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Review

Human Somatic Cell Nuclear Transfer

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Abstract

Human Somatic Cell Nuclear Transfer (hSCNT) is a required procedure before derivation of autologous embryonic stem cells (ESC) for clinical treatment. The debate between ethical concerns and the potential for clinical application is still ongoing. Currently, the milestone of deriving hSCNT blastocysts has been achieved. The developmental mechanism for SCNT is similar to the developmental mechanism for fertilized embryos without sperm fertilization. The hSCNT procedure consists of enucleating an oocyte and replacing with a donor somatic cell nucleus; this procedure is more difficult than a parthenogenetic procedure. The difficulties of SCNT involve the cell cycle synchronization between the donor nucleus and arrested ooplasm, suboptimal activation of reconstructed "zygotes", suboptimal culture condition, and incomplete genetic remodeling/epigenetic modification. There are protocols intended to overcome the difficulties, but no center has derived hSCNT ESC yet.

Key Words: human, SCNT, ESC, egg activation, embryo

Introduction

The study of human embryonic stem cells (hESC) is a relatively new field. It offers the potential for understanding such biomolecular events as cell differentiation and pathological mechanisms inherent to human disease processes. The hope to apply the study of human embryonic stem cells to the medical field has created a new category of medicine - regenerative medicine. The basic idea is to use stem cell derivatives to repair damaged tissues in such chronic disease states as heart failure, Parkinson's disease, spinal cord injury, diabetes, cancer, aging, and so on. Any form of human tissue replacement would require tissue typing and the use of anti-rejection medication. To prevent the rejection problem, autologous transplantation is the most ideal approach. Currently, the only way to derive autologous hESC is through a process known as somatic cell nuclear transfer (SCNT).

SCNT has been called a cloning procedure. It was such a procedure that Wilmut's team used to produce Dolly in 1996 (8). This form of SCNT is called reproductive cloning. Reproductive cloning may yield some advantages in the field of animal husbandry. For humans, reproductive cloning is universally condemned by society and its scientists because of religious and ethical considerations.

On the other hand, the so-called therapeutic cloning is deemed a valuable procedure. SCNT, utilized as therapeutic cloning, is the procedure used to derive hESC from cloned embryos. No attempt is made at replicating a living human individual. It is characterized by taking the somatic cell nucleus of an adult and placing it in the enucleated oocyte (2) in an attempt to reprogram the nucleus into forming a developing embryo. Stem cells are derived from the developing blastocyst in the usual way. To date, the successful creation of an hESC line has not been accomplished. But recently, the derivation of ESC from a non-human primate SCNT embryo was successfully reported (6). Though SCNT appears to be the "traditional" technique, the gold standard, if

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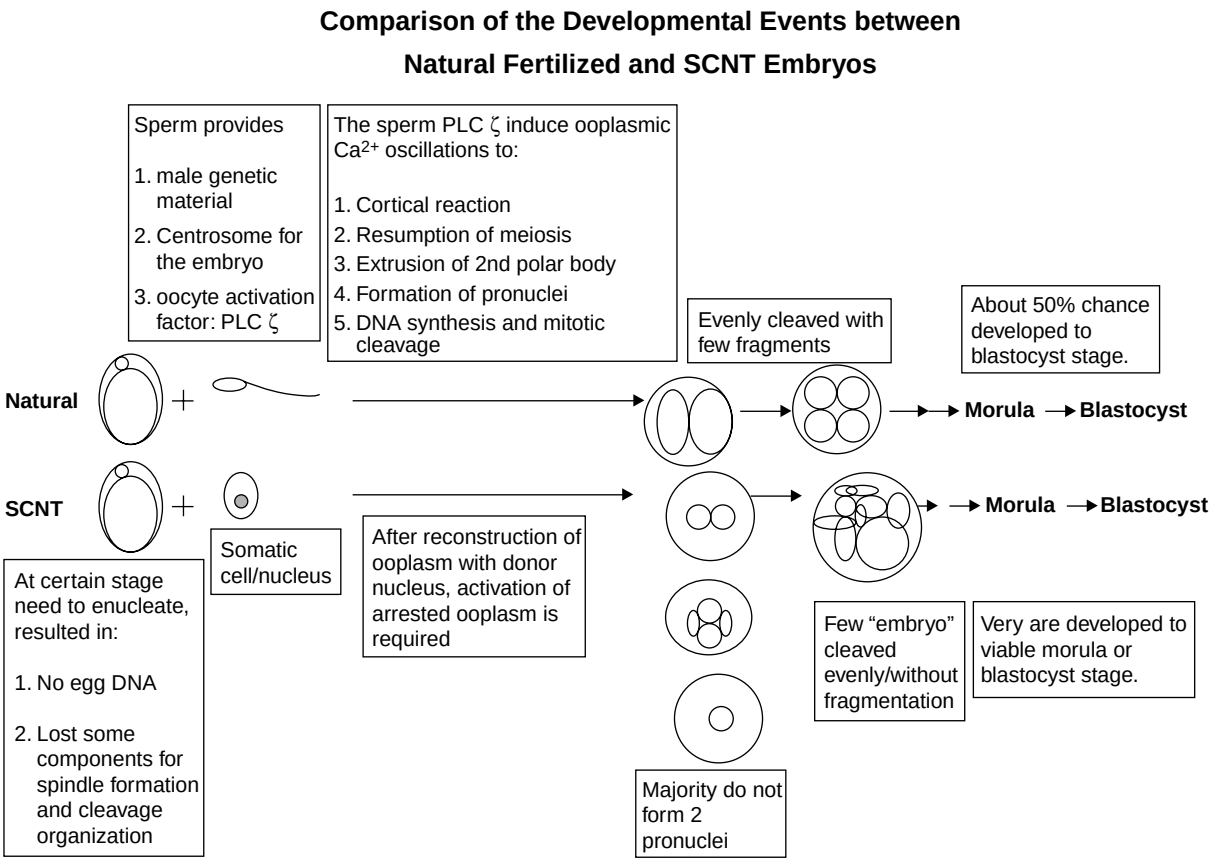

Fig. 1. Sketched figure to compare the developmental events between naturally fertilized and SCNT embryos. The inserted boxes indicated the major differences.

you will, another technique for producing patient-specific or autologous hESC's has been created by Yamanaka's team, at the Kyoto Institute (37) and by Thomason's group at the Genome Center of Wisconsin (40). The procedure uses somatic cells and viral vectors to reprogram the differentiation state of the somatic cells back to pluripotent cells resembling those derived from the blastocysts. This new line of stem cells is known as induced Pluripotent Stem cells or iPS. This step is a significant one in stem cell research. It solves the complication of the ethical debate and the difficulty of procuring human eggs as reviewed in "The status of human nuclear transfer" by Hall and Stojkovic (11). The future application of iPS cells in regenerative medicine awaits further clarification.

There are two major concerns about the current iPS technique: efficacy and safety. The efficacy consideration is that the process of ESC differentiation and its regulation mechanism are still not fully understood. One still requires a gold standard for comparisons, *i.e.* SCNT ESC *vs.* iPS. Secondly, one must be concerned with the safety issue. Due to the teratogenic risk of ESC, the theoretical application in regenerative medicine tends to use differentiated precursor cells. Transfection with viral vectors undoubtedly adds one more layer of safety issues in regenerative medicine, *i.e.* cancer incidence, stability of differentiation and physiological regulation. Because of these concerns about the current iPS technique, SCNT will continue to be an active area of research.

What, exactly, is the SCNT procedure? How successful is this procedure in animal models? And, most importantly, what are the major difficulties encountered in human SCNT?

Comparison of Developmental Events between Natural Fertilized and SCNT Embryos

The knowledge of obtaining SCNT embryos is based on the process of oocyte maturation and "natural" fertilization. This section will review the "natural" process, followed by describing the SCNT process and comparison between the two, as shown in Fig. 1.

Phases of SCNT

During the oocyte maturation process, the developing cell accumulates all necessary machinery

for fertilization and for early embryo development. Maturation promotion factor (MPF) is a general term for a complex regulatory system, which is composed of cyclin B and $p34^{cdc2}$(2, 7, 26). It promotes the oocyte's progression to the Metaphase II stage and arrests it at the Metaphase II stage until fertilized. Fertilization activates $p34^{cdc2}$ kinase, thus triggering a cascade reaction to decrease the MPF concentration, followed by the initiation of mitosis or meiosis. MPF is gradually acquired during the oocyte maturation process. At the germinal vesicle stage, MPF is low. The breakdown of the germinal vesicle to metaphase I (egg without germinal vesicle and without first polar body) stage is due to the transient peak of MPF (7). The MPF gradually increases and remains at a plateau during the Metaphase II stage (egg with first polar body). Gleaned from the experience of human intra-cytoplasmic sperm injection (ICSI), one finds that the high concentration of MPF in ooplasm has the ability to dissolve the sperm cell membrane and nuclear membrane. The dissolution of the sperm acrosomal membrane exposes the sperm's specific oocyte activation factor, phospholipase C ζ (PLCζ) (26, 35) in the perinuclear matrix at the post-acrosomal region (Fujimoto 2004). The PLCζ is released into the ooplasm within 90 minutes after ICSI, at least for bull and mouse models (39). PLCζ activates the oocyte through the pulsatile release of Ca^{2+} from the endoplasmic reticulum with a specific temporal pattern (22, 35). The initial phase involves cortical granule release and a decrease in the MPF concentration. With the decrease of MPF, the arrested spindle apparatus proceeds to separate and expel the second polar body. The oocyte haploid number of chromosomes gradually forms a female pronucleus. The sperm head gradually decondenses because of glutathione and replaces the sperm chromosomal protamine with oocyte histone, thus gradually forming a male pronucleus. The centrosome introduced with the sperm starts to form aster fiber, then gradually projecting to the female pronucleus and pulling it to abut with the male pronucleus. About 13-19 hours after ICSI, the 2 pronuclei are observable under the microscope (31). After pronucleus formation, the genomes from both parents are subjected to differential epigenetic modification that basically involves DNA methylation and histone acetylation (1, 34). After the pronuclei abut, the pronuclear membrane dissolves and gradually forms the spindle apparatus with the male centrosome. About 30 hours after ICSI, the embryo starts to cleave and become a 2-cell stage embryo. A viable embryo usually cleaves to 6-8 cells on day 3. It progresses to the morula stage by day 4 and the blastocyst stage by day 5.

The underlying principle of SCNT is the use of the machinery in the ooplasm to perform the reprogramming, followed by supporting the donor nucleus' development as an embryo (cleaved embryo or blastocyst). The current concept of deriving SCNT ESC is based on using the inner cell mass of a SCNT blastocyst (38) or a cleaved embryo blastomere (17). One may discuss the problems inherent to SCNT in three areas. [1] Enucleation is the removal of the oocyte's genetic material at a certain stage to prevent the risk of mixture between recipient and donor genetic materials; [2]. The length of time of reprogramming involving two time segments. [3] Activation techniques used to stimulate the natural process initiated by the union of male and female germ cells.

In the majority of studies enucleation is performed on Metaphase II oocytes. This is the mature oocyte adequately equipped with all required machinery, and the spindle apparatus is organized visibly so that it may be seen through a DIC microscope or polarized light microscope (Spindle view microscope). Neither Metaphase I nor germinal vesicle stage oocytes are good candidates for enucleation. Metaphase I oocytes are hard to find, and the genetic materials are not packed properly. Removal of the germinal vesicle also depletes a lot of components for cell cleavage.

The stage known as programming is a complex one. Reprogramming is a long process. It goes from the transfer of the donor nucleus to the egg before activation, to after activation and the entirety of embryogenesis, all the way up to the blastocyst stage. For better understanding, one must examine the reprogramming in sections. The first section is the duration from egg retrieval to the time of donor nucleus transfer. Immediately after egg retrieval (about 36 hours after hCG injection), nuclear maturity may be achieved at the Metaphase II stage, but cytoplasmic maturity is still ongoing *via* intracellular calcium ion concentration and gradually gaining the capability to respond to sperm activation, *i.e.* increase the sensitivity of IP3 (Inositol triphosphate) receptors to stimuli and the storage of calcium in the endoplasmic reticulum for long term Ca^{2+} oscillations (5, 14). This is the reason that human IVF usually inseminates 3-6 hours after egg retrieval (32). For SCNT, Stojkovic *et al.* (36) reported better successful data within 1 hour of egg retrieval. The preliminary data for this study showed that 2-4 hours after egg retrieval yields better "pronucleus" formation and cleavage than the results from the group within 1 hour after egg retrieval (unpublished data).

The second section is the duration in donor nucleus transfer to egg activation. Mitalipov *et al.* (23) examined the effect of activation of monkey cytoplasts on SCNT outcome. Pre-activation (4 hours before somatic nucleus transfer), instant activation, and post-activation (3 hours after somatic nucleus transfer) gave different outcomes. Only post-activation supported the SCNT embryos' development to blastocysts (23). This study suggested a post-activation

approach when using Metaphase II oocytes. This duration is usually 2 hours in primate studies (25, 27). During this time, there is an occurrence of premature chromosome condensation due to the high concentration of MPF. The donor nucleus goes through an incomplete genetic reprogramming process, *i.e.* deacetylation, then genetic imprinting (27, 33).

The third section is the activation of the reconstructed cell/embryo. The reconstructed cell/embryo will remain arrested without activation. There are ways to activate the ooplasm, *i.e.* calcium ionophore (ionomycin, A23147), Sr^{2+}, 7% ethanol, thimerosal, electro-activation, and so on (21). All activation protocols act through increasing the free Ca^{2+} in the ooplasm. Calcium ionophore gives a massive Ca^{2+} influx from the culture medium, along with an efflux from the endoplasmic reticulum during the treatment period. The treatment usually involves a sequence of or accompaniment with MPF reduction maneuvering, *i.e.* dimethylaminopurine (DMAP; 12, 23), cycloheximide (17), cytochalasin (33, 42). The Sr^{2+} (41) and thimerosal (13) treatments can mimic the pulsatile Ca^{2+} release pattern as sperm do. The 7% ethanol and electro-activation treatments result in a bolus of intracellular free Ca^{2+} increase as the calcium ionophore does (29, 30). All ooplasm activation protocols aim to break the ooplasm arresting status by decreasing the MPF *via* cytoplasmic Ca^{2+} oscillations (22). After activation, a viable SCNT embryo will proceed to form a "pronucleus", followed by cleavage. The reprogramming is continuously progressing during the DNA replication up to the blastocyst stage (19).

Difficulties of and Possible Working Hypothesis for hSCNT

Even though SCNT has succeeded in many mammalian species, such as mouse, rabbit, bovine, porcine, equine, non-human primates, the process in humans has proven to be difficult to perform (12). The first human SCNT ESC report in 2004 (Hwang *et al.*, withdrew report from Science journal) proved to be flawed. It was actually the first time that stem cells had been derived from human parthenogenesis (15), not from SCNT. In early 2008, French *et al.* (10) first succeeded in producing human blastocysts from SCNT. This achievement was a mile stone. That success takes us closer to the goal of the derivation of hESC's using SCNT. Currently, there has been no research team that has succeeded in procuring stable human SCNT ESC. As shown in Fig. 2, morulae were produced from SCNT, but there have not been ESC derived from blastomeres yet. From Fig. 2, it is obvious that fragmentation is prevalent in these SCNT embryos. The fragmentation is the surface phenomenon. The defect mechanism is still not clear. It could be an apoptotic process, secondary to missing or insufficient

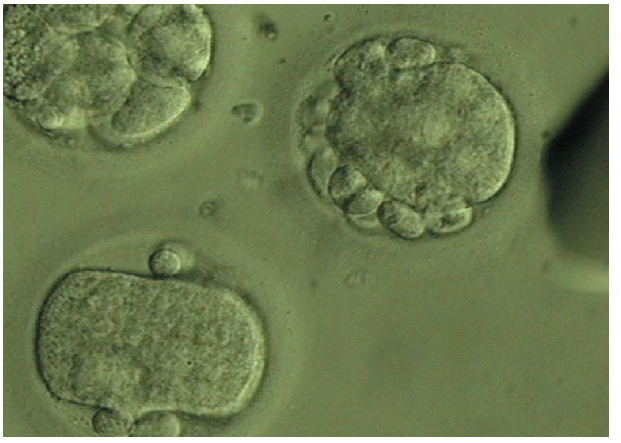

Fig. 2. Two human morulae derived from SCNT. The somatic fibroblast cell was obtained from neonatal baby foreskin tissue after circumcision. The pictures were taken 82 h after SCNT. On the top left corner is a cleaved and arrested embryo. There are multiple fragments in the top morulae. These 2 morulae did not progress to the blastocyst stage.

molecules resulting from the enucleation procedure, the dys-synchronization of ooplasm and donor nuclei, simple trauma due to the enucleation and donor nucleus transfer procedure, out-of-sequence gene expression, centrosome-related issues and so on. The other consideration is the inadequate activation regime of the reconstructed egg/embryo. Ozil *et al.* (28, 29, 30) reported that the modulation of Ca^{2+} signal modulation had a long-term impact on parthenotes and fertilized egg development. By using rabbit oocytes, the dynamics of parthenotes' early cleavage were not influenced by either frequency or amplitude of Ca^{2+} release regimes (28). At the post-implantation stage, the amplitude and temporal modulation of Ca^{2+} in the early minutes of activation does impact the parthenogenetic conceptus' developmental performance (28). In the mouse egg model, the activation events seemed to be regulated by the duration of sustained cytoplasmic Ca^{2+} signaling (29). In the fertilized mouse egg model, modulation of Ca^{2+} oscillatory patterns results in differential gene expression and the capability to develop to term (30). Although the incidence of development to blastocyst is not influenced by modulation of sperm-induced Ca^{2+} oscillations, reduced Ca^{2+} oscillations compromised the blastocyst's implantation capability and hyper-stimulated Ca^{2+} signals compromised the post-implantation development (30). The optimal protocol of Ca^{2+} signal regime for SCNT awaits further clarification. Incomplete genetic reprogramming and imprinting is another difficulty (3, 4, 16). Ng *et al.* (26) have observed that a majority of non-human primate SCNT embryos are arrested by the weakness of aneuploidy. It has been observed that the majority of

human SCNT embryos arrested also exhibit aneuploidy, as the Ng *et al*'s study shows (manuscript submitted). A SCNT embryo grows better in the somatic cell culture medium than the embryo culture medium (4). A SCNT fetus is usually of low birth weight, along with placenta hypertrophy (16). These observations suggest that incomplete reprogramming is the defect. Currently, the majority of researchers believe that the reprogramming is the central theme of SCNT (3, 24). During gametogenesis, a considerable length of time is required to prepare the sperm or egg chromosomes for effective deacetylation and acetylation for genetic reprogramming and imprinting. The SCNT nucleus has never been properly prepared for epigenetic remodeling. The epigenetic reprogramming is always incomplete or insufficient.

Worldwide, all human SCNT centers have different working hypotheses to solve the problems. The ability to culture human SCNT embryo to blastocyst stage is very encouraging (10). The final goal of producing stable hESC is waiting to be fulfilled. One of the working models to bypass this difficulty is to derive the hESC from cleaved embryos instead of inner cell mass from blastocysts. Recently, Lanza's team at Advanced Cell Technology (ACT) (17) has succeeded in developing techniques to derive ESC from cleaved embryos. Working with ACT's team and utilizing there techniques (9), stable hESC still could not be derived due to the "epidemic" aneuploidy found in hSCNT blastomeres (manuscript submitted).

There are protocols in which concepts to prepare the donor nucleus, making it easier to be reprogrammed. The majority of working models are concentrated upon the genetic reprogramming process (3). The second model is to "reprogram" the donor cells, *i.e*. Trichostatin A (16). The third model aims at destabilizing the supercoil structure of chromosomes by a heating treatment to enhance the efficiency of reprogramming (20). The fourth model works on the serial nuclear transfer to enhance reprogramming (27). Other creative working hypotheses are also being formulated.

Since SCNT ESC have already been successfully derived in other mammals (including non-human primates), it is reasonable to expect that deriving human SCNT ESC is a workable project. With enough resources and eager engagement, it is just a matter of time for human SCNT ESC to succeed and to realize the potential of clinical application.

References

1. Adenot, P.G., Mercier, Y., Renard, J.P. and Thompson, E.M. Differential H4 acetylation of parternal and maternal chromatin precedes DNA replication and differential transcriptional activity in pronuclei of 1- cell mouse embryos. *Development* 124: 4615-4625, 1997.
2. Alberio, R., Zakhartchenko, V., Motlik, J. and Wolf, E. Mammalian oocyte activation:lessons from the sperm and implications for nuclear transfer. *Int. J. Dev. Biol.* 45: 797-809, 2001.
3. Armstrong, L., Lako,M., Dean, W. and Stojkovic, M. Epigenetic modification is central to genome reprogramming in somatic cell nuclear transfer. *Stem Cells* 24: 805-814, 2006.
4. Boiani, M., Gentile, L., Gambles, V.V., Cavaleri, F., Redi, C. and Scholer, H. Variable reprogramming of the pluripotent stem cell marker Oct4 in mouse clones: distinct developmental potentials in different culture environments. *Stem Cells* 23: 1089-1104, 2005.
5. Boni, R., Gualtieri, R., Talevi, R. and Tosti, E. Calcium and other ion dynamic during gamete maturation and fertilization. *Theriogenology* 68 (suppl 1): S156-S164, 2007.
6. Byrne, J.A., Pedersen, D.A., Clepper, L.L., Nelson, M., Sanger, W.G., Gokhale, S., Wolf, D.P. and Mitalipov, S.M. Producing primate embryonic stem cells by somatic cell nuclear transfer. *Nature* 450: 497-502, 2007.
7. Campbell, K.H., Loi, P., Otaegui, P.J. and Wilmut , I. Cell cycle co-ordination in embryo cloning by nuclear transfer. *Rev. Reprod.* 1: 40-46, 1996.
8. Cambell, K.H., McWhir, J., Ritchie, W.A. and Wilmut, I. Sheep cloned by nuclear transfer from a cultured cell line. *Nature* 380: 64-66, 1996.
9. Chung, Y., Klimanskaya, I., Becker, S., Li, T., Maserati, M., Lu, S.J., Zdravkovic, T., Ilic, D., Genbacev, O., Fisher, S., Krtolica, A. and Lanza, R. Human embryonic stem cell lines generated without embryo desruction. *Cell Stem Cell* 2: 113-117, 2008.
10. French, J.A., Adams, C.A., Anderson L.S., Kitchen J.R., Hughes, M.R. and Wood, S.H. Development of human cloned blastocysts following somatic cell nuclear transfer (SCNT) with adult fibroblasts. *Stem Cells* 26: 485-493, 2008.
11. Hall, V.J. and Stojkvic, M. The status of human nuclear transfer. *Stem Cell Rev.* 2: 301-308. 2006.
12. Heindryckx, B., De Sutter, P., Gerris, J., Dhont, M. and Van der Elst, J. Embryo development after successful somatic cell nuclear transfer to *in vitro* matured human germinal vesicle oocytes. *Hum. Reprod.* 22: 1982-1990, 2007.
13. Herbert, M., Gillespie, J.I. and Murdoch, A.P. Development of calcium signaling mechanisms during maturation of human oocytes. *Mol. Hum. Reprod.* 3: 965-973, 1997.
14. Homa, S.T., Carrol, J. and Swann, K. The role of calcium in mammalian oocytes maturation and egg activation. *Hum. Reprod.* 8: 1274-1281, 1993.
15. Kim, K., Ng, K., Rugg-Gunn, P.J., Shieh, J-H., Kirak, O., Jaenisch, R., Wakayama, T., Moore, M.A., Pedersen R.A. and Daley, G. Recombination signatures distinguish embryonic stem cells derived by parthenogenesis and somatic cell nuclear transfer. *Cell Stem Cell* 1: 346-352, 2007.
16. Kishigami, S., Mizutani, E., Ohta, H., Hikichi,, T., Van Thuan, N., Wakayama, S., Bui, H-T. and Wakayam, T. Significant improvement of mouse cloning technique by treatment with Trichostatin A after somatic nuclear transfer. *Biochem. Biophy. Res. Communi.* 340: 183-189, 2006.
17. Klimanskaya, I., Chung, Y., Becker, S., Lu, S.J. and Lanza R. Human embryonic stem cell lines derived from single blastomeres. *Nature* 444: 481-485, 2006.
18. Kurosaka S., Nagao, Y., Minami, N., Yamada, M. and Imai, H. Dependence of DNA sysnthesis and invitro development of bovine nuclear transfer embryos on the stage of the cell cycle of donor cells and recipient cytoplasts. *Biol. Reprod.* 67: 643-647, 2002.
19. Latham, K.E. Early and delayed aspects of nuclear reprogramming during cloning. *Biol. Cell* 97: 119-132, 2005.
20. Loi, P., Clinton, M., Barboni, B., Fulka, J. Jr., Cappai, P., Feil, R., Moor, R.M. and Ptak, G. Nuclei of nonviable ovine somatic cells develop into lambs after nuclear transplantation. *Biol. Reprod.* 67: 126-132, 2002.
21. Machaty, Z. and Prather, R.S. Strategies for activating nuclear transfer embryos. *Reprod. Fertil. Dev.* 10: 599-613, 1998.

22. Malcuit, C., Kurokawa, M. and Fissore, R.A. Calcium oscillations and mammalian egg activation. *J. Cell. Physiol*. 206: 565-573, 2006.
23. Mitalipov, S.M., Yeoman R.R., Nusser, K.D. and Wolf, D.P. Rhesus monkey embryos produced by nuclear transfer from embryonic blastomeres or somatic cells. *Biol. Reprod*. 66: 1367-1373, 2002.
24. Mitalipov, S.M., Zhou, Q., Byrne, J.A., Ji, W.Z., Norgren, R.B. and Wolf, D.P. Reprogramming following somatic cell nuclear transfer in primate is dependent upon nuclear remodeling. *Hum. Reprod*. 22: 2232-2242, 2007.
25. Miyazaki, S. and Ito, M. Calcium signals for egg activation in mammals. *J. Pharmacol. Sci*. 100: 545-552, 2006.
26. Ng, S.-C., Chen, N., Yip, W.-Y., Liow, S.-L., Tong, G.-Q., Martelli, B., Tan, L.G. and Martelli, P. The first cell cycle after transfer of somatic cell nuclei in a non-human primate. *Development* 131: 2475-2484, 2004.
27. Ono, Y., Shimozawa, N., Ito, M. and Kono, T. Cloned mice from fetal fibroblast cells at metaphase by serial nuclear transfer. *Biol. Reprod*. 64: 44-50, 2001.
28. Ozil, J.P. and Huneau, D. Activatin of rabbit oocytes, the impact of the Ca^{2+} signal regime on development. *Development* 128: 917-928, 2001.
29. Ozil, J.-P., Markoulaki, S., Toth, S., Matson, S., Barnrezes, B., Knott, J.G., Schultz, R.M., Huneau, D. and Ducibella, T. Egg activation events are regulated by the duration of a sustained $[Ca^{2+}]_{cyt}$ signal in the mouse. *Dev. Biol.* 282: 39-54, 2005.
30. Ozil, J.P., Banrezes, B., Toth, S., Pan, H. and Schultz, R.M. Ca^{2+} oscillatory pattern in fertilized mouse eggs affects gene expression and development to term. *Dev. Biol.* 300: 534-544, 2006.
31. Payne, D., Flaherty, S.P., Barry, M.F. and Matthews, C.D. Preliminary observation on polar body extrusion and pronuclear formation in human oocytes using time-lapse video cinematography. *Hum. Reprod*. 12: 532-541, 1997.
32. Rienzi, L., Ubaldi, F., Anniballo, R., Cerulo, G. and Greco, E. Preincubation of human oocytes may improve fertilization and embryo quality after intracytoplasmic sperm injection. *Hum. Reprod.* 13: 1014-1019, 1998.
33. Rybouchkin, A., Kato, Y. and Tsunoda, Y. Role of histone acetylation in reprogramming of somatic nuclei following nuclear transfer. *Biol. Rerpod.* 74: 1083-1089, 2006.
34. Santos, F., Hendrich, B., Reik, W. and Dean, W. Dynamic reprogramming of DNA methylation in the early mouse embryo. *Dev. Biol.* 241: 172-182, 2002.
35. Saunders, C.M., Larman, M.G., Parrington, J., Cox, L.J., Royse, J., Blayney L.M., Swann, K. and Lai, F.A. PLCζ: a sperm-specific trigger of Ca^{2+} oscillation in eggs and embryo development. *Development* 129: 3533-3544, 2002.
36. Stojkovic, M., T. Stojkovic, P., Leary, C., Hall, V.J., Armstrong, L., Herbert, M., Nesbitt, M., Lako, M. and Murdoch, A. Derivation of a human blastocyst after herterologus nuclear transfer to donated oocytes. *Reprod. Biomed. Online* 11: 226-231, 2005.
37. Takahashi, K., Tanabe, K., Ohnuki, M., Narita, M., Ichisaka, T., Tomoda, K. and Yamanaka, S. Induction of pluripotent stem cells from adult human fibroblasts by defined factors. *Cell* 131: 861-872, 2007.
38. Thomson, J.A., Itskovitz-Eldor, J., Shapiro, S.S., Waknitz, M.A., Swiergiel, J.J., Marshall, V.S. and Jones, J.M. Embryonic stem cell lines derived from human blastocysts. *Science* 282: 1145-1147, 1998.
39. Yoon, S.Y. and Fissore, R.A. Release of phohpholipase C ζ and $[Ca^{2+}]_i$ oscillation-inducing activity during mammalian fertilization. *Reproduction* 134: 695-704, 2007.
40. Yu, J., Vodyanik, M.A., Smuga-Otto, K., Antosiewicz-Bourget, J., Frane, J.L., Tian, S., Nie, J., Jonsdottir, G.A. Ruotti, V., Stewart, I.I. and Thomson, J.A. Induced pluripotent stem cell lines derived from human somatic cells. *Science* 318: 1917-1920, 2007.
41. Zhang, D., Pan, L., He, X.K., Huang, X.Y. and Sun, F.Z. Strontium promotes calcium oscillations in mouse meiotic oocytes and early embryo through InsP3 receptors, and requires activation of phospholipase and the synergistic action of InsP3. *Hum. Reprod*. 20: 3053-3061, 2005.
42. Zhou, Q., Yang, S.H., Ding, C.H., He, X.C., Hildebrandt, T.B., Mitalipov, S.M., Tang, X.H., Wolf, D.P. and Ji, W.Z. A comparative approach to somatic cell nuclear transfer in the rhesus monkey. *Hum. Reprod*. 21: 2564-2571, 2006.