Acta Scientiae Veterinariae, 2011. 39(Suppl 1): s83 - s95.

ISSN 1679-9216 (Online)

Reprogramming somatic cells: pluripotency through gene induction and nuclear transfer

Fabiana Fernandes Bressan, Felipe Perecin, Juliano Rodrigues Sangalli & Flávio Vieira Meirelles

ABSTRACT

***Background*:** The understanding of nuclear reprogramming pathways provides important contributions to applied and basic sciences such as the development of autologous cellular therapies for the treatment of numerous diseases, the improved efficiency of animal-based biotechnology or the generation of functional gametes *in vitro*. Strategies such as nuclear transfer and induced reprogramming have been used to induce somatic cells into an embryonic-like pluripotent state. Both techniques have been routinely performed worldwide, and live offspring have been successfully derived from them, resulting in a proof of efficacy of both techniques. Detailed studies on cellular and molecular mechanisms that mediate reprogramming, however, still require further investigation to develop practical applications in veterinary and human medicine.

***Review*:** Studies on cell reprogramming, differentiation and proliferation have revealed that a core of transcription factors, as for example, OCT4, SOX2 and NANOG, act together promoting cell commitment or pluripotency. Mechanisms of induced reprogramming by pluripotency-related transcription factors forced expression or nuclear transfer seems to be mediated by the same pathways observed in fertilization, eliciting nuclear remodeling and modulating gene expression. However, abnormal chromatin conformation, often leading to disrupted imprinting and atypical gene expression patterns are frequently observed on *in vitro* reprogramming. Strategies used to facilitate nuclear remodeling, such as chromatin modifying agents, as for example, histone deacetilases inhibitors or DNA methyltransferases; or chemicals responsible for the inhibition of development-related pathways, as for example, MEK and GSK3 inhibitors, when used in the *in vitro* culture of cells or embryos, have proved to favors transcriptional regulation and improve reprogramming. Such alternatives are highly prone to enable the routine use of *in vitro* reprogramming in animal production and medical sciences, for example, by promoting the generation of functional male and female functional gametes capable of producing viable offspring. Thus, the properties, deficiencies and implications of induced reprogramming and nuclear transfer techniques in somatic cells were discussed in this review, as well as its probable outcomes.

***Conclusions*:** The combination of both reprogramming techniques - induced reprogramming and nuclear transfer, may be essential to clarify the mechanisms of gene expression that are responsible for induced pluripotency. As discussed here, the mechanisms responsible for triggering the pluripotency status of a somatic cell are probably closely related to the epigenetic changes and gene expression profiles present in early development following fertilization. We report here that the nuclear transfer of SOX2 expressing donor cells resulted in similar rates of embryo production when compared to control cells. A better understanding of the contribution of each reprogramming factor used in induced reprogramming may result in the establishment of strategies aiming to enhance *in vitro* reprogramming performance. Such knowledge will contribute to *in vitro* animal production by increasing the cloning efficiency and regenerative medicine through the derivation and adequate culture of reprogrammed embryonic stem cells.

Keywords: bovine, pluripotency, reprogramming, nuclear transfer.

Faculdade de Zootecnia e Engenharia de Alimentos, Universidade de São Paulo (USP), Pirassununga, SP, Brazil. CORRESPONDENCE: F.F. Bressan or F.V. Meirelles [fabianabressan@usp.br - meirellf@usp.br]. Faculty of Animal Sciences and Food Engineering - FZEA, University of São Paulo (USP), CEP 13635-900 Pirassununga, SP, Brazil.

I. INTRODUCTION

Increased efficiency and control of nuclear reprogramming is desirable for developing therapeutics to allow the derivation of autologous stem cells in cellular therapies and for animal production because increased efficiency and control are indispensible for the success of several assisted reproductive technologies (ARTs). Several studies and reviews have attempted to elucidate the molecular mechanisms involved in cellular pluripotency. However, the exact mechanism of cellular reprogramming is not completely understood.

The process of reprogramming into an undifferentiated stage similar to the embryonic pluripotency has been performed through the transfer of a somatic nucleus into an oocyte (somatic cell nuclear transfer (SCNT, or cloning [126]). Another process that is capable of reprogramming a cell is cellular fusion. In this technique, one somatic cell is fused with an embryonic cell, which reprograms the somatic cell into a pluripotent stage similar to the embryonic pluripotent stage [22]. More recently, the direct reprogramming of the somatic cell has been achieved through a revolutionary experiment that was based on the introduction and expression of known transcription factors, which have been designated as pluripotency induction factors [112].

II. REPROGRAMMING AND PLURIPOTENCY

In mammals, early embryonic development is characterized by the induction of totipotency, which reprograms male and female nuclei into an undifferentiated state, resulting in the formation of the zygote and the initiation of embryonic development. Consecutively, the process of embryonic development leads to the differentiation of cells into two different cell lineages, the inner cell mass and the trophectoderm, which are visually distinguishable at the blastocyst stage. The trophoblast originates part of extra-embryonic tissues, whereas cells from inner cell mass, which are classified as pluripotent cells, exhibit the ability to differentiate into one of the three following germ layers: endoderm, mesoderm and ectoderm. These cells will give rise to more than 200 different cellular types that constitute an organism [85]. Because no genetic modifications are involved in the developmental determination and cellular differentiation, these events depend on epigenetic modifications to drive the expression of specific genes [5].

Initial experiments on the mechanisms of cell differentiation and reprogramming have revealed that pluripotent cells express a group of genes that are responsible for the dedifferentiation or pluripotency status. *OCT4* and *NANOG* were first identified as fundamental transcription factors for early embryonic development and the maintenance of stem cell pluripotency. *SOX2*, which is another transcription factor, heterodimerizes with *OCT4* and regulates several genes in pluripotent cells [12]. *OCT4*, *SOX2* and *NANOG* are essential but not unique transcription factors to regulate the pluripotent status in embryonic cells. Aside from the triad*OCT4*, *SOX2* and *NANOG*, many pluripotent factors have been identified such as *SALL4*, *DAX1*, *ESSRB*, *TBX3*, *TCL1*, *RIF1* and *NAC1* [51]. A connected network of regulatory genes is responsible for the development or maintenance of the pluripotency status of embryos in a complex and probably species-dependent manner [6]. Even in primordial germ cells, several pluripotent genes, including *OCT4*, *SOX2* and *NANOG*, are expressed in embryonic cells. The expression of these genes is reduced with the progression of gonadal cell differentiation into gametes [2].

Not only the transcription factors bind their DNA target sites, the proteins interact with each other and also with other chromatin remodeling factors and enzymes that regulate histone modifications to modulate chromatin structure and, consequently, gene expression [17,124].

Transcriptional regulation is an essential mechanism for the differentiation, dedifferentiation or maintenance of pluripotency and acts on normal or induced processes of reprogramming such as those

mediated by the fertilization of the oocyte by spermatozoa, the cytoplasm in SCNT or the fusion of somatic and stem cells and in directly induced pluripotency.

III. REPROGRAMMING THROUGH SOMATIC CELL NUCLEAR TRANSFER

The factors that determine the ability of the oocyte cytoplasm to reprogram the somatic cell nucleus have been under investigation for a long time. The first experiment on nuclear transfer was performed over 50 years ago and demonstrated that the development of embryos or adult individuals may be obtained after the injection of cells in the embryonic stage or the injection of differentiated cells into enucleated oocytes of amphibians, respectively [14].

Notably, the reprogramming ability of the oocytes is not restricted to the embryonic nucleus. The birth of Dolly, which was the first viable mammal that was produced by SCNT, has shown that the oocyte can reprogram a highly differentiated nucleus into a pluripotent state and facilitate the development of viable animals.

The reprogramming of differentiated cells into pluripotent embryonic cells using SCNT has been successfully applied in several species, including humans [31]. Moreover, several farm, pet and laboratory animals have already been cloned, e.g., bovine, caprine, ovine, swine, equine, rabbits, mice, ferrets, camels, cats, dogs and wolves [3,53].

Although SCNT reprograms a differentiated nucleus, it remains an inefficient technique. Commonly, less than 5% of produced embryos generate healthy adult animals [19,125]. Several studies have demonstrated nuclear reprogramming deficiencies in cloned embryos [11,25] leading to problems such as placental dysfunctions, large offspring syndrome, and hepatic and respiratory complications [39,72].

Upon the formation of pluripotent cells after fertilization, chromatin that is derived from male and female gametes is independently demethylated. The paternal genome is actively demethylated, probably through the oxidation of 5-methylcitosine followed by the substitution for non-methylated cytosine [50], whereas the maternal genome is passively demethylated during first embryonic cleavages. For normal embryonic development, these processes are followed by *de novo*methylation, which is essential for establishing embryonic patterns of gene expression, X chromosome inactivation in females and the maintenance of genomic imprinting, which is established in germinal lineages [83].

Remodeled global patterns of chromatin methylation are designated as nuclear remodeling and are incomplete in SCNT-derived embryos. The nucleus frequently fails to reestablish an embryonic pattern of chromatin modifications, resulting in abnormal expression patterns of genes that are related to early development [7]. Cloned bovine embryos show an incomplete wave of demethylation followed by a precocious *de novo* methylation [128]. Therefore, the reprogramming of global methylation in cloned embryos is different from *in vivo*-derived embryos. The methylation patterns of cloned embryos are similar to somatic cells indicating that cloned embryos undergo only a partial remodeling of the somatic genome [9]. These abnormal patterns are frequently associated with the loss of monoallelic expression of imprinted genes, thus compromising the normal development, growth and placental function of these embryos.

IV. FACILITATING NUCLEAR REMODELING IN SCNT

Numerous factors play a role in SCNT efficiency, and the failure of the donor nucleus to promote epigenetic reprogramming seems to be a central cause of low SCNT efficiency [25,]. Because nuclear remodeling is not complete, strategies that facilitate the relaxation of chromatin may be useful to enable nuclear reprogramming. Several approaches that are mainly based on the effects of proteins and transcription factors on chromatin have been described. Such approaches include the potential transfer of chromosomes and chromatin by increasing the probability of cytoplasmic proteins that are freely exchanged from the oocyte to the introduced genetic material, reducing the amount of proteins from somatic origin. It is also highlighted the relaxation of chromatin chromatin-modifying agents (CMAs). CMAs such as histone deacetylases inhibitors (HDACi) and DNA methyltransferases have emerged as important tools to study nuclear reprogramming mechanisms, to correct epigenetic failures and

improve the production of cloned animals [48].

The inhibition of histone deacetylases in nuclei donor cells or reconstructed embryos with CMAs such as trichostatin A (TSA) or *Scriptaid* 6-(1,3-dioxo-1H,3H-benzo[de]isoquinolin-2-yl)-hexanoic acid hydroxyamide) have been effective to increase the global acetylation of histones, which facilitates transcription. In addition, modifying DNA methylation using chemicals such as 5-aza-2β-deoxycytidine is a possible approach to facilitate reprogramming in cultured donor cells or reconstructed embryos.

Numerous studies have reported the use of CMAs in cultured donor cells before SCNT or cloned embryos after activation. Some groups have reported increased blastocyst production [1,21,59], quality and *in vivo* development [120]. Adverse effects have been observed with high doses of CMAs.

The effects of these drugs on pregnancy rates and the development to term are rare in bovine. Reasons include the extended length of gestation and the high cost of recipients, which hinder further studies that evaluate the impact of these drugs on development in bovine. To our knowledge, one unique study has described a positive effect of CMAs on the development of viable cloned calves.

Similarly, it is postulated that cloning efficiency is inversely correlated to the differentiation status of the donor cell, suggesting that an undifferentiated nucleus is more likely to be remodeled and reprogrammed [36]. SCNT procedures using embryonic cells [47] or differentiated somatic cells such as lymphocytes and fibroblasts [35] have demonstrated that the use of undifferentiated cells results in higher efficiency [40], suggesting that using a compromised nucleus reduces success rates [8,47].

Data from our laboratory has indicated that efficient cloning is required for the production of transgenic animals with high and homogeneous transgene expression and it is improved by a second round of nuclear transfer. These data corroborated earlier studies, and we speculated that this effect was caused by the use of a cell lineage that has previously been successfully reprogrammed by SCNT [32,57].

Therefore, the selection of cell populations that are, somehow, amenable to reprogramming using the ooplasm may be important to increase the cloning efficiency [105].

V. GENETICALLY INDUCED PLURIPOTENCY – NEW POSSIBILITIES

In 2006, directly induced dedifferentiation was established through the incorporation of known transcription factors into the mouse and human genomes. Takahashi and Yamanaka have shown that the expression of four transcription factors, *OCT3/4*, *SOX2*, *KLF4* and *C-MYC* (represented by OSKM), is sufficient for the induction of fibroblasts into pluripotency. Induced pluripotent stem cells (iPSCs) retain most of the characteristics of embryonic stem cells, including a high proliferating rate and the ability to form many tissues from the three germinal layers *in vitro* and *in vivo*.

Hundreds of studies have been published since 2006 to confirm the consistency of induced pluripotency in mice and human somatic cells and, more recently, in rats, rabbits, dogs, pigs, non-human primates, sheep and cattle through the expression of the *OSKM* factors in several combinations, in addition to *NANOG*, *LIN28* and *TCL-1A*, which are genes that belong to a connected network of embryonic pluripotency as previously described [38,44,45,64,86, 108,130].

Interestingly, iPSCs have been characterized in some species such as bovine, ovine and equine, in which "true" embryonic stem cells had not been derived. The lack of defined characteristics in these species has been reported by several studies. Because these species do not show the same characteristics that are used to classify embryonic stem cells [114], the pluripotent cells are designated "stem cell-like". After the derivation of iPSCs, seems that the main mechanisms of pluripotency in mammals use a constitutive pathway independent of peculiarities within each species.

The iPS cells are extremely promising for the study and therapeutic development for human diseases [89] because their similarities to embryonic stem cells, regarding self-renewal and the development of originating tissues from the three germ layers. However, its clinical usage is limited for several reasons, including a low reprogramming efficiency and genomic alterations arising from viral integration. The derivation of these cells must be improved to allow its therapeutic use without restrictions [65]. On the other hand, iPSCs are a unique model to elucidate the mechanisms of the genetic induction into pluripotency and

differentiation as well as cellular commitment, which is usually represented by C. H. Waddington's developmental potential model (*epigenetic landscape model*; Waddington, 1957; [43]).

The induction of pluripotency without transgene integration in human and mouse genomes has been obtained through the continuous transfection of non-integrating OSKM cDNA in the genome of the cell, e.g., utilizing non-integrative adenovirus [107] or expression plasmids and episomes [87]. Recent strategies such as mRNA and protein approaches have also been effective [62] and represent an important step toward its use in cell therapy. Nonetheless, these strategies are less efficient when compared with lentiviral transduction.

VI. FACILITATING INDUCED PLURIPOTENCY

The generation efficiency of cells that are completely pluripotent after genetic induction is usually lower at 0.01% to 0.1% than that related to SCNT at less than 5% (reviewed by [43]). Microarray-based gene expression analysis has revealed that the pattern of the global gene expression of iPS cells is similar to those of embryonic stem cells (ESCs) and not those from fibroblasts. However, differences between iPSCs and ESCs are evident [112,134].

SCNT has a low efficiency that is mostly credited to the failure of donor cell epigenetic reprogramming. Unsurprisingly, the epigenetic reprogramming of iPSCs is considered abnormal. Methylation abnormalities of genes such as *OCT3/4* and *NANOG* have been reported. Studies on the imprinting of these cells have shown that, similarly to cloned embryos, a significant number of lineages exhibits an abnormal expression of imprinted genes, including *H19*, *IGF2R*, *PEG3* and *MEG3*[93]. However, similar to SCNT, the ability of these cells to generate mice to term has been demonstrated.

The "partially reprogrammed" status has been reported and characterized in iPSCs. In 2009, Chan and collaborators reported that the expression of commonly used stem-cell markers as follows: alkaline phosphatase, SSEA-4, GDF3, hTERT and NANOG. These markers do not distinguish between partially and fully reprogrammed colonies. In contrast, transgene silencing, TRA-1-60, DNMT3B and REX1 expression, are found in colonies that are considered "true" (completely reprogrammed colonies [16]).

The treatment of these partially reprogrammed cells with signaling cascade inhibitors, e.g., inhibitors against extracellular signal-related kinase (ERK) and glycogen synthase kinase 3 (GSK3) signaling pathways, have enabled the conversion of partially reprogrammed colonies into fully reprogrammed cells [104,129].

Interestingly, the utilization of inhibitors and CMAs seems to favors the derivation of iPSCs in species with ESCs that have not been reported as "true" ESCs. Two interesting examples occur in rat and bovine, in which probably the same factors that support induced pluripotency may exert favorable roles in nuclear transfer by becoming collaborators in reprogramming events or by increasing the success rates. These factors, then, could enable the routine use of inhibitors and CMAs on animal production. In rats, the derivation of "true" ESCs was not reported until 2008 [15], when the use of inhibitors against the FGF receptor, MEK (MEK*i*) and GSK3 pathways (GSK3*i*, designated 3i) or only MEK*i* and GSK3*i* (designated 2i) enabled the derivation. Therefore, the use of these and other chemicals has been tested on the derivation of iPSCs from unconventional species [79].

VII. WHEN REPROGRAMMING CLOSES THE CYCLE – BACK TO THE GAMETES

Male and female gametes are required for the continuation and evolution of species and are the main resource for a vast number of reproductive biotechnologies. Therefore, they are largely studied and manipulated directly in *in vitro* fertilization and culture or indirectly through hormonal stimulation in infertility treatments.

The specialization of the pluripotent inner cell mass occurs during cellular differentiation at post-blastocyst embryonic stage to form the epiblast and the hypoblast. A part of the epiblast is comprised of the cells that are highly related to the extra-embryonic ectoderm and acquires the property of contribution to the germ line [71]. This process is regulated by factors that are secreted by extra-embryonic cells (e.g., bone morphogenetic protein or BMP) which participate in a regulatory gene pathway, which will induce the development of primordial germ cells (PGCs) that are responsible for gamete generation.

Structures that are similar to male or female

gametes have been derived *in vitro* from human, mice and swine or from human and mouse stem cell populations, respectively (for a review, see). Embryonic stem cells, when cultured in monolayers or in suspension (hanging drops) without pluripotency-maintaining factors for days or weeks tend to spontaneously differentiate into cells that are compromised with one of the three germ layers. Amongst these structures, cells that are similar to PGCs may be observed [46,123]. PGCs have been derived from mouse ESC populations and the culture of swine fetal skin-derived cells that have been supplemented with follicular liquid, probably derived from a subpopulation that may be similar to other undifferentiated cells [26,].

These ESC-derived PGCs develop into structures that are similar to oogonia, which may undergo meiosis, recruit adjacent cells to form follicle-like structures, and mediate the embryonic development into blastocyst from spontaneous parthenogenesis [27]. Similar to female gametes, male germ cells have been reported to form blastocysts and viable adult animals after intracytoplasmic injections (ICSI) [33,115]. Despite the difficulties associated with the generation of human PGCs *in vitro* compared with that of mouse PGCs (revised by [90]), the possibility of using these cells as a tool for male and female infertilities and to provide autologous oocytes for many embryonic biotechnological techniques such as SCNT is invaluable.

The induction into germinal lineages may be a rare event, and the similarities between this process and the natural process requires further investigation. Interestingly, these cells seem to lack *Igf2r* and -*H19* imprints, which are representative characteristics of germ cells [33]. However, similar to studies on nuclear transfer reprogramming and the direct reprogramming by transcription factors, Nayernie and collaborators in 2006 identified remodeling events in DNA methylation with disruptions that lead to an incomplete epigenetic reprogramming of male germ cells *in vitro*.

Obtaining functional gametes *in vitro* may become as intriguing as the success of IVF babies 40 years ago. Using epigenetic studies of induced and cloned models, the ability of iPSCs to produce functional gametes may be used for the autologous treatment of several infertilities and avoid the ethical problems that are related to embryos that are produced using SCNT.

VIII. SCNT AND IPS CELLS: CONCLUSIONS AND FUTURE CHALLENGES

It is known that the reprogramming events are mediated by known transcription factors, which leads to chromatin remodeling and alter gene expression to create an ESC-like status and pluripotent cells. Induced reprogramming has raised questions concerning the manner by which reprogramming factors execute alterations in gene expression. These questions apply to reprogramming events that are induced by the oocyte cytoplasm during SCNT or the cytoplasm of ESCs after cellular fusion [22].

A better understanding of the contribution of each reprogramming factor and the detailed characterization of each step of reprogramming in cloned embryos and iPSCs are required for the elucidation of the molecular basis of nuclear reprogramming.

The bovine model provides great benefits to analyze reprogramming. The characterization of ESC-like cells in these species requires further investigation because several research groups have reported conflicting characterizations [34,119]. The production of bovine iPSCs has been recently reported. In addition, bovine species shows a reasonable rate of *in vivo* development after nuclear transfer, which is indicated by the thousands of animals that are produced to term. Therefore, the bovine model is useful for understand the reprogramming events that are induced by the cytoplasm or specific reprogramming factors.

The strategies for induced pluripotency and nuclear transfer should be studied together unearthing the deficiencies of both techniques. If the mechanisms of nuclear reprogramming through oocytes are understood, this information will be used to augment the conversion efficiency of fibroblasts into iPSCs. Conversely, if the mechanisms of gene expression that are responsible for induced pluripotency are elucidated, these mechanisms can be applied in nuclear transfer experiments. Moreover, iPSCs may be the next logical step for cloning if the concept of facility in reprogramming cells more amenable to reprogramming remains valid.

In fibroblast-derived iPSCs, the integration of 10 to 20 copies of each gene in the cellular genome and the varying proportions of each OKSM factor have been reported [68,112]. These studies have suggested that different relative quantities of each factor are important for reprogramming. Such assumptions have been confirmed by Papapetrou and collaborators in 2009, which have reported that a precise stoichiometry between each factor is critical for the efficient reprogramming of iPSCs through a mechanism that involved the use of reporter genes for each of the for pluripotency-inducing factors. This system has enabled the detailed study of the kinetics of the four transgenes silencing during reprogramming [88].

Based on such mechanisms, our group studied the effect of exogenous *SOX2* expression on nuclear reprogramming trough nuclear transfer (Bressan and collaborators, unpublished data). We observed that donor cells expressing exogenous *SOX2* did not differ from non-genetically modified cells in the production of cloned embryos *in vitro*. The effects of each pluripotency-related transcription factor in SCNT-produced embryos should reveal unique or common molecular mechanisms for both SCNT and iPS techniques.

REFERENCES

1 Akagi S., Matsukawa K., Mizutani E., Fukunari K., Kaneda M., Watanabee S. & Takahashi S. 2011. Treatment with a histone deacetylase inhibitor after nuclear transfer improves the preimplantation development of cloned bovine embryos. *Journal of Reproduction and Development*. 57(1): 120-126.

2 Avilion A.A., Nicolis S.K., Pevny L.H., Perez L., Viviane N. & Lovell-Badge R. 2003. Multipotent cell lineages in early mouse development depend on SOX2 function. *Gene and Development*. 17(1): 126-140.

3 Baguisi A., Behboodi E., Melican D.T., Pollock J.S., Destrempes M.M., Cammuso C., Williams J.L., Nims S.D., Porter D.A., Midura P., Palacios M.J., Ayres S.L., Denniston R.S., Hayes M.L., Ziomek C.A., Meade H.M., Godke R.A., Gavin W.G., Overstrome E.W. & Echelard Y. 1999. Production of goats by somatic cell nuclear transfer. *Nature Biotechnology*. 17(5): 456-461.

4 Bao L., He L., Chen J., Wu Z., Liao J., Rao L., Ren J., Li H., Zhu H., Qian L., Gu Y., Dai H., Xu X., Zhou J., Wang W., Cuie C. & Xiao L. 2011. Reprogramming of ovine adult fibroblasts to pluripotency via drug-inducible expression of defined factors. *Cell Research*. 21(4): 600-608.

5 Beisel C. & Paro R. 2009. Dissection of gene regulatory networks in embryonic stem cells by means of high-throughput sequencing. *Journal of Biological Chemistry*. 390(11): 1139-1144.

6 Berg D.K., Smith C.S., Pearton D.J., Wells D.N., Broadhurst R., Donnisone M. & Pfeffer P.L. 2011. Trophectoderm lineage determination in cattle.*Developmental Cell*. 20(2): 244-255.

7 Bird A. 2002. DNA methylation patterns and epigenetic memory. *Genes and Development*. 16(1): 6-21.

8 Blelloch R., Wang Z., Meissner A., Pollard S., Smithe A. & Jaenisch R. 2006. Reprogramming efficiency following somatic cell nuclear transfer is influenced by the differentiation and methylation state of the donor nucleus. *Stem Cells*. 24(9): 2007-2013.

9 Bonk A.J., Cheong H.T., Li R., Lai L., Hao Y., Liu Z., Samuel M., Fergason E.A., Whitworth K.M., Murphy C.N., Antonioue E. & Prather R.S. 2007. Correlation of developmental differences of nuclear transfer embryos cells to the methylation profiles of nuclear transfer donor cells in Swine.*Epigenetics*. 2(3): 179-186.

10 Bortvin A., Eggan K., Skaletsky H., Akutsu H., Berry D.L., Yanagimachi R., Pagee D.C. & Jaenisch R. 2003. Incomplete reactivation of Oct4-related genes in mouse embryos cloned from somatic nuclei. *Development*. 130(8): 1673-1680.

11 Bourc'his D., Le Bourhis D., Patin D., Niveleau A., Comizzoli P., Renarde J.P. & Viegas-Pequignot E. 2001. Delayed and incomplete reprogramming of chromosome methylation patterns in bovine cloned embryos. *Current Biology*. 11(19): 1542-1546.

12 Boyer L.A., Lee T.I., Cole M.F., Johnstone S.E., Levine S.S., Zucker J.P., Guenther M.G., Kumar R.M., Murray H.L., Jenner R.G., Gifford D.K., Melton D.A., Jaenische R. & Young R.A. 2005. Core transcriptional regulatory circuitry in human embryonic stem cells. *Cell*. 122(6): 947-956.

13 Bressan F.F., Dos Santos Miranda M., Perecin F., De Bem T.H., Pereira F.T., Russo-Carbolante E.M., Alves D., Strauss B., Bajgelman M., Krieger J.E., Binellie M. & Meirelles F.V. 2011. Improved production of genetically modified fetuses

with homogeneous transgene expression after transgene integration site analysis and recloning in cattle. *Cell Reprogram*. 13(1): 29-36.

14 Briggs R. & King T. J. 1952. Transplantation of living nuclei from blastula cells into enucleated frogs' eggs. *Proceedings of the National Academy of Science*. 38(5): 455-463.

15 Buehr M., Meek S., Blair K., Yang J., Ure J., Silva J., Mclay R., Hall J., Ying Q.L. & Smith A. 2008. Capture of authentic embryonic stem cells from rat blastocysts. *Cell*. 135(7): 1287-1298.

16 Chan E.M., Ratanasirintrawoot S., Park I.H., Manos P.D., Loh Y.H., Huo H., Miller J.D., Hartung O., Rho J., Ince T.A., Daleye G.Q. & Schlaeger T.M. 2009. Live cell imaging distinguishes bona fide human iPS cells from partially reprogrammed cells. *Nature Biotechnology*. 27(11): 1033-1037.

17 Chen L. & Daley G.Q. 2008. Molecular basis of pluripotency. *Human Molecular Genetics*. 17(R1): R23-27.

18 Cho H.J., Lee C.S., Kwon Y.W., Paek J.S., Lee S.H., Hur J., Lee E.J., Roh T.Y., Chu I.S., Leem S.H., Kim Y., Kang H.J., Park Y.B. & Kim H.S. 2010. Induction of pluripotent stem cells from adult somatic cells by protein-based reprogramming without genetic manipulation. *Blood*. 116(3): 386-395.

19 Cibelli J. 2007. Development. Is therapeutic cloning dead? *Science*. 318(5858): 1879-1880.

20 Cibelli J.B., Stice S.L., Golueke P.J., Kane J.J., Jerry J., Blackwell C., Ponce De Leone F.A. & Robl J.M. 1998. Cloned transgenic calves produced from nonquiescent fetal fibroblasts. *Science*. 280(5367): 1256-1258.

21 Costa-Borges N., Santaloe J. Ibanez E. 2011. Comparison between the effects of valproic acid and trichostatin A on the *in vitro* development, blastocyst quality, and full-term development of mouse somatic cell nuclear transfer embryos. *Cell Reprogram*. 12(4): 437-446.

22 Cowan C.A., Atienza J., Meltone D.A. & Eggan K. 2005. Nuclear reprogramming of somatic cells after fusion with human embryonic stem cells. *Science*. 309(5739): 1369-1373.

23 Cui X.S., Xu Y.N., Shen X.H., Zhang L.Q., Zhange J.B. & Kim J.H. 2011. Trichostatin a modulates apoptotic-related gene expression and improves embryo viability in cloned bovine embryos. *Cell Reprogram*. 13(2): 179-89.

24 Daley G.Q. 2007. Gametes from embryonic stem cells: a cup half empty or half full? *Science*. 316(5823): 409-410.

25 Dean W., Santos F., Stojkovic M., Zakhartchenko V., Walter J., Wolfe E. & Reik W. 2001. Conservation of methylation reprogramming in mammalian development: aberrant reprogramming in cloned embryos. *Proceeding of the National Academic of Science*. 98(24): 13734-13738.

26 Dyce P.W. & Li J. 2006. From skin cells to ovarian follicles? *Cell Cycle*. 5(13): 1371-1375.

27 Dyce P.W., Shen W., Huynh E., Shao H., Villagomez D.A., Kidder G.M., Kinge W.A. & Li J. 2011. Analysis of oocyte-like cells differentiated from porcine fetal skin-derived stem cells. *Stem Cells and Development*. 20(5): 809-819.

28 Eilertsen K.J., Power R.A., Harkinse L.L. & Misica 2007. Targeting cellular memory to reprogram the epigenome, restore potential, and improve somatic cell nuclear transfer. *Animal Reproduction Science*. 98(1-2): 129-146.

29 Enright B.P., Kubota C., Yange X. & Tian X.C. 2003. Epigenetic characteristics and development of embryos cloned from donor cells treated by trichostatin A or 5-aza-2'-deoxycytidine. *Biology of Reproduction*. 69(3): 896-901.

30 Esteban M.A., Xu J., Yang J., Peng M., Qin D., Li W., Jiang Z., Chen J., Deng K., Zhong M., Cai J., Laie L. & Pei D. 2009. Generation of induced pluripotent stem cell lines from Tibetan miniature pig. *Journal of Biological Chemistry*. 284(26): 17634-17640.

31 French A.J., Adams C.A., Anderson L.S., Kitchen J.R., Hughese M.R. & Wood S.H. 2008. Development of human cloned blastocysts following somatic cell nuclear transfer with adult fibroblasts. *Stem Cells*. 26(2): 485-493.

32 Fujimura T., Murakami H., Kurome M., Takahagi Y., Shigehisae T. & Nagashima H. 2008. Effects of recloning on the efficiency of production of alpha 1,3-galactosyltransferase knockout pigs. *Journal of Reproduction and Development*. 54(1): 58-62.

33 Geijsen N., Horoschak M., Kim K., Gribnau J., Eggan K. & Daley G.Q. 2004. Derivation of embryonic germ cells and male gametes from embryonic stem cells. *Nature*. 427(6970): 148-154.

34 Gjorret J.O. & Maddox-Hyttel P. 2005. Attempts towards derivation and establishment of bovine embryonic stem cell-like cultures. *Reproduction, Fertility and Development*. 17(1-2): 113-124.

35 Gong G., Dai Y., Zhu H., Wang H., Wang L., Li R., Wan R., Liue Y. & Li N. 2004. Generation of cloned calves from different types of somatic cells.*Science in China Series C: Life Science*. 47(5): 470-476.

36 Green A.L., Wellse D.N. & Oback B. 2007. Cattle cloned from increasingly differentiated muscle cells. *Biology of Reproduction*. 77(3): 395-406.

37 Gurdon J.B., Elsdalee T.R., Fischberg M. 1958. Sexually mature individuals of *Xenopus laevis* from the transplantation of single somatic nuclei. *Nature*. 182(4627): 64-65.

38 Han J.W. & Yoon Y.S. 2011. Induced Pluripotent Stem Cells: Emerging Techniques for Nuclear Reprogramming. *Antioxid Redox Signal*. May: 1-20.

39 Heyman Y., Chavatte-Palmer P., Lebourhis D., Camous S., Vignone X. & Renard J.P. 2002. Frequency and occurrence of late-gestation losses from cattle cloned embryos. *Biology of Reproduction*. 66(1): 6-13.

40 Hiiragi T. & Solter D. 2005. Reprogramming is essential in nuclear transfer. *Molecular Reproduction and Development*. 70(4): 417-421.

41 Hill J.R., Roussel A.J., Cibelli J.B., Edwards J.F., Hooper N.L., Miller N.W., Thompson J.A., Looney C.R., Westhusin M.E., Roble J.M. & Stice S.L. 1999. Clinical and pathologic features of cloned transgenic calves and fetuses (13 case studies). *Theriogenology*. 51(8): 1451-1465.

42 Hochedlinger K. & Jaenisch R. 2002. Nuclear transplantation: lessons from frogs and mice. *Current opinions in Cell Biology* 14(6): 741-748.

43 Hochedlinger K. & Plath K. 2009. Epigenetic reprogramming and induced pluripotency. *Development*. 136(4): 509-523.

44 Honda A., Hirose M., Hatori M., Matoba S., Miyoshi H., Inouee K. & Ogura A. 2010. Generation of induced pluripotent stem cells in rabbits: potential experimental models for human regenerative medicine. *Journal of Biological Chemistry*. 285(41): 31362-31369.

45 Huangfu D., Osafune K., Maehr R., Guo W., Eijkelenboom A., Chen S., Muhlesteine W. & Melton D.A. 2008. Induction of pluripotent stem cells from primary human fibroblasts with only Oct4 and Sox2. *Nature Biotechnology*. 26(11): 1269-1275.

46 Hubner K., Fuhrmann G., Christenson L.K., Kehler J., Reinbold R., De La Fuente R., Wood J., Strauss 3rd J.F., Boianie M. & Scholer H.R. 2003. Derivation of oocytes from mouse embryonic stem cells. *Science*. 300(5623): 1251-1256.

47 Humpherys D., Eggan K., Akutsu H., Hochedlinger K., Rideout W.M. 3rd, Biniszkiewicz D., Yanagimachie R. & Jaenisch R. 2001. Epigenetic instability in ES cells and cloned mice. *Science*. 293(5527): 95-97.

48 Iager A.E., Ragina N.P., Ross P.J., Beyhan Z., Cunniff K., Rodrigueze R.M. & Cibelli J.B. 2008. Trichostatin A improves histone acetylation in bovine somatic cell nuclear transfer early embryos. *Cloning Stem Cells*. 10(3): 371-379.

49 Inoue K., Wakao H., Ogonuki N., Miki H., Seino K., Nambu-Wakao R., Noda S., Miyoshi S., Koseki H., Taniguchie M. & Ogura A. 2005. Generation of cloned mice by direct nuclear transfer from natural killer T cells. *Current Biology*. 15(12): 1114-1118.

50 Iqbal K., Jin S.G., Pfeifere G.P. & Szabo P.E. 2011. Reprogramming of the paternal genome upon fertilization involves genome-wide oxidation of 5-methylcytosine. *Proceeding of the National Academy of Science*. 108(9): 3642-3647.

51 Ivanova N., Dobrin R., Lu R., Kotenko I., Levorse J., Decoste C., Schafer X., Lune Y. & Lemischka I.R. 2006. Dissecting self-renewal in stem cells with RNA interference. *Nature*. 442(7102): 533-538.

52 Johnson B.V., Shindo N., Rathjen P.D., Rathjene J. & Keough R.A. 2008. Understanding pluripotency: how embryonic stem cells keep their options open. *Molecular Human Reproduction*. 14(9): 513-520.

53 Kato Y., Tani T., Sotomaru Y., Kurokawa K., Kato J., Doguchi H., Yasuee H. & Tsunoda Y. 1998. Eight calves cloned from somatic cells of a single adult. *Science*. 282(5396): 2095-2098.

54 Kim J.B., Zaehres H., Wu G., Gentile L., Ko K., Sebastiano V., Arauzo-Bravo M.J., Ruau D., Han D.W., Zenkee M. & Scholer H.R. 2008. Pluripotent stem cells induced from adult neural stem cells by reprogramming with two factors. *Nature*. 454(7204): 646-650.

55 Kim M.K., Jang G., Oh H.J., Yuda F., Kim H.J., Hwang W.S., Hossein M.S., Kim J.J., Shin N.S., KangeS.K. & Lee B.C. 2007. Endangered wolves cloned from adult somatic cells. *Cloning Stem Cells*. 9(1): 130-137.

56 Kishigami S., Mizutani E., Ohta H., Hikichi T., Thuan N.V., Wakayama S., Buie H.T. & Wakayama T. 2006. Significant improvement of mouse cloning technique by treatment with trichostatin A after somatic nuclear transfer. *Biochemical and Biophysical Research Communications*. 340(1): 183-189.

57 Kuroiwa Y., Kasinathan P., Choi Y.J., Naeem R., Tomizuka K., Sullivan E.J., Knott J.G., Duteau A., Goldsby R.A., Osborne B.A., Ishidae I. & Robl J.M. 2002. Cloned transchromosomic calves producing human immunoglobulin. *Nature Biotechnology*. 20(9): 889-294.

58 Lee M.J., Kim S.W., Lee H.G., Im G.S., Yang B.C., Kime N.H. & Kim D.H. 2011. Trichostatin a promotes the development of bovine somatic cell nuclear transfer embryos. *Journal of Reproduction and Development*. 57(1): 34-42.

59 Li J., Svarcova O., Villemoes K., Kragh P.M., Schmidt M., Bogh I.B., Zhang Y., Du Y., Lin L., Purup S., Xue Q., Bolund L., Yang H., Maddox-Hyttel P. & Vajta G. 2008. High *in vitro* development after somatic cell nuclear transfer and trichostatin A treatment of reconstructed porcine embryos.*Theriogenology*. 70(5): 800-808.

60 Li P., Tong C., Mehrian-Shai R., Jia L., Wu N., Yan Y., Maxson R.E., Schulze E.N., Song H., Hsieh C.L., Pera M.F. & Ying Q.L. 2008. Germline competent embryonic stem cells derived from rat blastocysts. *Cell.* 135(7): 1299-1310.

61 Li Y., Cang M., Lee A.S., Zhange K. & Liu D. 2011. Reprogramming of sheep fibroblasts into pluripotency under a drug-inducible expression of mouse-derived defined factors. *PLoS One*. 6(1): e15947.

62 Li Z., Yang C.S., Nakashimae K. & Rana T.M. 2011. Small RNA-mediated regulation of iPS cell generation. *EMBO Journal*. 30(5): 823-834.

63 Linher K., Dyce P. & Li J. 2009. Primordial germ cell-like cells differentiated *in vitro* from skin-derived stem cells. PLoS One. 4(12): e8263.

64 Liu H., Zhu F., Yong J., Zhang P., Hou P., Li H., Jiang W., Cai J., Liu M., Cui K., Qu X., Xiang T., Lu D., Chi X., Gao G., Ji W., Dinge M. & Deng H. 2008. Generation of induced pluripotent stem cells from adult rhesus monkey fibroblasts. *Cell Stem Cell*. 3(6): 587-590.

65 Liu N., Lu M., Tiane X. & Han Z. 2007. Molecular mechanisms involved in self-renewal and pluripotency of embryonic stem cells. *Journal of Cellular Physiology.* 211(2): 279-286.

66 Loh Y.H., Wu Q., Chew J.L., Vega V.B., Zhang W., Chen X., Bourque G., George J., Leong B., Liu J., Wong K.Y., Sung K.W., Lee C.W., Zhao X.D., Chiu K.P., Lipovich L., Kuznetsov V.A., Robson P., Stanton L.W., Wei C.L., Ruan Y., Lime B. & Ng H.H. 2006. The Oct4 and Nanog transcription network regulates pluripotency in mouse embryonic stem cells. *Nature Genetics*. 38(4): 431-440.

67 Lucifero D., Suzuki J., Bordignon V., Martel J., Vigneault C., Therrien J., Filion F., Smithe L.C. & Trasler J.M. 2006. Bovine SNRPN methylation imprint in oocytes and day 17 *in vitro*-produced and somatic cell nuclear transfer embryos. Biology of Reproduction. 75(4): 531-538.

68 Maherali N., Sridharan R., Xie W., Utikal J., Eminli S., Arnold K., Stadtfeld M., Yachechko R., Tchieu J., Jaenisch R., Plath K. & Hochedlinger K. 2007. Directly reprogrammed fibroblasts show global epigenetic remodeling and widespread tissue contribution. *Cell Stem Cell.* 1(1): 55-70.

69 Mann M.R., & Bartolomei M.S. 2002. Epigenetic reprogramming in the mammalian embryo: struggle of the clones. *Genome Biology*. 3(2): Reviews 1003-1004.

70 Martins D.S., Ambrosio C.E., Saraiva N.Z., Wenceslau C.V., Morini A.C., Kerkis I., Garcia J.M. & Miglino M.A. 2011. Early development and putative primordial germ cells characterization in dogs. *Reproduction in Domestic Animals*. 46(1): e62-66.

71 Mclaren A. 2003. Primordial germ cells in the mouse. *Developmental Biology*. 262(1): 1-15.

72 Meirelles F.V., Birgel E.H., Perecin F., Bertolini M., Traldi A.S., Pimentel J.R., Komninou E.R., Sangalli J.R., Neto P.F., Nunes M.T., Pogliani F.C., Meirelles F.D., Kubrusly F.S., Vannucchie C.I. & Silva L.C. 2010. Delivery of cloned offspring: experience in Zebu cattle (*Bos indicus*).*Reproduction, Fertility and Development*. 22(1): 88-97.

73 Mitsui K., Tokuzawa Y., Itoh H., Segawa K., Murakami M., Takahashi K., Maruyama M., Maedae M. & Yamanaka S. 2003. The homeoprotein Nanog is required for maintenance of pluripotency in mouse epiblast and ES cells. *Cell*. 113(5): 631-642.

74 Morgan H.D., Santos F., Green K., Deane W. & Reik W. 2005. Epigenetic reprogramming in mammals. *Human Molecular Genetics*. 14(1): R47-58.

75 Munoz M., Rodriguez A., De Frutos C., Caamano J.N., Diez C., Facal N. & Gomez E. 2008. Conventional pluripotency markers are unspecific for bovine embryonic-derived cell-lines. *Theriogenology*. 69(9): 1159-1164.

76 Munsie M.J., Michalska A.E., O'brien C.M., Trounson A.O., Perae M.F. & Mountford P.S. 2000. Isolation of pluripotent embryonic stem cells from reprogrammed adult mouse somatic cell nuclei. *Current Biology*. 10(16): 989-992.

77 Nafee T.M., Farrell W.E, Carroll W.D., Fryere A.A. & Ismail K.M. 2008. Epigenetic control of fetal gene expression. *BJOG: An International Journal of Obstetrics and Gynaecology*. 115(2): 158-168.

78 Nagano M.C. 2007. *In vitro* gamete derivation from pluripotent stem cells: progress and perspective. *Biology of Reproduction*. 76(4): 546-551.

79 Nagy K., Sung H.K., Zhang, P., Laflamme S., Vincent P., Agha-Mohammadi S., Woltjen K., Monetti C., Michael I.P., Smith L.C. & Nagy A. 2011. Induced Pluripotent Stem Cell Lines Derived from Equine Fibroblasts. Stem Cell Rev. [in press].

80 Nakagawa M., Koyanagi M., Tanabe K., Takahashi K., Ichisaka T., Aoi T., Okita T., Mochiduki Y., Takizawae N. & Yamanaka S. 2008. Generation of induced pluripotent stem cells without Myc from mouse and human fibroblasts. *Nature Biotechnology*. 26(1): 101-106.

81 Nayernia K., Nolte J., Michelmann H.W., Lee J.H., Rathsack K., Drusenheimer K., Dev A., Wulf G., Ehrmann I.E., Elliott D.J., Okpanyi V., Zechner U., Haaf T., Meinhardt A. & Engel W. 2006. *In vitro*-differentiated embryonic stem cells give rise to male gametes that can generate offspring mice. *Developmental Cell*. 11(1): 125-132.

82 Nettersheim D., Biermann K., Gillis A.J., Steger K., Looijengae L.H. & Schorle H. 2011. NANOG promoter methylation and expression correlation during normal and malignant human germ cell development. *Epigenetics*. 6(1): 114-122.

83 Ng H.H. & Bird A. 1999. DNA methylation and chromatin modification. *Current Opinion in Genetics and Development* 9(2): 158-163.

84 Nichols J., Zevnik B., Anastassiadis K., Niwa H., Klewe-Nebenius D., Chambers I., Scholere H. & Smith A. 1998. Formation of pluripotent stem cells in the mammalian embryo depends on the POU transcription factor Oct4. *Cell*. 95(3): 379-391.

85 Ohgane J., Yagie S. & Shiota K. 2008. Epigenetics: the DNA methylation profile of tissue-dependent and differentially methylated regions in cells.*Placenta*. 29 (Suppl A): S29-35.

86 Okita K., Ichisakae T. & Yamanaka S. 2007. Generation of germline-competent induced pluripotent stem cells. *Nature*. 448(7151): 313-317.

87 Okita K., Nakagawa M., Hyenjong H., Ichisakae T. & Yamanaka S. 2008. Generation of mouse induced pluripotent stem cells without viral vectors.*Science*. 322(5903): 949-953.

88 Papapetrou E.P., Tomishima M.J., Chambers S.M., Mica Y., Reed E., Menon J., Tabar V., Mo Q., Studer L. & Sadelain M. 2009. Stoichiometric and temporal requirements of Oct4, Sox2, Klf4, and c-Myc expression for efficient human iPSC induction and differentiation. *Proceeding of the National Academy of Science*. 106(31): 12759-12764.

89 Park I.H., Zhao R., West J.A., Yabuuchi A., Huo H., Ince T.A., Lerou P.H., Lensche M.W. & Daley G.Q. 2008. Reprogramming of human somatic cells to pluripotency with defined factors. *Nature*. 451(7175): 141-146.

90 Pelosi E., Forabosco A. & Schlessinger D. 2011. Germ cell formation from embryonic stem cells and the use of somatic cell nuclei in oocytes. *Annals of the New York Academy of Science*. 1221: 18-26.

91 Pesce M. & Scholer H.R. 2000. Oct-4: control of totipotency and germline determination. *Molecular Reproduction and Development*. 55(4): 452-457.

92 Picanco-Castro V., Russo-Carbolante E., Reis L.C., Fraga A.M., De Magalhaes D.A., Orellana M.D., Panepucci R.A., Pereirae L.V. & Covas D.T. 2011. Pluripotent reprogramming of fibroblasts by lentiviral mediated insertion of SOX2, C-MYC, and TCL-1A. *Stem Cells and Development*. 20(1): 169-180.

93 Pick M., Stelzer Y., Bar-Nur O., Mayshar Y., Edene A. & Benvenisty N. 2009. Clone- and gene-specific aberrations of parental imprinting in human induced pluripotent stem cells. *Stem Cells*. 27(11): 2686-2690.

94 Plath K. & Lowry W.E. 2011. Progress in understanding reprogramming to the induced pluripotent state. *Nature Reviews Genetics*. 12(4): 253-265.

95 Polejaeva I.A., Chen S.H., Vaught T.D., Page R.L., Mullins J., Ball S., Dai Y., Boone J., Walker S., Ayares D.L., Colmane A. & Campbell K.H. 2000. Cloned pigs produced by nuclear transfer from adult somatic cells. *Nature*. 407(6800): 86-90.

96 Rideout W.M. 3rd, Wakayama T., Wutz A., Eggan K., Jackson-Grusby L., Dausman J., Yanagimachie R. & Jaenisch R. 2000. Generation of mice from wild-type and targeted ES cells by nuclear cloning. *Nature Genetics*. 24(2): 109-110.

97 Rideout W.M., Eggane K. & Jaenisch R. 2001. Nuclear cloning and epigenetic reprogramming of the genome. *Science*. 293(5532): 1093-1098.

98 Roach M., Wang L., Yang X. & Tian X.C. 2006. Bovine embryonic stem cells. *Methods in Enzymology*. 418: 21-37.

99 Rossant J. 2001. Stem cells from the Mammalian blastocyst. *Stem Cells*. 19(6): 477-482.

100 Santos F. & Dean W. 2004. Epigenetic reprogramming during early development in mammals. *Reproduction*. 127(6): 643-651.

101 Santos F., Zakhartchenko V., Stojkovic M., Peters A., Jenuwein J., Wolf E., Reike W. & Dean W. 2003. Epigenetic marking correlates with developmental potential in cloned bovine preimplantation embryos. *Current Biology*. 13(13): 1116-1121.

102 Shi D., Lu F., Wei Y., Cui K., Yang S., Weie J. & Liu Q. 2007. Buffalos (Bubalus bubalis) cloned by nuclear transfer of somatic cells. *Biology of Reproduction*. 77(2): 285-291.

103 Shi L.H., Miao Y.L., Ouyang Y.C., Huang J.C., Lei Z.L., Yang J.W., Han Z.M., Song X.F., Sune Q.Y. & Chen D.Y. 2008. Trichostatin A (TSA) improves the development of rabbit-rabbit intraspecies cloned embryos, but not rabbit-human

interspecies cloned embryos. *Developmental Dynamics.* 237(3): 640-648.

104 Silva J., Barrandon O., Nichols J., Kawaguchi J., Theunissen T.W. & Smith A. 2008. Promotion of reprogramming to ground state pluripotency by signal inhibition. *PLoS Biology*. 6(10): e253.

105 Solter D. 2000. Mammalian cloning: advances and limitations. *Nature Reviews Genetics*. 1(3): 199-207.

106 Sridharan R., Tchieu J., Mason M.J., Yachechko R., Kuoy E., Horvath S., Zhou Q. & Plath K. 2009. Role of the murine reprogramming factors in the induction of pluripotency. *Cell*. 136(2): 364-377.

107 Stadtfeld M., Nagaya M., Utikal J., Weire G. & Hochedlinger K. 2008. Induced pluripotent stem cells generated without viral integration. *Science*. 322(5903): 945-949.

108 Sumer H., Liu J., Malaver Ortega L.F., Lim M.L., Khodadadie K. & Verma P.J. 2011. NANOG is a key factor for induction of pluripotency in bovine adult fibroblasts. *Journal of Animal Science*. [in press].

109 Surani M.A. 1998. Imprinting and the initiation of gene silencing in the germ line. *Cell*. 93(3): 309-312.

110 Suzuki J., Therrien J., Filion F., Lefebvre R., Goff A.K., Perecin F., Meirellese F.V. & Smith L.C. 2011. Loss of Methylation at H19 DMD Is Associated with Biallelic Expression and Reduced Development in Cattle Derived by Somatic Cell Nuclear Transfer. *Biology of Reproduction*. 84(5): 947-956.

111 Tada M. & Tada T. 2006. Nuclear reprogramming of somatic nucleus hybridized with embryonic stem cells by electrofusion. *Methods in Molecular Biology*. 329: 411-420.

112 Takahashi K. & Yamanaka S. 2006. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell*. 126(4): 663-76.

113 Takahashi K., Tanabe K., Ohnuki M., Narita M., Ichisaka T., Tomodae K. & Yamanaka S. 2007. Induction of pluripotent stem cells from adult human fibroblasts by defined factors. *Cell*. 131(5): 861-872.

114 Telugu B.P., Ezashie T. & Roberts R.M. 2010. The promise of stem cell research in pigs and other ungulate species. *Stem Cell Reviews*. 6(1): 31-41.

115 Toyooka Y., Tsunekawa N., Akasu R. & Noce T. 2003. Embryonic stem cells can form germ cells *in vitro*. *Proceeding of the National Academy of Science.* 100(20): 11457-11462.

116 Wakayama T. & Yanagimachi R. 2001. Mouse cloning with nucleus donor cells of different age and type. *Molecular Reproduction and Development*. 58(4): 376-383.

117 Wakayama T., Perry A.C., Zuccotti M., Johnsone K.R. & Yanagimachi R. 1998. Full-term development of mice from enucleated oocytes injected with cumulus cell nuclei. *Nature*. 394(6691): 369-374.

118 Wakayama T., Rodriguez I., Perry A.C., Yanagimachie R. & Mombaerts P. 1999. Mice cloned from embryonic stem cells. *Proceeding of the National Academy of Science*. 96(26): 14984-14989.

119 Wang L., Duan E., Sung L.Y., Jeong B.S., Yang X. & Tian X.C. 2005. Generation and characterization of pluripotent stem cells from cloned bovine embryos. *Biology of Reproduction.* 73(1): 149-155.

120 Wang Y.S., Xiong X.R., An Z.X., Wang L.J., Liu J., Quan F.S., Huae S. & Zhang Y. 2011. Production of cloned calves by combination treatment of both donor cells and early cloned embryos with 5-aza-2/-deoxycytidine and trichostatin A. *Theriogenology*. 75(5): 819-825.

121 Wani N.A., Wernery U., Hassan F.A., Wernerye R. & Skidmore J.A. 2010. Production of the first cloned camel by somatic cell nuclear transfer. Biology of Reproduction. 82(2): 373-379.

122 Wernig M., Meissner A., Foreman R., Brambrink T., Ku M., Hochedlinger K., Bernsteine B.E. & Jaenisch R. 2007. *In vitro* reprogramming of fibroblasts into a pluripotent ES-cell-like state. *Nature*. 448(7151): 318-324.

123 West J.A., Park I.H., Daleye G.Q. & Geijsen N. 2006. *In vitro* generation of germ cells from murine embryonic stem cells. *Nature Protocols*. 1(4): 2026-2036.

124 Whitworth K.M. & Prather R.S. 2010. Somatic cell nuclear transfer efficiency: how can it be improved through nuclear remodeling and reprogramming*Molecular Reproduction and Development*. 77(12): 1001-1015.

125 Wilmut I., 2002. Cloning and stem cells. *Cloning Stem Cells*. 4(2): 103-104.

126 Wilmut I., Schnieke A.E., Mcwhir J., Kinde A.J. & Campbell K.H. 1997. Viable offspring derived from fetal and adult mammalian cells. *Nature*. 385(6619): 810-813.

127 Wu Z., Chen J., Ren J., Bao L., Liao J., Cui C., Rao L., Li H., Gu Y., Dai H., Zhu H., Teng X., Chenge L. & Xiao L. 2009. Generation of pig induced pluripotent stem cells with a drug-inducible system. *Journal of Molecular Cell Biology*. 1(1): 46-54.

128 Yang X., Smith S.L., Tian X.C., Lewin H.A., Renarde J.P. & Wakayama T. 2007. Nuclear reprogramming of cloned embryos and its implications for therapeutic cloning. *Nature Genetics*. 39(3): 295-302.

129 Ying Q.L., Wray J., Nichols J., Batlle-Morera L., Doble B., Woodgett J., Cohen P. & Smith A. 2008. The ground state of embryonic stem cell self-renewal. *Nature*. 453(7194): 519-523.

130 Yu J., Hu K., Smuga-Otto K., Tian S., Stewart R., Slukvin, I.I. & Thomson J.A. 2009. Human induced pluripotent stem cells free of vector and transgene sequences. *Science*. 324(5928): 797-801.

131 Yu J., Vodyanik M.A., Smuga-Otto K., Antosiewicz-Bourget J., Frane J.L., Tian S., Nie J., Jonsdottir G.A., Ruotti V., Stewart R., Slukvin I.I. & Thomson J.A. 2007. Induced pluripotent stem cell lines derived from human somatic cells. *Science*. 318(5858): 1917-1920.

132 Zhao J., Hao Y., Ross K.W., Spate L.D., Walters E.M., Samuel M.S., Rieke A., Murphye C.N. & Prather R.S. 2010. Histone deacetylase inhibitors improve *in vitro* and *in vivo* developmental competence of somatic cell nuclear transfer porcine embryos. *Cell Reprogram*. 12(1): 75-83.

133 Zhao J., Ross J.W., Hao Y., Spate L.D., Walters E.M., Samuel M.S., Rieke A., Murphye C.N. & Prather R.S. 2009. Significant improvement in cloning efficiency of an inbred miniature pig by histone deacetylase inhibitor treatment after somatic cell nuclear transfer. *Biology of Reproduction*. 81(3): 525-530.

134 Zhao R. & Daley G.Q. 2008. From fibroblasts to iPS cells: induced pluripotency by defined factors. *Journal of Cellular Biochemistry*. 105(4): 949-955.

135 Zhao X.Y., Li W., Lv Z., Liu L., Tong M., Hai T., Hao J., Guo C.L., Ma Q.W., Wang L., Zeng F. & Zhou Q. 2009. iPS cells produce viable mice through tetraploid complementation. *Nature*. 461(7260): 86-90.