Review

Nucleus reprogramming/remodeling through selective enucleation (SE) of immature oocytes and zygotes: a nucleolus point of view

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This article is dedicated to our colleagues and friends, Jacek A. Modlinski (1944–2021) and Olga Zatsepina (1951–2020), who passed away recently. Both these scientists were born behind the "The Iron curtain – Poland and USSR" and not everybody can imagine how difficult it was to work with old equipment, insufficient supply of chemicals and information, and limited possibilities to travel abroad. Jacek invented the "selective enucleation" method whilst Olga significantly contributed to our understanding of the role of specific nucleoli in mammalian oocytes and zygotes. As this article is dedicated to JM and OZ, we attempted to extrapolate their contributions, albeit sometimes indirect, to SCNT (somatic cell nucleus transfer).

Abstract. It is now approximately 25 years since the sheep Dolly, the first cloned mammal where the somatic cell nucleus from an adult donor was used for transfer, was born. So far, somatic cell nucleus transfer, where G1-phase nuclei are transferred into cytoplasts obtained by enucleation of mature metaphase II (MII) oocytes followed by the activation of the reconstructed cells, is the most efficient approach to reprogram/remodel the differentiated nucleus. In general, in an enucleated oocyte (cytoplast), the nuclear envelope (NE, membrane) of an injected somatic cell nucleus breaks down and chromosomes condense. This condensation phase is followed, after subsequent activation, by chromatin decondensation and formation of a pseudo-pronucleus (i) whose morphology should resemble the natural postfertilization pronuclei (PNs). Thus, the volume of the transferred nuclei increases considerably by incorporating the content released from the germinal vesicles (GVs). In parallel, the transferred nucleus genes must be reset and function similarly as the relevant genes in normal embryo reprogramming. This, among others, covers the relevant epigenetic modifications and the appropriate organization of chromatin in pseudo-pronuclei. While reprogramming in SCNT is often discussed, the remodeling of transferred nuclei is much less studied, particularly in the context of the developmental potential of SCNT embryos. It is now evident that correct reprogramming mirrors appropriate remodeling. At the same time, it is widely accepted that the process of rebuilding the nucleus following SCNT is instrumental to the overall success of this procedure. Thus, in our contribution, we will mostly focus on the remodeling of transferred nuclei. In particular, we discuss the oocyte organelles that are essential for the development of SCNT embryos.
Key words: Nucleus, Remodeling, Reprogramming, Selective enucleation

Introduction

In recent years, some factors responsible for reprogramming of somatic nuclei following somatic cell nucleus transfer (SCNT) have been defined, which has led to a marked increase in overall efficiency [1, 2]. However, several factors and important processes remain to

be identified. Although it is widely accepted that complete rebuilding of the nucleus following SCNT is essential, the oocyte (cytoplast) organelles and components that are needed for correct remodeling and rebuilding of a transferred nucleus are not known. While the majority of studies have focused on the process of reprogramming, incorrect nuclear function might have more profound consequences. For example, every cell in the body is exposed to DNA-damaging agents (50,000–500,000 insults per day) and not all lesions are sufficiently repaired [3, 4]. This enables the somatic cell to survive but does not support optimal development when its nucleus is used for nuclear transfer. Multiple nuclear proteins are known to be involved in DNA repair, including typical nuclear envelope structural proteins such as lamins [5, 6]. Therefore, only a correctly assembled nucleus may support additional functions that are not directly linked

to reprogramming. This is another limitation of SCNT that is often not considered.

In addition to the classical NT scheme, where the oocyte nuclear components are made available to the transferred somatic nucleus due to their release into the cytoplasm following germinal vesicle breakdown (GVBD), there are other approaches that can help us to understand the mechanisms of reprogramming/remodeling in detail [7, 8].

1/ **Transfer of nuclei into germinal vesicles** – The permeabilized nuclei are directly injected into germinal vesicles (GVs), where they are partially reprogrammed. Concomitantly, the size of the transferred nuclei also increases, and their morphology is modified [9]. This approach is mainly used in amphibians and only exceptionally in mammals as their oocytes are much smaller and can be easily damaged. As the somatic nucleus remains intact, we may expect a limited turnover of nuclear structural components. Another aspect that we must take into account is that amphibian GVs are transcriptionally active, but the same activity has ceased in fully grown mammalian oocytes.

2/ **Reprogramming by cell fusion** – The differentiated cell is typically fused to embryonic stem cells (ESCs) (or alternatively only to its cytoplast-cybrids). Under the influence of ESC factors, differentiated nuclei are at least partially reprogrammed and remodeled [10, 11].

3/ **Treating permeabilized cells with cell extracts from pluripotent cells** – Permeabilized differentiated cells are partially reprogrammed when exposed to protein extracts prepared from pluripotent (ESC) cells or germ cells [12, 13].

4/ **Induced overexpression of specific transcription factors** – In differentiated cells, the overexpression of key transcription factors (Oct 3/4, Sox 2, Klf 4, c-Myc) is induced. If successful, these cells are converted into pluripotent ES cell-like state-induced pluripotent stem cells (iPSCs). This scheme has several modifications [14, 15]. Changes in the composition of nuclei under the influence of reprogramming factors are unknown.

5/ **Alternative nuclear transfer approaches** – In general, these approaches are modifications of a commonly used scheme which involves nucleus transfer into enucleated metaphase II oocytes. Typically, ES cells or somatic cell nuclei (M or G2 phase) are transferred into zygotic M-phase or into two-cell embryo M-phase cytoplasts, where they are reprogrammed/remodeled and the reconstructed cells are allowed to develop. Offspring are obtained here, but only when ES cells were used as karyoplasts [16, 17]. Nevertheless, as in the commonly used scheme, transferred nuclei are disassembled and rebuilt with relevant zygotic or embryonic nuclear components.

6/ **NT into selectively enucleated (SE) zygotes or immature oocytes** – PN or GV nuclear envelope is removed along with the associated chromatin, but the PN (GV) soluble content, nucleoli, and probably other intranuclear organelles are expelled into the zygote/oocyte cytoplasm. Nuclei are then transferred to these cytoplasts. Under these conditions, only a limited pool of nuclear structural components is available to the transferred nucleus. Therefore, this approach helps investigate the extent to which certain structural nuclear components participate in reprogramming and remodeling processes.

In the next section, we focus exclusively on SE (Fig. 1). The biological materials (FG- fully grown oocytes, and 1-cell stage embryos) and their gross morphological characteristics, as well as SE relevant activities, are briefly described.

Oocytes

To date, only completely developmentally competent oocytes have been used for selective enucleation. These oocytes can mature in culture and develop up to the blastocyst stage after fertilization or parthenogenetic activation – "fully grown oocytes (FG)." Their diameters in mice are approximately 70–80 µm. These oocytes contain a prominent nucleus (germinal vesicle, GV) with a well-visible nucleolus (NLB, nucleolus-like body) [18]. Compared to somatic cell nucleoli, the composition of NLBs is only partially characterized [19–23]. It must be noted that NLBs in live oocytes are clearly visible in mice, rats, pigs, and humans, while they are not visible in cattle and sheep. The reason for this species-specific difference or its biological significance, if any, remains unknown.
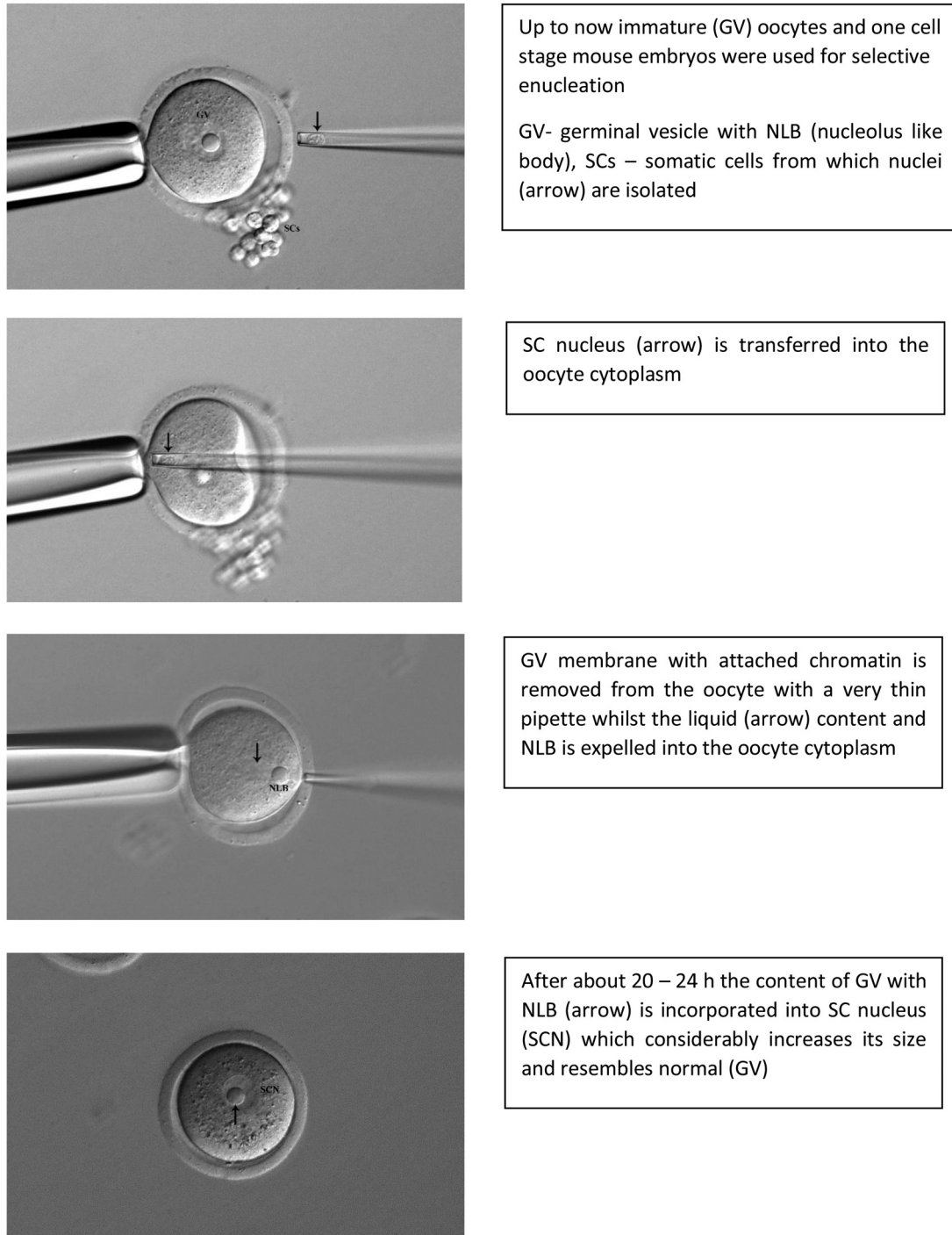
Compared with somatic cells, the organization of chromatin in FG GVs is distinct. Here, NLBs are enclosed by a prominent ring of heterochromatin (major/minor satellites), which are also attached to the NE, but to a much lesser extent. The nuclear lamina contains both lamin types (B and A/C) [24, 25]. Interestingly, meiosis-specific lamin isoforms have been identified in oocytes [26, 27]. The soluble GV content is not yet well characterized [28] but is essential for sperm head decondensation [29]. Although the presence of other typical intranuclear bodies (organelles) has not been systematically investigated in mammalian oocytes, some of them appear to be present, including speckles, Cajal bodies, and PLM bodies [30, 31].

Zygotes – One Cell Stage Embryos

In mice, normally fertilized oocytes contain two pronuclei, maternal/female (fPN) and paternal/male (mPN). However, the size of these pronuclei differs and the mPN is much larger. Both pronuclei typically contain several NLBs of maternal origin [32]. Besides the evident epigenetic asymmetry, some differences between the pronuclei can be detected when comparing the organization of chromatin. In fPN, centromeres surround the surface of NLBs, whereas in mPNs, they form rather unorganized clusters [33]. It is generally accepted that DNA replication begins earlier in mPN than in fPN [34], although a few authors have contradicted the same [35, 36]. Contradictory information concerning the presence of lamins as the major component of NE is often found in the literature as lamin B is consistently detected in both pronuclei [25, 37, 38]. On the other hand, some studies have reported the presence of lamin A/C in both pronuclei, while others could not detect the same [25, 37, 38]. Interestingly, it has been demonstrated that reprogramming activities are much higher in mPNs than in fPNs [39], and that paternal chromatin plays an important role in the regulation of transcriptional activities [40]. In addition, DNA repair activity is higher in mPN [41].

It must be noted that only minor transcriptional activity is detected in one-cell stage mouse embryos (minor zygotic genome activation), which is mirrored by the absence of bona fide speckles as the major site of RNA splicing [42–44].

In general, immature mammalian oocytes and zygotes contain nuclei that are unique compared to those of differentiated cells. These nuclei vary not only functionally (gene expression and transcription), but also morphologically. Moreover, the state of "differentiation/dedifferentiation" in embryos seems to be less stable compared to differentiated somatic cells and essentially changes with every embryonic cleavage [45]. This plasticity seems logical as during fertilization, two highly specialized cells, sperm and oocyte, meet. These germ cells are sometimes regarded as terminally differentiated cells but form a totipotent zygote [46].

Up to now immature (GV) oocytes and one cell stage mouse embryos were used for selective enucleation

GV- germinal vesicle with NLB (nucleolus like body), SCs – somatic cells from which nuclei (arrow) are isolated

SC nucleus (arrow) is transferred into the oocyte cytoplasm

GV membrane with attached chromatin is removed from the oocyte with a very thin pipette whilst the liquid (arrow) content and NLB is expelled into the oocyte cytoplasm

After about 20 – 24 h the content of GV with NLB (arrow) is incorporated into SC nucleus (SCN) which considerably increases its size and resembles normal (GV)

Fig. 1. The principle of selective enucleation (SE) and its use in nuclear transfer (NT) experiments.

Method of Selective Enucleation (SE) and Its Use in Reproductive and Developmental Biology

SE was invented by Jacek Modlinski in 1975 to produce haploid embryos in mice [47]. The author microsurgically removed either the paternal or the maternal pronuclear nuclear envelope (NE) using a thin pipette. Because chromatin is attached to NE, it is also removed. The soluble, or rather gel-like [48, 49], content of PNs, and some pronuclear organelles, NLBs, and probably others, are released into the zygote cytoplasm. Interestingly, the manipulated embryos developed

rather poorly, and when karyotyped, all were gynogenetic. This is attributed to inadequate manipulation and embryo culture conditions.

Borsuk later used the same approach in 1982 [50] to produce gynogenetic diploid mouse embryos. Borsuk first fertilized mouse oocytes and suppressed the extrusion of the second polar body with cytochalasin B (CB). Then, the male pronucleus NE with attached chromatin was removed by SE. The resulting embryos were diploid gynogenotes that developed to the blastocyst stage. Unfortunately, in these studies, no detailed characteristics of pronuclei that remained in the zygote cytoplasm were described. However, the complete

removal of pronuclear DNA attached to the NE was evident.

Mohammed *et al.* (2008) reported that fully grown immature (GV stage) mouse oocytes can be selectively enucleated [51]. The authors demonstrated that the replacement of GVs in SE cytoplasts with nuclei of different origins (cumulus cell, blastomere nuclei) was followed by maturation-like processes that were mostly abnormal, and the reconstructed oocytes showed abnormally-sized polar bodies and chaotic distribution of chromosomes. When the reconstructed and *in vitro*-matured oocytes were parthenogenetically activated or fertilized *in vitro*, the resulting PNs contained nucleoli, but their development was compromised.

About 30 years after Modlinski's original SE paper was published, Greda *et al.* (2006) used SE again [52]. In their experiments, by trapping the NE, PNs from fertilized mouse eggs were removed, and nuclei from eight-cell embryos were introduced into the cytoplasts. With SE NT (selective enucleation followed by nuclear transfer), it has been possible to obtain 70% of blastocysts and 8% of offspring. This has never been achieved before when eight-cell stage embryo nuclei were introduced into zygotes from which whole pronuclei, including all their content, were removed (CE, complete enucleation). These results indicate that certain reprogramming factors reside in PNs, and upon release, they can reprogram the advanced embryonic nuclei. This was later confirmed by Egli *et al.* (2007), who transferred ES or somatic cell mitotic groups into enucleated mitotic zygotes [16].

In 2015, Greda *et al.* [53] reported that cytoplasts produced by SE of zygotes support development to term even when 16 cell embryonic nuclei were used as karyoplasts. Full preimplantation development has been achieved even when embryonic fibroblasts or ES cell nuclei were used as karyoplasts. Unfortunately, these SE papers did not report how the morphology of the transferred nuclei changed.

Fulka *et al.* [54] studied the reprogramming and remodeling of somatic cell nuclei in immature, fully grown mouse oocytes. First, somatic cell (cumulus) G1-phase nuclei were injected into immature oocytes, followed by SE. Contrary to Mohammed *et al.* [51], these authors did not release the reconstructed oocytes from the maturation block immediately but prevented the onset of maturation by keeping the reconstructed cells in dbcAMP-supplemented medium. Surprisingly, the introduced cumulus cell nuclei considerably increased their volume within 18–20 h by incorporating the released GV content (including NLBs) and resembled normal intact oocyte GVs. No DNA replication was observed. When these oocyte-like cells were released from the dbcAMP block, a maturation-like process was observed with visible first polar bodies extruded after approximately 5–6 h. The metaphase II-like group contained a single chromatid chromosome. The envelope of the transformed nuclei seems to be fully functional, with no differences when compared to intact GVs (lamins B, A/C, NPC – nuclear pore complex). The somatic cell nuclei used for transplantation are transcriptionally active. However, transcription ceases in the SE cytoplasm. This does not happen if cumulus cell nuclei are injected either into intact or completely enucleated (whole GV removed) oocytes [30, 54]. Thus, the GV soluble material was able to silence RNA transcription.

The cytoplasm of SE oocytes can also promote epigenetic changes in the transferred nuclei. Immature GV oocytes were positive for histone variant H3.3 and negative for H3.1/3.2. On the other hand, somatic cells are highly positive for H3.1/H3.2, and much less positive for H3.3. The transformed somatic cell nuclei in SE cytoplasts became positive for H3.3, indicating that this variant was rapidly imported into the nuclei. In contrast, the other variants (H3.1/H3.2) were not completely removed in approximately half of the labeled reconstructed cells [54].

Collectively, these results demonstrate that GV karyoplasm can partially reprogram/remodel the transferred differentiated nuclei. It remains to be tested whether this pre-reprogramming/remodeling enhances the efficiency of SCNT when these transformed nuclei are used for NT into the MII cytoplast. From the perspective of the cell cycle, these nuclei would be suitable for NT as they are in the G1 phase. However, the exact roles of karyoplasm components in reprogramming and remodeling remain unclear. The only exception are oocyte nucleoli, which are discussed below.

Oocyte Nuclear Sub-structures Essential for SCNT Embryos Development

As mentioned above, SE removes GV/PN nuclear envelopes along with the attached chromatin, and gel-like nuclear content with nucleoli (NLBs) is released into the oocytes/zygote cytoplasm. In this context, it must be noted that at least one nuclear organelle, NLB (nucleolus like body - nucleolus), originating from the oocyte must be incorporated into the newly formed SCNT pseudo-pronucleus(i). Without this, the reconstructed embryo cleaves only once or twice, and then its development ceases. Interestingly, in the "early days of NT experiments", evaluation of nucleolar morphology served as an indicator of the successful nuclear transfer. In brief, when the transferred nucleus was first exposed to maturation promoting factor (MPF) and chromosomes condensed, the post-activation pseudo-pronuclei typically contained nucleoli similar to those detected in the zygote. On the other hand, when nuclei were transferred into cytoplasts obtained by the removal of whole pronuclei (PNs), i.e., including nucleoli [55, 56], the morphology of somatic or blastomere nucleoli remained unchanged [57].

The concept that nucleoli in pronuclei in fertilized, parthenogenetically activated, or pseudo-pronuclei of SCNT embryos (transfer into MII cytoplasts) are exclusively of maternal (oocyte) origin arises from the "enucleolation" experiments where nucleoli (NLBs) were microsurgically removed from immature (GV stage) oocytes [58]. The manipulated oocytes matured well in culture and reached MII. However, after activation or ICSI (intracytoplasmic sperm injection), the newly formed pronuclei did not contain nucleoli. Nucleolus-less zygotes cleaved only once or twice, and their development ceased [59]. These observations were further expanded, and it has been shown that the nucleoli of SCs or ESCs cannot substitute for the original oocyte NLBs. When either cumulus cells or ESC nuclei were transferred into cytoplasts originating from the enucleolated and then *in vitro*-matured oocytes, the reconstructed embryos contained well-formed pseudo-pronuclei that lacked detectable nucleoli [32]. These reconstructed embryos did not develop beyond the first stages of embryogenesis.

The re-injection of oocyte and even two-cell-stage embryo nucleoli into enucleolated oocytes rescued their developmental potential after fertilization or parthenogenetic activation [60]. It must also be noted here that when NLBs are re-injected into previously enucleolated or intact oocytes (zygotes), it is not necessary to inject them directly into GVs or PNs. When nucleoli are injected into the cytoplasm, they first disperse, but the nucleolar material is gradually reincorporated into the nucleus [61]. This is understandable because of the presence of nuclear/nucleolar localization signals in proteins known to constitute NLBs [62, 63]. Some results have shown that the embryo tolerates surplus nucleolar material rather than its reduced volume [64, 65].

Taken together, these experiments confirmed the commonly accepted view that oocyte nucleoli serve as deposits of material that is gradually transformed into typical tripartite nucleoli after fertilization.

However, these two experiments challenged this dogma. First, Ogushi and Saitou (2010) re-injected isolated GV NLBs into previously enucleolated oocytes or zygotes that originated from oocytes without NLBs (enucleolated and then matured) [66]. The injected oocytes formed PNs with nucleoli and developed well after fertilization. On the other hand, NLBs injected into fertilized oocytes (previously enucleolated and then matured) also contained PNs with nucleoli, but only zygotes that were NLB injected soon after fertilization developed well and gave rise to offspring. The development of zygotes from enucleolated oocytes injected with NLBs approximately 8–10 h post fertilization was greatly compromised. In 2014, Kyogoku *et al.* [67] removed nucleoli from mononucleolar zygotes at different times post-ICSI. Surprisingly, the zygotes enucleolated approximately 10 h post ICSI developed well and the embryos exhibited normal nucleologenesis and gave rise to offspring. These results indicate that nucleoli in zygotes, at least in mice, do not serve as a repository of material(s) that is used for the formation of nucleoli. Instead, they are essential only for a very short time period very soon after fertilization (see below).

Although several intranuclear organelles have been characterized in somatic cells (polycomb bodies, PML bodies, paraspeckles, clastosomes, nuclear speckles, Cajal bodies, and histone locus body) [68], to date, only the oocyte nucleolus (NLB) has been characterized as an essential intranuclear organelle inherited from the oocyte, which, once removed, cannot be formed *de novo*. This is because only the oocyte nucleoli are sufficiently large and visible (but not in all mammals) under a light microscope and can thus be manipulated relatively easily.

The Gain of Remodeling Capacity and the Suitability of Atypical Cytoplasts

In summary, the SE approach demonstrated that certain reprogramming factors were present in immature mammalian oocyte nuclei (GV). This confirms the classical experiments of John Gurdon [69], who injected differentiated nuclei into amphibian germinal vesicles. Similar experiments with mammalian oocytes are rather complicated because of their smaller size, fragility, and distinct physiological characteristics, such as transcription. Therefore, the differentiated cell nuclei were incubated in GV extracts and then evaluated or eventually injected into enucleated oocytes. If directly injected into GVs, a certain degree of dedifferentiation has been observed [70].

Interestingly, decondensation of sperm heads was also observed when mouse sperm heads were injected into GVs. Unfortunately, these decondensed sperm nuclei have not been characterized further [71].

Due to the above-mentioned technical difficulties, particularly the fragility of mammalian oocytes, some indirect approaches have been used to demonstrate that reprogramming factors are mostly localized in GVs or PNs [72]. Thus, GVs from oocytes are used for the preparation of extracts in which somatic cell nuclei are incubated or zygotes are periodically observed. As soon as they enter the M-phase, the condensed chromosomes are removed, and a donor-differentiated cell nuclear material (either G2-stage or condensed chromosomes) is transferred into these cytoplasts. The eventual disadvantage is that some reprogramming (or other) factors may be bound to condensed chromosomes, and are thus removed with the metaphase plate [73].

As mentioned above, with the exception of the oocyte nucleolar (NLB) material [32], it is not known which oocyte (zygote) nuclear components are used as building blocks in the process of transferred somatic cell nucleus remodeling. After SE, it does not matter if immature oocytes or zygotes are used the nucleoli are released into the cytoplasm and then dissolved in it. These nucleoli are then incorporated into the transferred nuclei [74, 75].

This probably also occurred when "universal cytoplasts" were used. These cytoplasts originate from enucleated MII oocytes, which are then parthenogenetically activated and after approximately 6 h fused with an intact cell, (blastomere from 8–16 cell embryo). Thus, they do not contain MPF/CCA and the transferred nucleus remains intact. However, it can be assumed that certain intranuclear organelles (bodies) and soluble factors are still present in these cytoplasts and move into the transferred nucleus through the intact membrane [76]. Unfortunately, these NT embryos, mostly in sheep, were not characterized in detail (only DNA replication), but we can expect that their nuclei contained extra nucleolar material as well as soluble nuclear content that was released into the oocyte cytoplasm when the oocytes began to mature.

Thus, in zygotes and in NT embryos, NLBs are exclusively derived from oocytes, either intact or enucleated (metaphase chromosomes removed), that are used as cytoplasts. Once the NLBs are removed, they cannot be formed. In mice, it has also been demonstrated that the oocyte nucleolus is essential during the early stages of PN formation [20, 77, 78]. We can also expect that M-phase zygotes or two-cell stage embryos can be used as a source of cytoplasts, when the nucleolar material originating from PNs or blastomere nuclei is dissolved in the cytoplasm. Nucleoli in newly formed NT nuclei originate from this material.

When an immature oocyte is selectively enucleated and its GV membrane is completely removed, the introduced somatic cell nucleus dramatically increases in size (volume), and its nuclear envelope seems to be functional, as shown by the presence of lamins (A/C, B) or the nuclear pore complex [54]. As demonstrated recently by Mukherjee *et al.* [79], the perinuclear endoplasmic reticulum participates in nuclear transformation and nuclear membrane formation [80]. This is not surprising, as the close association between ER and NE has been well described [81].

The Possible Function of the Nuclear Components and Their Potential Use for Embryo Selection

The correct organization of chromatin and normal overall architecture of nuclei are essential for embryo development [82]. In somatic cells, the major chromatin organizing structures are the nuclear periphery and the nucleoli. Although sequences interacting with the nuclear lamina have recently been mapped in mouse zygotes [83], the significance of these interactions with respect to normal development is unclear. The results using SE cytoplasts as recipients indicate that perhaps the chromatin-NE interactions during early embryonic development are less important than those in somatic cells. This indicates that chromatin-nucleoli (NLBs) association is more essential than the chromatin-NE association. It has been demonstrated that chromatin organization is highly aberrant in zygotes originating from enucleolated oocytes, and aberrant (pseudo) pronuclear morphology is also frequently detected in NT embryos [84, 85]. Thus, nucleoli (NLBs) represent a central structural platform for organizing specific sequences and possibly their remodeling, establishing totipotency [86]. However, while the role of nucleoli has been intensively studied in mouse oocytes and embryos, little is known about their function in other mammals. Thus, it is unclear whether the results obtained in mice can be extrapolated to other species where the SCNT procedure is highly relevant. This includes livestock, where individual animals can be extremely valuable both

economically and genetically. However, appropriate criteria for embryo selection are currently lacking.

Nevertheless, in bovine zygotes, as in other species, it seems that appropriate organization of chromosomes is essential for healthy embryo development [87]. In human embryos, the nucleoli in PNs are visible, they can be relatively easily tracked and closely mirror the organization of pronuclear chromatin [87–89]. On the other hand, in bovine zygotes, the nucleoli are not visible under a light microscope [18]. This prevents the use of nucleolar dynamics in zygotes of species such as bovine or sheep as potentially attractive noninvasive indicators of development, as has been suggested for human embryos [88, 89].

This problem can potentially be overcome by transferring the nucleoli (NLBs) from oocytes/zygotes of those species, where these organelles are well visible, into the oocytes/zygotes with no visible nucleoli. We have previously demonstrated that NLBs from mouse GV-stage oocytes can rescue the embryonic development of porcine enucleolated oocytes and vice versa [90, 91]. Xenogenic NLB material did not seem to seriously hamper embryonic development. Thus, when bovine oocytes/zygotes are injected with compact nucleoli isolated from mouse oocytes, they translocate into GVs/PNs where they are clearly visible. Thus, in theory, this approach could be used for more detailed analyses of certain processes that occur in PNs in one-cell-stage embryos (Figs. 2–5). However, more experiments are needed, as it is unclear whether mouse NLB material indeed reflects the position of chromatin.

Conclusion

Reprogramming in SCNT is well-understood due to advanced methods that have helped understand the overall chromatin organization in transferred nuclei and define those differences that may be responsible for aberrant development. In general, these observations demonstrate that the reprogramming SCNT processes are basically similar to those processes that can be seen in fertilized oocytes. However, many aberrations have been detected, including stronger TADs boundaries (topologically associated domains) and distinct super-enhancer and promoter interactions [92, 93]. Moreover, the essential role of NLBs in the transition from pluripotency to totipotency and the possibility of modulating it has been demonstrated, albeit
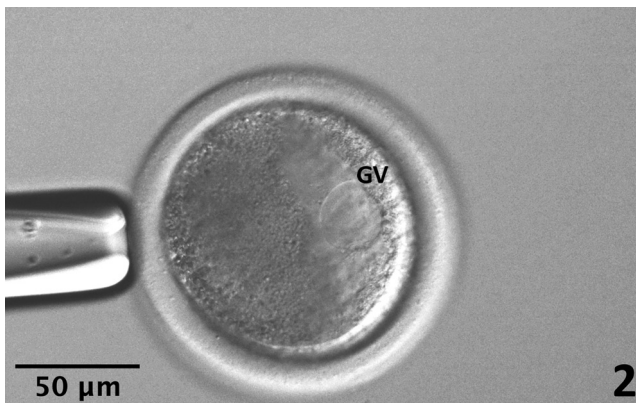

Fig. 2. Live immature bovine oocyte with germinal vesicle (GV) where the nucleolus like body is not visible.
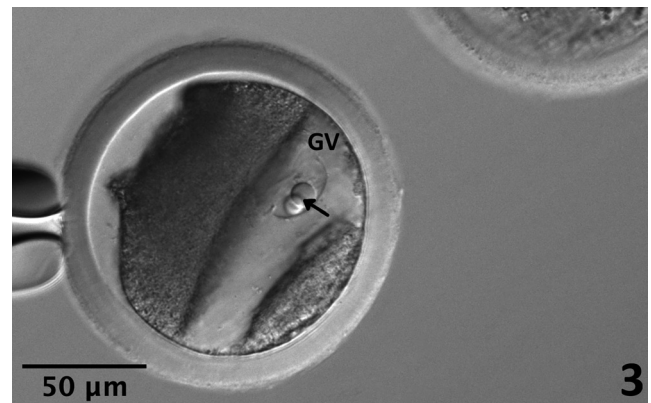
Fig. 3. Live immature bovine oocyte injected with mouse NLB that was isolated from an immature GV stage oocyte. NLB was injected into the bovine oocyte cytoplasm and rapidly moved into the bovine GV (GV – germinal vesicle, NLB – arrow).
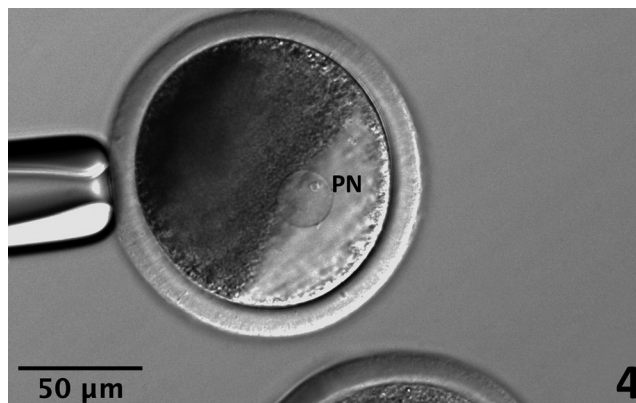
Fig. 4. Live bovine oocytes that are parthenogetically activated contain pronuclei without visible NLBs (PN – pronucleus).
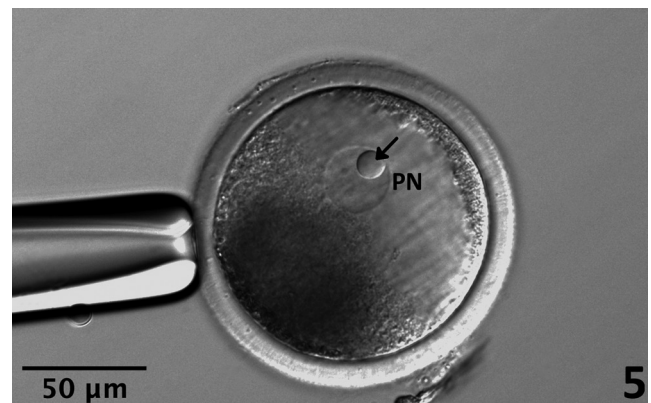
Fig. 5. When mature bovine oocytes (MII – metaphase II) are injected with mouse oocyte NLBs and these injected oocytes are then activated, the newly formed pronuclei contain visible nucleoli of mouse origin (arrow). In all above cases, the oocytes without cumulus cells were incubated in medium with cytochalasin B (5 µg/ml) for 10 min and then centrifuged for 10 min (9000 RCF).

indirectly, in ESCs [94].

The internal organization inside the somatic cell nucleus is not chaotic, and the same seems to be true for mammalian oocytes and embryos, as well as for embryos that are either produced by ICSI or somatic cell nucleus transfer. The size of mammalian oocytes, zygotes, early cleavage stage embryos, and advances in micromanipulation give us an excellent opportunity to explore and clarify the processes regulating intranuclear organization in more detail, and this will certainly lead to improvements in assisted reproduction and SCNT approaches.

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