Front. Agr. Sci. Eng. 2014, 1(2): 104–113
DOI: 10.15302/J-FASE-2014003

REVIEW

Oocyte-associated transcription factors in reprogramming after somatic cell nuclear transfer: a review

Fengxia YIN, Hui LIU, Shorgan BOU, Guangpeng LI (✉)

The Key Laboratory of National Education Ministry for Mammalian Reproductive Biology and Biotechnology, the College of Life Sciences, Inner Mongolia University, Hohhot 010070, China

Abstract Oocytes are unique cells with the inherent capability to reprogram nuclei. The reprogramming of the somatic nucleus from its original cellular state to a totipotent state is essential for term development after somatic cell nuclear transfer. The nuclear-associated factors contained within oocytes are critical for normal fertilization by sperm or for somatic cell nuclear reprogramming. The chromatin of somatic nuclei can be reprogrammed by factors in the egg cytoplasm whose natural function is to reprogram sperm chromatin. The oocyte first obtains its reprogramming capability in the early fetal follicle, and then its capacity is enriched in the late growth phase and reaches its highest capability for reprogramming as fully-grown germinal vesicle oocytes. The cytoplasmic milieu most likely contains all of the specific transcription and/or reprogramming factors necessary for cellular reprogramming. Certain transcription factors in the cytoplast may be critical as has been demonstrated for induced pluripotent stem cells. The maternal pronucleus exerts a predominant, transcription-dependent effect on embryo cytofragmentation, with a lesser effect imposed by the ooplasm and the paternal pronucleus. With deep analysis of transcriptomics in oocytes and early developmental stage embryos more maternal transcription factors inducing cellular reprogramming will be identified.

Keywords nuclear reprogramming, somatic cell, transcription factors, transcriptomics

1 Introduction

Fetal genesis is a unidirectional process that is accompanied by a loss of developmental potential of various cell lineages. Starting as a unicellular zygote, the progression of changes ends in the formation of 220 specialized cell types and a living organism [1]. Specific terms such as unipotency, pluripotency, multipotency and totipotency have been ascribed to specific populations of cells that arise during development [1–3]. Epigenetic signatures have been identified with these cell populations and correlated with differentiation potentials [4].

The nucleus of differentiated cells can revert to a less differentiated state by a process known as nuclear reprogramming [5]. Briggs, King [6] and Gurdon [7] demonstrated in an amphibian model during the 1950s and 1960s that genomes of adult cells, or terminally differentiated cells, can generate viable cloned animals upon nuclear transfer (NT) [2,3]. The birth of Dolly in 1997, the first cloned mammal [8], and other animal species to follow, indicates that differentiated cells, even those terminally differentiated, can revert to their original nuclear state (the zygotic state) and support term development of a live birth [9].

Although many cloned animal species have produced normal offspring, the somatic cell nuclear transfer (SCNT) efficiency is still extremely low ranging from 1% to 5% of live births [10,11]. The fact that somatic cells do not fully support pseudo-zygote (nuclear transfer embryos) development suggests that nuclear reprogramming is incomplete and that the donor cell nucleus does not fully revert to a pluripotent state. Although the causes are poorly understood, any reprogramming errors are most likely to be caused by genetic and epigenetic insufficiencies. The more that is learned about nuclear reprogramming, the more apparent is its complexity and the potential for as yet unknown factors. This review highlights the current state-of-the-art of somatic cellular reprogramming used in animal cloning, and discusses various nuclear-associated and transcription factors.

Received March 6, 2014; accepted March 26, 2014

Correspondence: gpengli@imu.edu.cn

2 Nuclear reprogramming begins from oogenesis

The vertebrate egg is the only known animal cell that can be reprogrammed with a somatic cell nucleus and undergoes embryonic development [12]. At birth, a mouse ovary contains clusters with thousands of oocytes. The vast majority of the oocytes have already entered meiosis. At, or around the time of birth, some of the oocytes will have entered the transitory stages of prophase (pachytene, early and late diplotene and dictyate) where they remain until meiosis resumes just before ovulation [13]. At the transition of primordial follicles to primary follicles on the third postnatal day in mice, the surrounding granulosa cells revert to a squamous to cuboidal shape with the diameter of the oocyte increasing to more than 20 μm. Numerous oocyte-specific genes that are critical for folliculogenesis and embryogenesis are initiated during the transition of the primordial follicle to primary follicle [14].

The follicular growth phase of the murine oocyte undergoes a considerable increase in diameter from less than 20 μm in the primordial follicle to more than 70 μm in the antral follicle. Transcription factors occurring during folliculogenesis have been shown to maintain critical roles in accumulating transcripts required for follicular growth (e.g., Bmp15 and Gdf9) and from fertilization to embryonic development (e.g., Mater and Zar1) [12].

Oocyte growth, even during the final stages of the growth phase, is critical for full developmental competence. This is a time when the development-associated materials accumulate. The nucleus of the oocyte becomes a large storage organelle (germinal vesicle, GV) containing histones, lamins, pore complexes, transcriptional factors and various small ribosomal nuclear proteins [15]. The cytoplasm of the oocyte drastically increases recruiting and accumulating the maternal origin contents such as certain meiosis-associated cell signals, mRNAs, nucleoplasms, proteins and rRNAs.

Oocytes that approach full size are then competent to complete maturation, which are about 75 μm in mice [16] and 130 μm in bovine [17]. Smaller oocytes will not support meiosis [18] and fail to induce the sperm nuclei to form pronuclei [15]. However, when smaller oocytes were fused to mitotically competent oocytes they underwent maturation, which strongly supports the suggestion that reprogramming modifications and the acquisition of epigenetic changes necessary for oocyte meiosis occur at a late in the oocyte growth phase [19].

Maternal epigenetic modifications can also occur during oogenesis [20]. It has been shown that the maternal genome gradually achieved developmental competence beginning at about 50 μm diameter in mouse [21]. In adult ovary the epigenetic modifications of maternal chromatin necessary for development to term appear to be established earlier in the oocyte growth phase. At this phase and during the maturation phase, oocytes have the capacity to extensively reprogram heterologous chromatin [22].

Gonadotropin hormone stimulated oocytes at GV stage complete meiosis by disassembling the nuclear membrane, undergoing chromatin condensation, assembling the metaphase spindle, completing the first meiotic division and ending at the second metaphase stage (MII). During meiotic resumption, nuclear membrane-associated compounds and nuclear matrix factors diffuse into the ooplasm. These compounds reassemble as the pronucleus forms at fertilization. During nuclear envelope breakdown (NEBD), the disassembling of the endoplasmic reticulum generates vesicular components for pronuclear development [15,23]. The fully developed GV contains essential materials and factors for the reassembly of the nuclear membrane and expedites chromatin decondensation and recondensation in sperm [15]. Oocyte specific transcription factors have been shown most likely to provide the switches directing the meiotic processes [24].

3 Germinal vesicle breakdown materials are needed for cellular reprogramming

During and after GV breakdown (GVBD) its contents are dispersed throughout the cytoplasm of maturing oocytes. The distributed nuclear materials are necessary for sperm chromatin decondensation and cleavage in fertilized eggs [23]. Nucleoplasmin removes sperm protamines and decondenses the sperm chromatin to allow for the assembly of the paternal pronucleus [25]. Iwashita et al. [26] performed a series of nucleocytoplasm mixing and enucleation experiments and observed that the GV contains a factor or factors that seemingly contribute(s) to the initial maturation-promoting factor (MPF) activation during meiosis I. Some nuclear factors were absolutely necessary for MPF reactivation and entry into meiosis II. Aside from the importance of MPF, mitogen-activated protein kinase (MAPK) also seemed to contribute to the regulation of meiotic maturation in the mammalian oocytes [27,28].

Given the various findings presented above, the general view that meiotic control in the vertebrate oocytes depends solely on cytoplasmic factors may need to be reconsidered [26]. In *Xenopus*, Gurdon et al. ruptured the GV by a needle to promote the mixing of GV contents with the oocyte cytoplast prior to nuclear transfer and significantly increased the efficiency of nuclear reprogramming [29]. The transfer of mouse somatic cells into GV oocytes confirmed that GV material was essential for nuclear reprogramming [30]. Nuclear components extracted from the GVs have been demonstrated to be important in facilitating reprogramming of the introduced foreign nuclei in mammals [31]. Polanski et al. showed that GV

components released into the cytoplasm of an oocyte after GVBD appear to be incorporated into the growing pronuclei [32]. These findings suggest that exchanges of materials between nuclear and cytoplast compartments play important roles during oocyte growth, maturation and embryonic mitosis. It was confirmed that the putative regulating factors were crucial for embryonic development [33,34]. Such factors may be localized to the pronuclei during interphase, which are partially or completely removed during the enucleation and subsequent nuclear transfer process. The nature of these various nuclear components still needs to be resolved. If animal cloning is to become a practical tool in the arsenal of assisted reproduction technologies, then the identification of such factors is essential for understanding the mechanism of meiotic control and the processes involved in somatic cell reprogramming.

Enucleated GV stage oocytes as recipient cytoplasts for somatic cell nuclear transfer failed to produce term pregnancies. When somatic cells were transferred into rabbit enucleated GV stage oocytes, the donor nuclei remained condensed or fragmented and did not form pronuclei (unpublished data). The results imply that GV stage cytoplasts are incompetent to support cloned embryo development. An earlier study by Mohammed et al. also concluded that GV stage oocytes were unsuitable as recipients for nuclear transfer [30]. It is thought that the reprogramming factor(s) responsible for embryonic stem cells reside in the nucleus [35,36]. To determine if the genomic reprogramming factors reside in the nucleus or cytoplasm of GV stage oocytes, and whether they can reprogram somatic cell nuclei, Bui et al. treated differentiated adult somatic cells with lysates of GV stage oocyte cytoplasm and successfully produced cloned mice [37]. The cloning efficiency was significantly increased from about 0.6% to 3.1%, which supports the hypothesis that genomic reprogramming factors are present in the cytoplasm of the GV stage oocyte and therefore can improve cloning technology [37]. The results of the study also suggested that GV stage oocytes are rich in materials necessary for nuclear reprogramming and embryo development, and the deletion of these factors, results in impaired nuclear programming and developmental failure of *in vitro* fertilized or nuclear reconstructed embryos/fetuses.

More recently, Wang et al. using semiquantitative mass spectrometry (MS) analysis confirmed that protein compositions were correlated with various oocyte developmental stages [38].

4 Zygote cytoplasts as recipients for somatic cell nuclear transfer

Modliński micro-surgically introduced mouse CBA/H T6T6 strain 8-cell stage nuclei into intact zygotes and identified the presence of two T6 marker chromosomes in resulting tetraploid blastocysts [39]. McGrath and Solter reported in their study the inability of mouse blastomere nuclei transferred into enucleated zygotes to support *in vitro* development [40]. Upon analyzing causes leading to failure of development of reconstituted zygotes, in which karyoplasts containing intact pronuclei were removed, Greda et al. stated that the failure might have been caused by the enucleation protocol [33]. It is known that the nucleolus in the zygote and early embryo is maternally inherited and originates from material sources that are present in the oocyte GV [41]. The nucleoli originating from a somatic cell or even from an embryonic stem cell cannot substitute for the original oocyte nucleolar material [41]. These studies suggest that zygote nuclear factors are critically important for the development of cloned embryos with zygote cytoplasts as recipients.

5 Second metaphase stage cytoplasts as recipients

To date almost all animals cloned from somatic cell nuclei have been produced using enucleated MII stage oocytes as recipient cytoplasts [33]. The MII stage oocyte creates a better environment for SCNT than either the GV or metaphase I stage oocytes [30,42,43].

As discussed above, GV materials diffuse and become distributed throughout the cytoplasm. Intensive mixing occurs during GVBD and when oocytes enter first and second meiosis. MII cytoplasts amass the entire nuclear reprogramming machinery and embryo development associated factors that are necessary to support early embryo development after fertilization or nuclear transfer.

The active forms of MPF and MAPK in MII oocytes are maintained at high levels and induce NEBD, which then disperses the nucleoli and causes premature chromosome condensation (PCC) in transferred somatic cell nuclei. Nuclear reprogramming will not occur without MFP and MAPK [44,45]. PCC most likely facilitates the formation of nuclear swelling. Enlargement of the nucleus has been correlated with successful nuclear reprogramming [46,47]. In contrast to GV stage and maturing oocytes, MII oocytes promote PCC and nuclear swelling of introduced nuclei, which provides a more suitable environment for nuclear reprogramming.

As previously mentioned, the prevailing problem with SCNT is the extremely low efficiency with only 1% to 5% of transferred embryos developing to term. Whether demethylation and remethylation are essential for development varies among studies in mice [48–50] and cattle [51–55]. Histone acetylation has been shown to be aberrant in bovine embryos made by NT [55,56]. Incomplete epigenetic reprogramming is the predominant cause of aberrant gene expression that contributes to the high incidence of pregnancy failure [57].

Higher efficiency cloning will require a greater understanding of the events involved in reprogramming by NT. Attempts are currently underway to improve the efficiency of NT by manipulating the donor cell prior to NT, which include treating cells with DNA methyltransferase inhibitors or histone deacetylase inhibitors [56,58,59]. The preimplantation embryo made by NT could be manipulated to reduce the DNA methylation level and increase the histone acetylation level. The transfer success of these epigenetically manipulated embryos has not been investigated. The reprogramming process of donor cell has been shown to be initiated by the recipient cytoplast and the cytoplast has a greater impact on NT than the donor cell [58]. In early stage bovine embryos, rRNAs are not transcribed until the 4-cell stage. Abundant nuclear transcriptional activity first occurs at the 8- to 16-cell stage [60]. Maternal transcripts and oocyte proteins are essential for embryogenic progression [58].

Enucleation is one of the key steps of nuclear transfer cloning and is accomplished by removing the MII spindle and a small volume of surrounding cytoplasm from the matured oocyte [43]. A problem associated with the procedure is that incomplete enucleation results in aneuploidy. This then leads to genetic interference within the recipient cytoplasm and abnormal development [61]. The removal of genetic content from the recipient cyotoplast also tends to delete factors that are important for nuclear reprogramming.

Using non-enucleated oocytes as nuclear recipients it was found that donor cell PCC occurred more readily in the intact oocyte compared to that in an enucleated one [62]. In the presence of oocyte nuclei, the transferred cell nuclei underwent NEBD and entered pre-metaphase I stage 5 times faster than the enucleated group. This phenomenon was also observed in mice by using an intact oocyte as the recipient cytoplast [63]. To verify oocyte nucleus capability for cell reprogramming, a reverse nuclear transfer (RNT) procedure was performed. The cell was first fused to an intact oocyte. After a short interaction between oocyte and somatic nuclei, the oocyte nucleus was then removed. The transferred nucleus was swollen, deployed and reorganized to form a pronuclear-like structure, which occurred faster than in the control group. The *in vitro* development capacity of embryos produced by RNT was significantly higher than the control group. From comparison of these results with similar studies [45], it is suggested that oocyte MII chromosomes can initiate PCC in the donor cell nuclei and the chromosomes of the oocyte maintain MPF at high levels. It is known that high levels of MPF contribute to the formation of PCC in the donor cell [64,65].In the enucleation process, much of the MPF was removed. The effect of MPF in the cytoplast was therefore much less than in the intact oocyte [45]. The reduced level of MPF in the enucleated cytoplast, however, still affects PCC, although at a slower rate [64].

Studies using extracts from GV stage oocytes or MII-oocytes have shown that oocytes at different stages had distinctive and unique mechanisms for somatic cell reprogramming, which differed from reprogramming techniques used to produce induced pluripotent stem cells with defined transcription factors [66–68]. It has been shown that treatment with MII extract could induce at least partial reprogramming in somatic cells [69]. The fact that MII extract is beneficial for somatic cell nuclear reprogramming may eventually lead to the identification of specific reprogramming factors in oocytes. However, using GV-extract treatment to promote somatic cell nuclear reprogramming may not lead to the identification of the various reprogramming factors, because the GV and cytoplasm components become artificially mixed [3].

6 Oocyte maternal nuclear material controls embryo quality

Embryo quality and developmental competence are associated with oocyte maturation [70–72]. Mammalian embryos are destined to die unless crucial early developmental events take place to suppress cellular death. When suppression is inadequate, apoptotic-like events occur in the early embryo including cytoplasmic fragmentation or blebbing (cytofragmentation), DNA fragmentation, and other changes commonly associated with apoptosis [73]. In humans, cytofragmentation often occurs at the 6- to 8-cell stages, immediately before or at the time of genome activation [74]. Cytofragmentation events in mice occur as early as the mid 2-cell stage, which is just before the major genome activation event [75,76]. Studies of cytofragmentation in mouse embryos indicated that the maternal pronucleus exerted a predominant, transcription-dependent effect on the phenotype, with lesser effects from the ooplasm and paternal pronucleus [75–77]. The parental origin effect is the result of a transgenerational epigenetic modification, whereby the inherited maternal grandpaternal contribution interacts with the fertilizing paternal genome and the ooplasm. Some epigenetic information related to grandparental origins of chromosomes is retained through oogenesis and transmitted to progeny, where it affects gene expression from the maternal pronucleus and subsequent embryo phenotype [77,78].

7 Localization and distribution of transcriptional factors in oocytes

The mammalian oocyte has a maternal gene expression profile that is unique. In mice, transcription arrest occurs at GV stage and is maintained until zygotic genome activation takes place approximately 9–10 h after fertilization [79–81]. Between cessation and resumption of

transcription, the maternal transcription profile is reprogrammed to that observed in the embryos [82,83]. The mechanisms underlying transcription-reprogramming still warrant further investigation. A clearer understanding of the molecular mechanisms responsible for the reprogramming process will undoubtedly lead to a higher efficiency in cloning technology [84,85].

Three hypotheses have been proposed to explain transcription silencing during embryogenesis: (1) transcription before the mid-blastula transition is prevented by rapid cell cycling as shown in *Xenopus* during early development; (2) the presence of inhibitory factors in eggs represses transcription; and (3) a deficiency in, or absence of, critical transcriptional factors leads to transcriptional silencing [82,83]. It has been reported in mice that dynamic changes in transcriptional activity occurred when a non-surrounding nucleolus changes to a surrounding nucleolus configuration [86,87]. In non-surrounding nuclei, transcription was active with the presence of eight general transcription factors (TFs), BRF1, PolII, TAF1, TAF4, TBP, TFIIA, TFIIB, and TRF3. The transcriptional machinery was still functional at this stage and was present with the chromatin. The preliminary data (authors' unpublished data) showed that chromatin factors, HDAC1, HDAC2, HP1, HP1®, TAFIIP250, TFIIB and Topo II, were associated with the DNA template during chromatin condensation in mice and bovine. At GVBD, these factors gradually dissociated from the chromatin and then attached to the spindle during meiotic processing. Several transcription factors such as BAF155, Brg-1, TFIID and Topo II® were dispersed throughout the nucleoplasm. This group of factors did not co-locate with the chromatin even though the chromatin condenses. They could only be detected in the cytoplasm after GVBD (authors' unpublished data). These data suggested that most general transcription molecules dissociated from chromatin and dispersed before the oocyte reached metaphase I stage. When oocytes advanced to meiosis MII, part of the TFs were shunted to the polar body while most retained their original distribution pattern.

The first event in the development of any vertebrate embryo is under maternal control until the zygotic genome becomes activated. Transcription starts at the 1-cell stage in mice and significantly increases by the 2-cell stage [88]. Once the pronuclear state is formed, DNA replication and transcription begins. TFs are synchronously synthesized and/or recovered and become re-associated with the chromatin. The zygotic genome then becomes activated and directs early embryogenesis.

Nuclear swelling, chromatin dispersal, and protein exchange between a transplanted nucleus and oocyte cytoplasm were essential for embryo development and reprogramming of the SCNT embryo [83]. The reprogramming of transplanted somatic nuclei retraced events similar to normal early development [83,89]. TFs were critical for transcription activation and regulation in the mouse [83]. TFs exhibited a characteristic pattern of dissociation from somatic cell nuclei, followed by a duplication process in the maternal chromatin. TFs became re-associated with the chromatin/pronuclei in SCNT and parthenogenesis, similar to normal fertilized embryos. Gao et al. concluded that somatic cell reprogramming had two phases: in the first, a broad range of TFs moved out of the somatic nucleus soon after nuclear transfer [83], and the second occurred shortly after pronuclear formation with nearly all of the TFs being reloaded back to chromatin.

TFs were observed to display a global pattern of dissociation and re-association from chromatin. The process of dissociation and re-association occurred in tandem with other reprogramming mechanisms such as DNA demethylation [9] and histone deacetylation [90]. The combination of these molecular processes reset the somatic cell nuclei to that of the normal embryonic state. Most TFs were reloaded back to nuclei shortly after pronuclear formation. Similar timing and dynamic changes were present for normal developed, SCNT and parthenogenesis embryos. Eggs produced by these methods seemed to follow the same pathway of development regardless of how they were created. Zygotic development is a process independent of the way eggs are produced [83]. The general nature of the development is determined by the egg rather than the chromatin. This becomes more apparent with parthenogenotes where the normal path for developmental is initiated and retraced without any addition(s) to the egg, e.g., sperm or somatic nucleus. The egg alone appears to contain most, if not all, the essential components for directing zygotic developmental and transcription [82,83]. Inoue et al. reported that deletion of *Xist*, a non-coding RNA that inactivated one of the two X chromosomes in females on Xa, resulted in an 8- to 9-fold increase in cloning efficiency accompanied with normal global gene expression [91]. Therefore, it is concluded that nonrandom reprogramming errors could be corrected to a point where SCNT will eventually become an economically viable procedure. Results from this study reinforce the basic premise that transcription factors play a critical role in nuclear reprogramming.

8 Transcriptomics may dissect the detailed gene regulatory mechanisms in cellular reprogramming

The most advanced single-cell sequencing technique makes it possible to dissect the detail of genetic regulatory mechanisms in a valuable resource, e.g., progressively developmental embryo. The transcriptome analysis of the different developmental stages from oocyte to morula in both mouse and human reveal a sequential order of transcriptional changes in pathways of cell cycle, gene

regulation, translation and metabolism, acting in a stepwise fashion from cleavage to morula [92]. In cattle, RNA sequencing analysis of transcripts in GV and M II oocytes, and in 4-, 8-, 16-cell and blastocyst stage embryos indicated that the largest proportion of gene activation was found in 8-cell embryos [93]. Cross-species comparisons between mouse and human showed that significant difference in developmental specificity and timing existed [92]. It has been reported that the maternal glucocorticoid receptor transcript and protein participated in the maternal reprogramming of zebrafish development [94]. Ces5/Ooep was suggested to be a maternal-effect gene directly associated with the oocyte cytoplasmic developmental patterns [95]. Transcription factor gene *MSX1* played important roles in bovine preimplantation embryo development. Injection of long double-stranded RNA and small interfering RNA to oocytes at GV stage reduced *MSX1* mRNA expression, which significantly affected oocyte maturation and the subsequent embryo development [96]. In *Xenopus*, the conserved long non-coding RNAs were expressed in a developmental stage-specific fashion [97]. It has been demonstrated that miRNAs played regulatory roles during early embryo development [98]. The Gli-like transcription factor Glis1 was enriched in unfertilized oocytes and embryos at the 1-cell stage. Glis1 was observed to promote multiple pro-reprogramming pathways, including Myc, Nanog, Lin28, Wnt, Essrb and the mesenchymal-epithelial transition [99]. Glis1, as a maternal transcription factor effectively promoted the direct reprogramming of somatic cells during iPSC generation [99]. With deep analysis of transcriptomics in oocytes and early developmental stage embryos, more maternal transcription factors inducing cellular reprogramming will be identified. Figure 1 illustrates the possible roles of transcriptional factors in oocyte maturation, fertilization, nuclear transfer reprogramming and embryo development.

9 Concluding remarks

Oocytes are special cells and have the inherent capability to reprogram nuclei. The ability to reprogram somatic cell nuclei is a critical step for deriving pluripotent stem cells from a differentiated cell. The reprogramming of the somatic nucleus from its original cellular state to a totipotent state is essential for term development after SCNT [100]. The nuclear-associated factors contained within oocytes are critical for normal fertilization by sperm or for somatic cell nuclear reprogramming. The chromatin of somatic nuclei can be reprogrammed by factors in the egg cytoplasm whose natural function is to reprogram sperm chromatin [66]. Oocyte cytoplast and nuclear materials jointly contribute to embryo development both from fertilization and SCNT. Incomplete reprogramming of a transferred somatic cell may be primarily due to insufficient amounts of reprogramming factors in the host

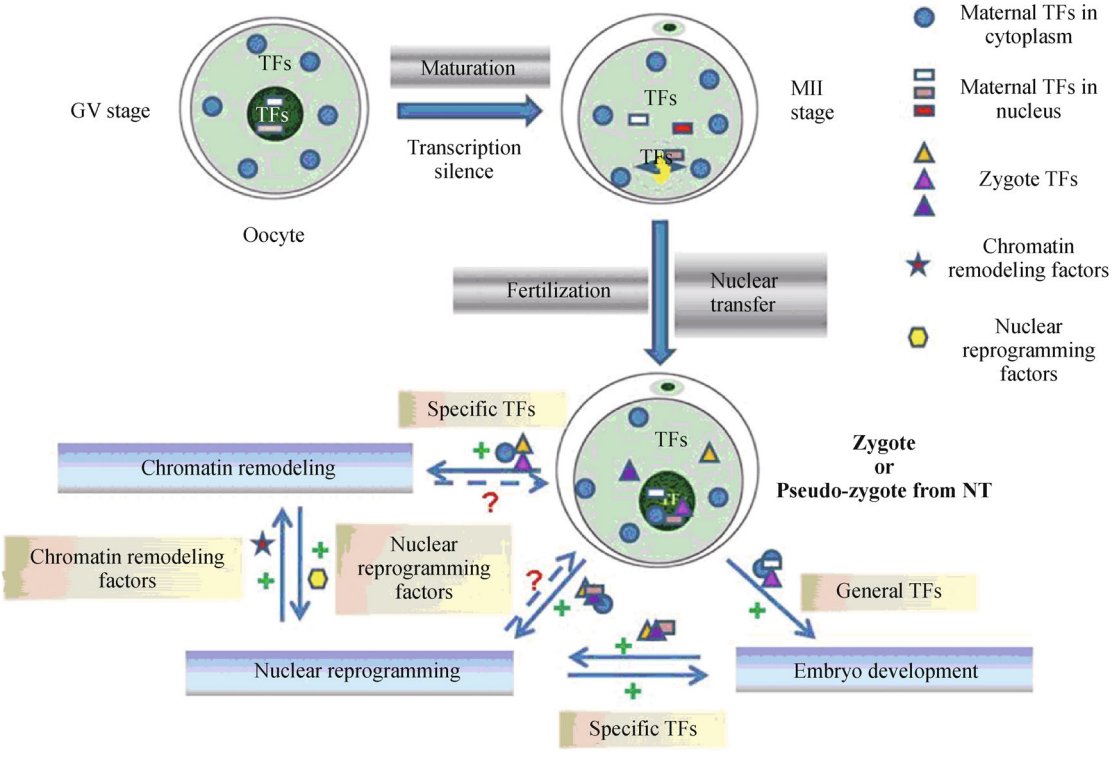

Fig. 1 The possible roles of transcription factors in oocyte maturation, fertilization, nuclear transfer and embryo development

cytoplast. Transcriptomics analyses of oocytes and early developmental stage embryos will identify more maternal transcription factors involved in inducing cellular reprogramming.

Acknowledgements Dr. Thomas D. Bunch, Department of Animal, Dairy and Veterinary Sciences, Utah State University, and Dr. Zhiming Han, Institute of Zoology, Chinese Academy of Sciences are thanked for their critical reviews of this manuscript. This work was supported by the National Basic Research Program of China (2012CB22306) and the National Natural Science Foundation of China (31372289).

Compliance with ethics guidelines Fengxia Yin, Hui Liu, Shorgan Bou and Guangpeng Li declare that they have no conflict of interest or financial conflicts to disclose.

This article is a review and does not contain any studies with human or animal subjects performed by any of the authors.

References

1. Hochedlinger K, Plath K. Epigenetic reprogramming and induced pluripotency. *Development*, 2009, 136(4): 509–523
2. Gurdon J B, Melton D A. Nuclear reprogramming in cells. *Science*, 2008, 322(5909): 1811–1815
3. Jaenisch R, Young R. Stem cells, the molecular circuitry of pluripotency and nuclear reprogramming. *Cell*, 2008, 132(4): 567–582
4. Reik W, Dean W, Walter J. Epigenetic reprogramming in mammalian development. *Science*, 2001, 293(5532): 1089–1093
5. Rideout W M 3rd, Eggan K, Jaenisch R. Nuclear cloning and epigenetic reprogramming of the genome. *Science*, 2001, 293 (5532): 1093–1098
6. Briggs R, King T J. The transplantation of living nuclei from blastula cells into enucleated frogs' eggs. *Proceedings of the National Academy of Sciences of the United States of America*, 1952, 38(5): 455–463
7. Gurdon J B. The developmental capacity of nuclei taken from intestinal epithelium cells of feeding tadpoles. *Journal of Embryology and Experimental Morphology*, 1962, 10: 622–640
8. Wilmut I, Schnieke A E, McWhir J, Kind A J, Campbell K H S. Viable offspring derived from fetal and adult mammalian cells. *Nature*, 1997, 385(6619): 810–813
9. Hanna J, Markoulaki S, Schorderet P, Carey B W, Beard C, Wernig M, Creyghton M P, Steine E J, Cassady J P, Foreman R, Lengner C J, Dausman J A, Jaenisch R. Direct reprogramming of terminally differentiated mature B lymphocytes to pluripotency. *Cell*, 2008, 133(2): 250–264
10. Hill J R. Abnormal in utero development of cloned animals: implications for human cloning. *Differentiation*, 2002, 69(4–5): 174–178
11. Wilmut I, Beaujean N, de Sousa P A, Dinnyes A, King T J, Paterson L A, Wells D N, Young L E. Somatic cell nuclear transfer. *Nature*, 2002, 419(6909): 583–587
12. Pangas S A, Rajkovic A. Transcriptional regulation of early oogenesis: in search of masters. *Human Reproduction Update*, 2006, 12(1): 65–76
13. McLaughlin E A, McIver S C. Awakening the oocyte: controlling primordial follicle development. *Reproduction*, 2009, 137(1): 1–11
14. Nandedkar T, Dharma S, Modi D, Dsouza S. Differential gene expression in transition of primordial to preantral follicles in mouse ovary. *Society of Reproduction and Fertility Supplement*, 2007, 63: 57–67
15. Bao S, Obata Y, Ono Y, Futatsumata N, Niimura S, Kono T. Nuclear competence for maturation and pronuclear formation in mouse oocytes. *Human Reproduction*, 2002, 17(5): 1311–1316
16. Eppig J J, Schultz R M, O'Brien M, Chesnel F. Relationship between the developmental programs controlling nuclear and cytoplasmic maturation of mouse oocytes. *Developmental Biology*, 1994, 164(1): 1–9
17. Motlík J. Cytoplasmic aspects of oocyte growth and maturation in mammals. *Journal of Reproduction and Fertility-Supplement*, 1989, 38: 17–25
18. Bao S, Obata Y, Carroll J, Domeki I, Kono T. Epigenetic modifications necessary for normal development are established during oocyte growth in mice. *Biology of Reproduction*, 2000, 62 (3): 616–621
19. Obata Y, Kaneko-Ishino T, Koide T, Takai Y, Ueda T, Domeki I, Shiroishi T, Ishino F, Kono T. Disruption of primary imprinting during oocyte growth leads to the modified expression of imprinted genes during embryogenesis. *Development*, 1998, 125(8): 1553–1560
20. Tomizawa S, Nowacka-Woszuk J, Kelsey G. DNA methylation establishment during oocyte growth: mechanisms and significance. *International Journal of Developmental Biology*, 2012, 56(10–12): 867–875
21. Obata Y, Kono T, Hatada I. Oogenesis: maturation of mouse fetal germ cells *in vitro*. *Nature*, 2002, 418(6897): 497
22. Byrne J A, Simonsson S, Western P S, Gurdon J B. Nuclei of adult mammalian somatic cells are directly reprogrammed to *oct-4* stem cell gene expression by amphibian oocytes. *Current Biology*, 2003, 13(14): 1206–1213
23. Bałakier H, Tarkowski A K. The role of germinal vesicle karyoplasm in the development of male pronucleus in the mouse. *Experimental Cell Research*, 1980, 128(1): 79–85
24. Nakaki F, Hayashi K, Ohta H, Kurimoto K, Yabuta Y, Saitou M. Induction of mouse germ-cell fate by transcription factors *in vitro*. *Nature*, 2013, 501(7466): 222–226
25. Laskey R A, Mills A D, Philpott A, Leno G H, Dilworth S M, Dingwall C, Lindquist S, Horwich A, Green N M, Gething M J, Neupert W, Ellis R J. The role of nucleoplasmin in chromatin assembly and disassembly. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 1993, 339(1289): 263–269
26. Iwashita J, Hayano Y, Sagata N. Essential role of germinal vesicle material in the meiotic cell cycle of *Xenopus* oocytes. *Proceedings of the National Academy of Sciences of the United States of America*, 1998, 95(8): 4392–4397
27. Choi T, Rulong S, Resau J, Fukasawa K, Matten W, Kuriyama R, Mansour S, Ahn N, Vande Woude C F. Mos/mitogen-activated protein kinase can induce early meiotic phenotypes in the absence of maturation-promoting factor: a novel system for analysing spindle formation during meiosis I. *Proceedings of the National*

Academy of Sciences of the United States of America, 1996, 93 (10): 4730–4735
28. Inoue M, Naito K, Nakayama T, Sato E. Mitogen-activated protein kinase translocates into the germinal vesicle and induces germinal vesicle breakdown in porcine oocytes. *Biology of Reproduction*, 1998, 58(1): 130–136
29. Gurdon J B, Laskey R A, De Robertis E M, Partington G A. Reprogramming of transplanted nuclei in amphibia. *International Review of Cytology-Supplement*, 1979, 9(9): 161–178
30. Mohammed A A, Karasiewicz J, Kubacka J, Greda P, Modliński J A. Enucleated GV oocytes as recipients of embryonic nuclei in the G1, S, or G2 stages of the cell cycle. *Cell Reprogram*, 2010, 12(4): 427–435
31. Bui H T, Kwon D N, Kang M H, Oh M H, Park M R, Park W J, Paik S S, Van Thuan N, Kim J H. Epigenetic reprogramming in somatic cells induced by extract from germinal vesicle stage pig oocytes. *Development*, 2012, 139(23): 4330–4340
32. Polanski Z, Hoffmann S, Tsurumi C. Oocyte nucleus controls progression through meiotic maturation. *Developmental Biology*, 2005, 281(2): 184–195
33. Greda P, Karasiewicz J, Modliński J A. Mouse zygotes as recipients in embryo cloning. *Reproduction*, 2006, 132(5): 741–748
34. Egli D, Rosains J, Birkhoff G, Eggan K. Developmental reprogramming after chromosome transfer into mitotic mouse zygotes. *Nature*, 2007, 447(7145): 679–685
35. Tada M, Takahama Y, Abe K, Nakatsuji N, Tada T. Nuclear reprogramming of somatic cells by *in vitro* hybridization with ES cells. *Current Biology*, 2001, 11(19): 1553–1558
36. Cowan C A, Atienza J, Melton D A, Eggan K. Nuclear reprogramming of somatic cells after fusion with human embryonic stem cells. *Science*, 2005, 309(5739): 1369–1373
37. Bui H T, Wakayama S, Kishigami S, Kim J H, Van Thuan N, Wakayama T. The cytoplasm of mouse germinal vesicle stage oocytes can enhance somatic cell nuclear reprogramming. *Development*, 2008, 135(23): 3935–3945
38. Wang S, Kou Z, Jing Z, Zhang Y, Guo X, Dong M, Wilmut I, Gao S. Proteome of mouse oocytes at different developmental stages. *Proceedings of the National Academy of Sciences of the United States of America*, 2010, 107(41): 17639–17644
39. Modliński J A. Transfer of embryonic nuclei to fertilised mouse eggs and development of tetraploid blastocysts. *Nature*, 1978, 273 (5662): 466–467
40. McGrath J, Solter D. Completion of mouse embryogenesis requires both the maternal and paternal genomes. *Cell*, 1984, 37(1): 179–183
41. Ogushi S, Palmieri C, Fulka H, Saitou M, Miyano T, Fulka Jr J. The maternal nucleolus is essential for early embryonic development in mammals. *Science*, 2008, 319(5863): 613–616
42. Campbell K H S, Fisher P, Chen W C, Choi I, Kelly R D W, Lee J H, Xhu J. Somatic cell nuclear transfer: past, present and future perspectives. *Theriogenology*, 2007, 68(Suppl 1): S214–S231
43. Li G P, White K L, Bunch T D. Review of enucleation methods and procedures used in animal cloning: state of the art. *Cloning and Stem Cells*, 2004, 6(1): 5–13
44. Collas P, Pinto-Correia C, Ponce de Leon F A, Robl J M. Effect of donor cell cycle stage on chromatin and spindle morphology in nuclear transplant rabbit embryos. *Biology of Reproduction*, 1992, 46(3): 501–511
45. Lee J H, Campbell K H S. Effects of enucleation and caffeine on maturation-promoting factor (MPF) and mitogen-activated protein kinase (MAPK) activities in ovine oocytes used as recipient cytoplasts for nuclear transfer. *Biology of Reproduction*, 2006, 74 (4): 691–698
46. Czołowska R, Modliński J A, Tarkowski A K. Behaviour of thymocyte nuclei in non-activated and activated mouse oocytes. *Journal of Cell Science*, 1984, 69: 19–34
47. Tani T, Kato Y, Tsunoda Y. Reprogramming of bovine somatic cell nuclei is not directly regulated by maturation promoting factor or mitogen-activated protein kinase activity. *Biology of Reproduction*, 2003, 69(6): 1890–1894
48. Chung Y G, Ratnam S, Chaillet J R, Latham K E. Abnormal regulation of DNA methyltransferase expression in cloned mouse embryos. *Biology of Reproduction*, 2003, 69(1): 146–153
49. Mann M R, Chung Y G, Nolen L D, Verona R I, Latham K E, Bartolomei M S. Disruption of imprinted gene methylation and expression in cloned preimplantation stage mouse embryos. *Biology of Reproduction*, 2003, 69(3): 902–914
50. Shi W, Haaf T. Aberrant methylation patterns at the two-cell stage as an indicator of early developmental failure. *Molecular Reproduction and Development*, 2002, 63(3): 329–334
51. Bordignon V, Clarke H J, Smith L C. Factors controlling the loss of immunoreactive somatic histone H1 from blastomere nuclei in oocyte cytoplasm: a potential marker of nuclear reprogramming. *Developmental Biology*, 2001, 233(1): 192–203
52. Dean W, Santos F, Stojkovic M, Zakhartchenko V, Walter J, Wolf E, Reik W. 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*, 2001, 98(24): 13734–13738
53. Kang Y K, Park J S, Koo D B, Choi Y H, Kim S U, Lee K K, Han Y M. Limited demethylation leaves mosaic-type methylation states in cloned bovine pre-implantation embryos. *The EMBO Journal*, 2002, 21(5): 1092–1100
54. Wu X, li Y, Li G P, Yang D S, Yue Y L, Wang L L, Li K H, Xin P H, Bou S, Yu H Q. Trichostatin A reduced genome DNA methylation of transgenic cells but not improved subsequent cloned embryo development. *Animal Biotechnology*, 2008, 19(4): 211–224
55. Wu X, Li Y, Wang L, Yue Y, Li K, Bao S, Li G P, Yu H. Multiple sites modifications of histone in somatic cell nuclear transfer and *in vitro* fertilized embryos in bovine. *Zygote*, 2010, 8: 1–15
56. Enright B P, Kubota C, Yang X, Tian X C. Epigenetic characteristics and development of embryos cloned from donor cells treated by trichostatin A or 5-aza-2′-deoxycytidine. *Biology of Reproduction*, 2003, 69(3): 896–901
57. Santos F, Zakhartchenko V, Stojkovic M, Peters A, Jenuwein T, Wolf E, Reik W, Dean W. Epigenetic marking correlates with developmental potential in cloned bovine preimplantation embryos. *Current Biology*, 2003, 13(13): 1116–1121
58. Aston K I, Li G P, Hicks B A, Sessions B R, Pate B J, Hammon D, Bunch T D, White K L. Effect of the time interval between fusion

and activation on nuclear state and development *in vitro* and *in vivo* of bovine somatic cell nuclear transfer embryos. *Reproduction*, 2006, 131(1): 45–51

59. Shi W, Hoeflich A, Flaswinkel H, Stojkovic M, Wolf E, Zakhartchenko V. 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*, 2003, 69(1): 301–309
60. Bilodeau-Goeseels S, Panich P. Effects of oocyte quality on development and transcriptional activity in early bovine embryos. *Animal Reproduction Science*, 2002, 71(3–4): 143–155
61. Dominko T, Chan A, Simerly C, Luetjens C M, Hewitson L, Martinovich C, Schatten G. Dynamic imaging of the metaphase II spindle and maternal chromosomesin bovine oocytes: implications for enucleation efficiency verification, avoidanceof parthenogenesis, and successful embryogenesis. *Biology of Reproduction*, 2000, 62(1): 150–154
62. Meng Q, Bai C, Liu Y, Wu X, Bunch T D, Li G P. *In vitro* development and chromosomal configuration of bovine somatic cloned embryos with nonenucleated metaphase II oocytes. *Cellular Reprogramming*, 2010, 12(4): 481–490
63. Yang H, Shi L, Zhang S, Ling J, Jiang J, Li J. High-efficiency somatic reprogramming induced by intact MII oocytes. *Cell Research*, 2010, 20(9): 1034–1042
64. Fulka J Jr, First N L, Moor R M. Nuclear transplantation in mammals: remodelling of transplanted nuclei under the influence of maturation promoting factor. *BioEssays*, 1996, 18(10): 835–840
65. Sung L Y, Shen P C, Jeong B S, Xu J, Chang C C, Cheng W T, Wu J S, Lee S N, Broek D, Faber D, Tian X C, Yang X, Du F. Premature chromosome condensation is not essential for nuclear reprogramming in bovine somatic cell nuclear transfer. *Biology of Reproduction*,2007, 76(2): 232–240
66. Bian Y, Alberio R, Allegrucci C, Campbell K H S, Johnson A D. Epigenetic marks in somatic chromatin are remodelled to resemble pluripotent nuclei by amphibian oocyte extracts. *Epigenetics*, 2009, 4(3): 194–202
67. Ji G, Ruan W, Liu K, Wang F, Sakellariou D, Chen J, Yang Y, Okuka M, Han J, Liu Z, Lai L, Gagos S, Xiao L, Deng H, Li N, Liu L. Telomere reprogramming and maintenance in porcine iPS cells. *PLoS ONE*, 2013, 8(9): e74202
68. Ruan W, Han J, Li P, Cao S, An Y, Lim B, Li N. A novel strategy to derive iPS cells from porcine fibroblasts. *Science China Life Sciences*, 2011, 54(6): 553–559
69. Miyamoto K, Tsukiyama T, Yang Y, Li N, Minami N, Yamada M, Imai H. Cell-free extracts from mammalian oocytes partially induce nuclear reprogramming in somatic cells. *Biology of Reproduction*, 2009, 80(5): 935–943
70. Kanka J, Nemcova L, Toralova T, Vodickova-Kepkova K, Vodicka P, Jeseta M, Machatkova M. Association of the transcription profile of bovine oocytes and embryos with developmental potential. *Animal Reproduction Science*, 2012(1–2): 29–35
71. Labrecque R, Sirard M A. The study of mammalian oocyte competence by transcriptome analysis: progress and challenges. *Molucular Human Reproduction*, 2014, 20(2): 103–116
72. Jiao Z X, Woodruff T K. Detection and quantification of maternal-effect gene transcripts in mouse second polar bodies: potential markers of embryo developmental competence. *Fertility and Sterility*, 2013, 99(7): 2055–2061
73. Antczak M, Van Blerkom J. Temporal and spatial aspects of fragmentation in early human embryos: possible effects on developmental competence and association with the differential elimination of regulatory proteins from polarized domains. *Human Reproduction*, 1999, 14(2): 429–447
74. Hardy K, Spanos S, Becker D, Iannelli P, Winston R M, Stark J. From cell death to embryo arrest: mathematical models of human preimplantation embryo development. *Proceedings of the National Academy of Sciences of the United States of America*, 2001, 98(4): 1655–1660
75. Hawes S M, Gie Chung Y, Latham K E. Genetic and epigenetic factors affecting blastomere fragmentation in two-cell stage mouse embryos. *Biology of Reproduction*, 2001, 65(4): 1050–1056
76. Jurisicova A, Latham K E, Casper R F, Varmuza S L. Expression and regulation of genes associated with cell death during murine preimplantation embryo development. *Molecular Reproduction and Development*, 1998, 51(3): 243–253
77. Han Z, Chung Y G, Gao S, Latham K E. Maternal factors controlling blastomere fragmentation in early mouse embryos. *Biology of Reproduction*, 2005, 72(3): 612–618
78. Han Z, Mtango N R, Zhong Z, Vassena R, Latham K E. Early transcription from the maternal genome controlling blastomere integrity in mouse two-cell-stage embryos. *American Journal of Physiology-Cell Physiology*, 2010, 298(5): C1235–C1244
79. Aoki F, Worrad D M, Schultz R M. Regulation of transcriptional activity during the first and second cell cycles in the preimplantation mouse embryo. *Developmental Biology*, 1997, 181(2): 296–307
80. Bouniol C, Nguyen E, Debey P. Endogenous transcription occurs at the 1-cell stage in the mouse embryo. *Experimental Cell Research*, 1995, 218(1): 57–62
81. Schultz R M. The molecular foundations of the maternal to zygotic transition in the preimplantation embryo. *Human Reproduction Update*, 2002, 8(4): 323–331
82. Sun F, Fang H, Li R, Gao T, Zheng J, Chen X, Ying W, Sheng H Z. Nuclear reprogramming: the zygotic transcription program is established through an “erase-and-rebuild” strategy. *Cell Research*, 2007, 17(2): 117–134
83. Gao T, Zheng J, Xing F, Fang H, Sun F, Yan A, Gong X, Ding H, Tang F, Sheng H Z. Nuclear reprogramming: the strategy used in normal development is also used in somatic cell nuclear transfer and parthenogenesis. *Cell Research*, 2007, 17(2): 135–150
84. Surani M A. Nuclear reprogramming by human embryonic stem cells. *Cell*, 2005, 122(5): 653–654
85. Gurdon J B, Byrne J A. The first half-century of nuclear transplantation. *Bioscience Reports*, 2004, 24(4-5): 545–557
86. Mattson B A, Albertini D F. Oogenesis: chromatin and microtubule dynamics during meiotic prophase. *Molecular Reproduction and Development*, 1990, 25(4): 374–383
87. Zuccotti M, Piccinelli A, Giorgi Rossi P, Garagna S, Redi C A. Chromatin organization during mouse oocyte growth. *Molecular Reproduction and Development*, 1995, 41(4): 479–485
88. Torres-Padilla M E, Zernicka-Goetz M. Role of TIF1α as a

modulator of embryonic transcription in the mouse zygote. The *Journal of Cell Biology*, 2006, 174(3): 329–338

89. Gurdon J B. Nuclear transplantation in eggs and oocytes. *Journal of Cell Science-Supplement*, 1986, 4: 287–318
90. Aoi T, Yae K, Nakagawa M, Ichisaka T, Okita K, Takahashi K, Chiba T, Yamanaka S. Generation of pluripotent stem cells from adult mouse liver and stomach cells. *Science*, 2008, 321(5889): 699–702
91. Inoue K, Kohda T, Sugimoto M, Sado T, Ogonuki N, Matoba S, Shiura H, Ikeda R, Mochida K, Fujii T, Sawai K, Otte A P, Tian X C, Yang X, Ishino F, Abe K, Ogura A. Impeding *Xist* expression from the active X chromosome improves mouse somatic cell nuclear transfer. *Science*, 2010, 330(6003): 496–499
92. Xue Z, Huang K, Cai C, Cai L, Jiang C, Feng Y, Liu Z, Zeng Q, Cheng L, Sun Y E. Liu J, Horvath S, Fan G. Genetic programs in human and mouse early embryos revealed by single-cell RNA sequencing. *Nature*, 2013, 500(7464): 593–597 PMID:23892778
93. Graf A, Krebs S, Zakhartchenko V, Schwalb B, Blum H, Wolf E. Fine mapping of genome activation in bovine embryos by RNA sequencing. *Proceedings of the National Academy of Sciences of the United States of America*, 2014, 111(11): 4139–4144
94. Pikulkaew S, Benato F, Celeghin A, Zucal C, Skobo T, Colombo L, Dalla Valle L. The knockdown of maternal glucocorticoid receptor mRNA alters embryo development in zebrafish. *Developmental Dynamics*, 2011, 240(4): 874–889
95. Tashiro F, Kanai-Azuma M, Miyazaki S, Kato M, Tanaka T, Toyoda S, Yamato E, Kawakami H, Miyazaki T, Miyazaki J. Maternal-effect gene *Ces5/Ooep/Moep19/Floped* is essential for oocyte cytoplasmic lattice formation and embryonic development at the maternal-zygotic stage transition. *Genes to Cells*, 2010, 15 (8): 813–828
96. Tesfaye D, Regassa A, Rings F, Ghanem N, Phatsara C, Tholen E, Herwig R, Un C, Schellander K, Hoelker M. Suppression of the transcription factor *MSX1* gene delays bovine preimplantation embryo development *in vitro*. *Reproduction*, 2010, 139(5): 857–870
97. Paranjpe S S, Jacobi U G, van Heeringen S J, C Veenstra G J. A genome-wide survey of maternal and embryonic transcripts during *Xenopus tropicalis* development. *BMC Genomics*, 2013, 14(1): 762
98. Mondou E, Dufort I, Gohin M, Fournier E, Sirard M A. Analysis of microRNAs and their precursors in bovine early embryonic development. *Molecular Human Reproduction*, 2012, 18(9): 425–434
99. Maekawa M, Yamaguchi K, Nakamura T, Shibukawa R, Kodanaka I, Ichisaka T, Kawamura Y, Mochizuki H, Goshima N, Yamanaka S. Direct reprogramming of somatic cells is promoted by maternal transcription factor Glis1. *Nature*, 2011, 474(7350): 225–229
100. Meissner A, Jaenisch R. Mammalian nuclear transfer. *Developmental Dynamics*, 2006, 235(9): 2460–2469