, pp. 662–673, 2002.
- [17] E. Bernstein and C. D. Allis, "RNA meets chromatin," *Genes and Development*, vol. 19, no. 14, pp. 1635–1655, 2005.
- [18] M. A. Surani, "Imprinting and the initiation of gene silencing in the germ line," *Cell*, vol. 93, no. 3, pp. 309–312, 1998.
- [19] H. H. Ng and A. Bird, "DNA methylation and chromatin modification," *Current Opinion in Genetics and Development*, vol. 9, no. 2, pp. 158–163, 1999.
- [20] W. Reik and W. Dean, "DNA methylation and mammalian epigenetics," *Electrophoresis*, vol. 22, no. 14, pp. 2838–2843, 2001.
- [21] S. Simonsson and J. Gurdon, "DNA demethylation is necessary for the epigenetic reprogramming of somatic cell nuclei," *Nature Cell Biology*, vol. 6, no. 10, pp. 984–990, 2004.
- [22] T. H. Bestor, "The DNA methyltransferases of mammals," *Human Molecular Genetics*, vol. 9, no. 16, pp. 2395–2402, 2000.
- [23] M. G. Goll and T. H. Bestor, "Eukaryotic cytosine methyltransferases," *Annual Review of Biochemistry*, vol. 74, pp. 481–514, 2005.
- [24] M. Okano, D. W. Bell, D. A. Haber, and E. Li, "DNA methyltransferases Dnmt3a and Dnmt3b are essential for de novo methylation and mammalian development," *Cell*, vol. 99, no. 3, pp. 247–257, 1999.
- [25] E. Li, T. H. Bestor, and R. Jaenisch, "Targeted mutation of the DNA methyltransferase gene results in embryonic lethality," *Cell*, vol. 69, no. 6, pp. 915–926, 1992.
- [26] W. Reik, W. Dean, and J. Walter, "Epigenetic reprogramming in mammalian development," *Science*, vol. 293, no. 5532, pp. 1089–1093, 2001.
- [27] W. Fischle, Y. Wang, and C. D. Allis, "Histone and chromatin cross-talk," *Current Opinion in Cell Biology*, vol. 15, no. 2, pp. 172–183, 2003.
- [28] K. Struhl, "Histone acetylation and transcriptional regulatory mechanisms," *Genes and Development*, vol. 12, no. 5, pp. 599–606, 1998.
- [29] J. Tazi and A. Bird, "Alternative chromatin structure at CpG islands," *Cell*, vol. 60, no. 6, pp. 909–920, 1990.
- [30] P. Jeppesen and B. M. Turner, "The inactive X chromosome in female mammals is distinguished by a lack of histone H4 acetylation, a cytogenetic marker for gene expression," *Cell*, vol. 74, no. 2, pp. 281–289, 1993.
- [31] T. Kouzarides, "Chromatin Modifications and Their Function," *Cell*, vol. 128, no. 4, pp. 693–705, 2007.
- [32] S. L. Berger, "Gene activation by histone and factor acetyltransferases," *Current Opinion in Cell Biology*, vol. 11, no. 3, pp. 336–341, 1999.
- [33] M.-H. Kuo and C. D. Allis, "Roles of histone acetyltransferases and deacetylases in gene regulation," *BioEssays*, vol. 20, no. 8, pp. 615–626, 1998.
- [34] J. Nakayama, J. C. Rice, B. D. Strahl, C. D. Allis, and S. I. S. Grewal, "Role of histone H3 lysine 9 methylation in epigenetic control of heterochromatin assembly," *Science*, vol. 292, no. 5514, pp. 110–113, 2001.
- [35] C. Martin and Y. Zhang, "The diverse functions of histone lysine methylation," *Nature Reviews Molecular Cell Biology*, vol. 6, no. 11, pp. 838–849, 2005.
- [36] B. D. Strahl and C. D. Allis, "The language of covalent histone modifications," *Nature*, vol. 403, no. 6765, pp. 41–45, 2000.
- [37] A. P. Wolffe and D. Guschin, "Review: chromatin structural features and targets that regulate transcription," *Journal of Structural Biology*, vol. 129, no. 2-3, pp. 102–122, 2000.
- [38] S. McGraw, C. Robert, L. Massicotte, and M.-A. Sirard, "Quantification of histone acetyltransferase and histone deacetylase transcripts during early bovine embryo development," *Biology of Reproduction*, vol. 68, no. 2, pp. 383–389, 2003.
- [39] T. Kouzarides, "Histone acetylases and deacetylases in cell proliferation," *Current Opinion in Genetics and Development*, vol. 9, no. 1, pp. 40–48, 1999.
- [40] F. Fuks, W. A. Burgers, N. Godin, M. Kasai, and T. Kouzarides, "Dnmt3a binds deacetylases and is recruited by a sequence-specific repressor to silence transcription," *The EMBO Journal*, vol. 20, no. 10, pp. 2536–2544, 2001.
- [41] F. Fuks, P. J. Hurd, D. Wolf, X. Nan, A. P. Bird, and T. Kouzarides, "The methyl-CpG-binding protein MeCP2 links DNA methylation to histone methylation," *The Journal of Biological Chemistry*, vol. 278, no. 6, pp. 4035–4040, 2003.
- [42] A. K. Robertson, T. M. Geiman, U. T. Sankpal, G. L. Hager, and K. D. Robertson, "Effects of chromatin structure on the enzymatic and DNA binding functions of DNA methyltransferases DNMT1 and Dnmt3a in vitro," *Biochemical and Biophysical Research Communications*, vol. 322, no. 1, pp. 110–118, 2004.
- [43] W. Dean, F. Santos, and W. Reik, "Epigenetic reprogramming in early mammalian development and following somatic nuclear transfer," *Seminars in Cell and Developmental Biology*, vol. 14, no. 1, pp. 93–100, 2003.
- [44] P. G. Adenot, Y. Mercier, J.-P. Renard, and E. M. Thompson, "Differential H4 acetylation of paternal and maternal chromatin precedes DNA replication and differential transcriptional activity in pronuclei of 1-cell mouse embryos," *Development*, vol. 124, no. 22, pp. 4615–4625, 1997.
- [45] J.-M. Kim, H. Liu, M. Tazaki, M. Nagata, and F. Aoki, "Changes in histone acetylation during mouse oocyte meiosis," *Journal of Cell Biology*, vol. 162, no. 1, pp. 37–46, 2003.
- [46] O. F. Sarmiento, L. C. Digilio, Y. Wang et al., "Dynamic alterations of specific histone modifications during early murine development," *Journal of Cell Science*, vol. 117, no. 19, pp. 4449–4459, 2004.
- [47] M. Spinaci, E. Seren, and M. Mattioli, "Maternal chromatin remodeling during maturation and after fertilization in mouse oocytes," *Molecular Reproduction and Development*, vol. 69, no. 2, pp. 215–221, 2004.
- [48] F. Santos, V. Zakhartchenko, M. Stojkovic et al., "Epigenetic marking correlates with developmental potential in cloned bovine preimplantation embryos," *Current Biology*, vol. 13, no. 13, pp. 1116–1121, 2003.

- [49] W. E. Maalouf, R. Alberio, and K. H. S. Campbell, "Differential acetylation of histone H4 lysine during development of in vitro fertilized, cloned and parthenogenetically activated bovine embryos," *Epigenetics*, vol. 3, no. 4, pp. 199–209, 2008.
- [50] W. Reik, F. Santos, and W. Dean, "Mammalian epigenomics: reprogramming the genome for development and therapy," *Theriogenology*, vol. 59, no. 1, pp. 21–32, 2003.
- [51] C. J. Brown, "Equality of the sexes: mammalian dosage compensation," *Seminars in Reproductive Medicine*, vol. 19, no. 2, pp. 125–132, 2001.
- [52] F. Xue, X. C. Tian, F. Du et al., "Aberrant patterns of X chromosome inactivation in bovine clones," *Nature Genetics*, vol. 31, no. 2, pp. 216–220, 2002.
- [53] N. Takagi and M. Sasaki, "Preferential inactivation of the paternally derived X chromosome in the extraembryonic membranes of the mouse," *Nature*, vol. 256, no. 5519, pp. 640–642, 1975.
- [54] N. Takagi, O. Sugawara, and M. Sasaki, "Regional and temporal changes in the pattern of X-chromosome replication during the early post-implantation development of the female mouse," *Chromosoma*, vol. 85, no. 2, pp. 275–286, 1982.
- [55] I. Okamoto, D. Arnaud, P. Le Baccon et al., "Evidence for de novo imprinted X-chromosome inactivation independent of meiotic inactivation in mice," *Nature*, vol. 438, no. 7066, pp. 369–373, 2005.
- [56] J. L. Thorvaldsen, R. I. Verona, and M. S. Bartolomei, "X-tra! X-tra! News from the mouse X chromosome," *Developmental Biology*, vol. 298, no. 2, pp. 344–353, 2006.
- [57] H. D. Morgan, F. Santos, K. Green, W. Dean, and W. Reik, "Epigenetic reprogramming in mammals," *Human Molecular Genetics*, vol. 14, no. 1, pp. R47–R58, 2005.
- [58] M. Zuccotti, S. Garagna, and C. A. Redi, "Nuclear transfer, genome reprogramming and novel opportunities in cell therapy," *Journal of Endocrinological Investigation*, vol. 23, no. 9, pp. 623–629, 2000.
- [59] L. Armstrong, M. Lako, W. Dean, and M. Stojkovic, "Epigenetic modification is central to genome reprogramming in somatic cell nuclear transfer," *Stem Cells*, vol. 24, no. 4, pp. 805–814, 2006.
- [60] Y.-K. Kang, D.-B. Koo, J.-S. Park et al., "Aberrant methylation of donor genome in cloned bovine embryos," *Nature Genetics*, vol. 28, no. 2, pp. 173–177, 2001.
- [61] J. Ohgane, T. Wakayama, Y. Kogo et al., "DNA methylation variation in cloned mice," *Genesis*, vol. 30, no. 2, pp. 45–50, 2001.
- [62] B. P. Enright, C. Kubota, X. Yang, and X. C. Tian, "Epigenetic characteristics and development of embryos cloned from donor cells treated by trichostatin A or 5-aza-2'-deoxycytidine," *Biology of Reproduction*, vol. 69, no. 3, pp. 896–901, 2003.
- [63] Y. Kato, T. Tani, and Y. Tsunoda, "Cloning of calves from various somatic cell types of male and female adult, newborn and fetal cows," *Journal of Reproduction and Fertility*, vol. 120, no. 2, pp. 231–237, 2000.
- [64] C. Kubota, H. Yamakuchi, J. Todoroki et al., "Six cloned calves produced from adult fibroblast cells after long-term culture," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 97, no. 3, pp. 990–995, 2000.
- [65] X. Vignon, P. Chesne, D. LeBourhis, Y. Heyman, and J. P. Renard, "Developmental potential of bovine embryos reconstructed with somatic cell nuclei from cultured skin and muscle fetal cells," *Theriogenology*, vol. 49, no. 1, p. 392, 1998.
- [66] D. Bourc'his, D. Le Bourhis, D. Patin et al., "Delayed and incomplete reprogramming of chromosome methylation patterns in bovine cloned embryos," *Current Biology*, vol. 11, no. 19, pp. 1542–1546, 2001.
- [67] Y. M. Han, Y. K. Kang, D. B. Koo, and K. K. Lee, "Nuclear reprogramming of cloned embryos produced *in vitro*," *Theriogenology*, vol. 59, no. 1, pp. 33–44, 2003.
- [68] Y.-K. Kang, S. P. Jung, D.-B. Koo et al., "Limited demethylation leaves mosaic-type methylation states in cloned bovine pre-implantation embryos," *The EMBO Journal*, vol. 21, no. 5, pp. 1092–1100, 2002.
- [69] N. Rodriguez-Osorio, Z. Wang, P. Kasinathan, G. P. Page, J. M. Robl, and E. Memili, "Transcriptional reprogramming of gene expression in bovine somatic cell chromatin transfer embryos," *BMC Genomics*, vol. 10, article 190, 2009.
- [70] S. Biliya and L. A. Bulla Jr., "Genomic imprinting: the influence of differential methylation in the two sexes," *Experimental Biology and Medicine*, vol. 235, no. 2, pp. 139–147, 2010.
- [71] G. Wee, D.-B. Koo, B.-S. Song et al., "Inheritable histone H4 acetylation of somatic chromatins in cloned embryos," *The Journal of Biological Chemistry*, vol. 281, no. 9, pp. 6048–6057, 2006.
- [72] M. R. DeBaun, E. L. Niemitz, and A. P. Feinberg, "Association of in vitro fertilization with Beckwith-Wiedemann syndrome and epigenetic alterations of LIT1 and H19," *American Journal of Human Genetics*, vol. 72, no. 1, pp. 156–160, 2003.
- [73] S. Zhang, C. Kubota, L. Yang et al., "Genomic imprinting of H19 in naturally reproduced and cloned cattle," *Biology of Reproduction*, vol. 71, no. 5, pp. 1540–1544, 2004.
- [74] K. Inoue, T. Kohda, J. Lee et al., "Faithful expression of imprinted genes in cloned mice," *Science*, vol. 295, no. 5553, p. 297, 2002.
- [75] L. Yang, P. Chavatte-Palmer, C. Kubota et al., "Expression of imprinted genes is aberrant in deceased newborn cloned calves and relatively normal in surviving adult clones," *Molecular Reproduction and Development*, vol. 71, no. 4, pp. 431–438, 2005.
- [76] S. L. Smith, R. E. Everts, X. C. Tian et al., "Global gene expression profiles reveal significant nuclear reprogramming by the blastocyst stage after cloning," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 102, no. 49, pp. 17582–17587, 2005.
- [77] D. R. Arnold, V. Bordignon, R. Lefebvre, B. D. Murphy, and L. C. Smith, "Somatic cell nuclear transfer alters peri-implantation trophoblast differentiation in bovine embryos," *Reproduction*, vol. 132, no. 2, pp. 279–290, 2006.
- [78] R. De La Fuente, A. Hahnel, P. K. Basrur, and W. A. King, "X inactive-specific transcript (Xist) expression and X chromosome inactivation in the preattachment bovine embryo," *Biology of Reproduction*, vol. 60, no. 3, pp. 769–775, 1999.
- [79] D. S. Goodsell, "The molecular perspective: histone deacetylase," *Stem Cells*, vol. 21, no. 5, pp. 620–621, 2003.
- [80] G. Lagger, D. O'Carroll, M. Rembold et al., "Essential function of histone deacetylase 1 in proliferation control and CDK inhibitor repression," *The EMBO Journal*, vol. 21, no. 11, pp. 2672–2681, 2002.

- [81] J.-H. Lee, S. R. L. Hart, and D. G. Skalnik, "Histone deacetylase activity is required for embryonic stem cell differentiation," *Genesis*, vol. 38, no. 1, pp. 32–38, 2004.
- [82] N. Saunders, A. Dicker, C. Popa, S. Jones, and A. Dahler, "Histone deacetylase inhibitors as potential anti-skin cancer agents," *Cancer Research*, vol. 59, no. 2, pp. 399–404, 1999.
- [83] P. A. Marks, V. M. Richon, R. Breslow, and R. A. Rifkind, "Histone deacetylase inhibitors as new cancer drugs," *Current Opinion in Oncology*, vol. 13, no. 6, pp. 477–483, 2001.
- [84] R. W. Johnstone, "Histone-deacetylase inhibitors: novel drugs for the treatment of cancer," *Nature Reviews Drug Discovery*, vol. 1, no. 4, pp. 287–299, 2002.
- [85] G. Zupkovitz, J. Tischler, M. Posch et al., "Negative and positive regulation of gene expression by mouse histone deacetylase 1," *Molecular and Cellular Biology*, vol. 26, no. 21, pp. 7913–7928, 2006.
- [86] G. Wee, J.-J. Shim, D.-B. Koo, J.-I. Chae, K.-K. Lee, and Y.-M. Han, "Epigenetic alteration of the donor cells does not recapitulate the reprogramming of DNA methylation in cloned embryos," *Reproduction*, vol. 134, no. 6, pp. 781–787, 2007.
- [87] A. E. Iager, N. P. Ragina, P. J. Ross et al., "Trichostatin A improves histone acetylation in bovine somatic cell nuclear transfer early embryos," *Cloning and Stem Cells*, vol. 10, no. 3, pp. 371–379, 2008.
- [88] S. Kishigami, E. Mizutani, H. Ohta et al., "Significant improvement of mouse cloning technique by treatment with trichostatin A after somatic nuclear transfer," *Biochemical and Biophysical Research Communications*, vol. 340, no. 1, pp. 183–189, 2006.
- [89] Y. Tsuji, Y. Kato, and Y. Tsunoda, "The developmental potential of mouse somatic cell nuclear-transferred oocytes treated with trichostatin A and 5-aza-2-deoxycytidine," *Zygote*, vol. 17, no. 2, pp. 109–115, 2009.
- [90] X. Li, Y. Kato, Y. Tsuji, and Y. Tsunoda, "The effects of trichostatin A on mRNA expression of chromatin structure-, DNA methylation-, and development-related genes in cloned mouse blastocysts," *Cloning and Stem Cells*, vol. 10, no. 1, pp. 133–142, 2008.
- [91] Y. Zhang, J. Li, K. Villemoes, A. M. Pedersen, S. Purup, and G. Vajta, "An epigenetic modifier results in improved in vitro blastocyst production after somatic cell nuclear transfer," *Cloning and Stem Cells*, vol. 9, no. 3, pp. 357–363, 2007.
- [92] J. Li, O. Svarcova, K. Villemoes et al., "High in vitro development after somatic cell nuclear transfer and trichostatin A treatment of reconstructed porcine embryos," *Theriogenology*, vol. 70, no. 5, pp. 800–808, 2008.
- [93] J. Zhao, J. W. Ross, Y. Hao et al., "Significant improvement in cloning efficiency of an inbred miniature pig by histone deacetylase inhibitor treatment after somatic cell nuclear transfer," *Biology of Reproduction*, vol. 81, no. 3, pp. 525–530, 2009.
- [94] J. Zhao, Y. Hao, J. W. Ross et al., "Histone deacetylase inhibitors improve in vitro and in vivo developmental competence of somatic cell nuclear transfer porcine embryos," *Cellular Reprogramming*, vol. 12, no. 1, pp. 75–83, 2010.
- [95] M. A. Martinez-Diaz, L. Che, M. Albornoz et al., "Pre- and postimplantation development of swine-cloned embryos derived from fibroblasts and bone marrow cells after inhibition of histone deacetylases," *Cellular Reprogramming*, vol. 12, no. 1, pp. 85–94, 2010.
- [96] L. H. Shi, J. S. Ai, Y. C. OuYang et al., "Trichostatin A and nuclear reprogramming of cloned rabbit embryos," *Journal of Animal Science*, vol. 86, no. 5, pp. 1106–1113, 2008.
- [97] Q. Meng, Z. Polgar, J. Liu, and A. Dinnyes, "Live birth of somatic cell-cloned rabbits following trichostatin a treatment and cotransfer of parthenogenetic embryos," *Cloning and Stem Cells*, vol. 11, no. 1, pp. 203–208, 2009.
- [98] A. Rybouchkin, Y. Kato, and Y. Tsunoda, "Role of histone acetylation in reprogramming of somatic nuclei following nuclear transfer," *Biology of Reproduction*, vol. 74, no. 6, pp. 1083–1089, 2006.
- [99] C. S. Oliveira, N. Z. Saraiva, M. M. De Souza, T. A.D. Tetzner, M. R. De Lima, and J. M. Garcia, "Effects of histone hyperacetylation on the preimplantation development of male and female bovine embryos," *Reproduction, Fertility and Development*, vol. 22, no. 6, pp. 1041–1048, 2010.
- [100] F. D. Jousan, L. A. De Castro E Paula, A. M. Brad, Z. Roth, and P. J. Hansen, "Relationship between group II caspase activity of bovine preimplantation embryos and capacity for hatching," *Journal of Reproduction and Development*, vol. 54, no. 3, pp. 217–220, 2008.
- [101] G. Antunes, A. Chaveiro, P. Santos, A. Marques, H. S. Jin, and F. M. da Silva, "Influence of apoptosis in bovine embryo's development," *Reproduction in Domestic Animals*, vol. 45, no. 1, pp. 26–32, 2010.
- [102] A. Yakovlev, M. Khafizova, Z. Abdullaev, D. Loukinov, and A. Kondratyev, "Epigenetic regulation of caspase-3 gene expression in rat brain development," *Gene*, vol. 450, no. 1–2, pp. 103–108, 2009.
- [103] Y. Koyama, M. Adachi, M. Sekiya, M. Takekawa, and K. Imai, "Histone deacetylase inhibitors suppress IL-2-mediated gene expression prior to induction of apoptosis," *Blood*, vol. 96, no. 4, pp. 1490–1495, 2000.
- [104] S. Ikeda, A. Tatemizo, D. Iwamoto et al., "Enhancement of histone acetylation by trichostatin a during in vitro fertilization of bovine oocytes affects cell number of the inner cell mass of the resulting blastocysts," *Zygote*, vol. 17, no. 3, pp. 209–215, 2009.
- [105] K. Miyoshi, H. Mori, Y. Mizobe et al., "Valproic acid enhances in vitro development and oct-3/4 expression of miniature pig somatic cell nuclear transfer embryos," *Cellular Reprogramming*, vol. 12, no. 1, pp. 67–74, 2010.
- [106] Z. C. Das, M. K. Gupta, S. J. Uhm, and H. T. Lee, "Increasing histone acetylation of cloned embryos, but not donor cells, by sodium butyrate improves their in vitro development in pigs," *Cellular Reprogramming*, vol. 12, no. 1, pp. 95–104, 2010.
- [107] J. Jentink, M. A. Loane, H. Dolk et al., "Valproic acid monotherapy in pregnancy and major congenital malformations," *The New England Journal of Medicine*, vol. 362, no. 23, pp. 2185–2193, 2010.
- [108] B. M. Kluger and K. J. Meador, "Teratogenicity of antiepileptic medications," *Seminars in Neurology*, vol. 28, no. 3, pp. 328–335, 2008.
- [109] G. Wu, C. Nan, J. C. Rollo, X. Huang, and J. Tian, "Sodium valproate-induced congenital cardiac abnormalities in mice are associated with the inhibition of histone deacetylase," *Journal of Biomedical Science*, vol. 17, no. 1, article 16, 2010.
- [110] W. Dean, F. Santos, M. Stojkovic et al., "Conservation of methylation reprogramming in mammalian development: aberrant reprogramming in cloned embryos," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 98, no. 24, pp. 13734–13738, 2001.

- [111] B. P. Enright, L.-Y. Sung, C.-C. Chang, X. Yang, and X. C. Tian, "Methylation and acetylation characteristics of cloned bovine embryos from donor cells treated with 5-aza-2'-deoxycytidine," *Biology of Reproduction*, vol. 72, no. 4, pp. 944–948, 2005.
- [112] W. Shi, A. Hoefflich, H. Flaswinkel, M. Stojkovic, E. Wolf, and V. Zakhartchenko, "Induction of a senescent-like phenotype does not confer the ability of bovine immortal cells to support the development of nuclear transfer embryos," *Biology of Reproduction*, vol. 69, no. 1, pp. 301–309, 2003.