Chapter 4
Nuclear–Centrosome Relationships During Fertilization, Cell Division, Embryo Development, and in Somatic Cell Nuclear Transfer Embryos

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Abstract Nuclear–centrosome relationships are critical for synchronized cell cycle progression. During fertilization and syngamy, centrosome–nuclear relationships are important for pronuclear migration and to allow synchronized maturation of the sperm nucleus into the male pronucleus and of the sperm's centriole–centrosome complex into a division-competent centrosome that is able to separate chromosomes precisely into the dividing daughter cells. Abnormalities in nuclear–centrosome interactions are among the underlying causes for male and female factor infertility, for developmental disorders, and disease. Centrosome–nuclear abnormalities are also encountered in somatic cell nuclear transfer (SCNT) embryos when centrosome reprogramming is defective. The present review is focused on 1) The sperm centriole–centrosome complex and associations with the sperm nucleus before fertilization; 2) Centrosome–nuclear relationships during pronuclear/zygote stage, cell division, and embryo development; and 3) Centrosome–nuclear interactions and centrosome reprogramming abnormalities in SCNT embryos.

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4.1 Introduction

Structural and functional relationships between the nucleus and centrosomes are critically important for successful fertilization, accurate cell division, and proper embryo development. In somatic interphase cells, centrosomes are closely associated with the nuclear surface and structural centrosome–nuclear relationships have also been shown for *C. elegans* embryos (Meyerzon et al. 2009) while studies to explore the structural and functional centrosome–nuclear relationships in mammalian embryo cells are still only at the beginning.

Is has been well shown in somatic cell systems that precise nuclear–centrosome synchrony is essential for mitosis and cytokinesis when centrosomes, microtubules, and chromosomes need to interact precisely to fulfill mitotic checkpoint licensing and coordinate chromosome separation for accurate cell division (reviewed in Schatten 2008 and Chaps. 8, 9, 10, and 11 of this book). In the developing embryo, centrosomes and the nucleus have to coordinate both symmetric and asymmetric cell divisions to distribute cellular components and cell fate determinants to the dividing daughter cells which is important for cell differentiation. As our previous reviews have addressed the role of centrosomes in oocyte maturation (Schatten and Sun 2011b), fertilization (Schatten and Sun 2009a, 2009b, 2010, 2011a, 2011c), and male factor infertility (Schatten et al. 2011) we will focus the present review on 1) The sperm centriole–centrosome complex and associations with the sperm nucleus before fertilization; 2) Centrosome–nuclear− synchrony during pronuclear/zygote stage, cell division, and embryo development; and 3) Centrosome–nuclear interactions and reprogramming abnormalities in somatic cell nuclear transfer (SCNT) embryos.

4.2 The Sperm Centriole–Centrosome Complex and Associations with the Sperm Nucleus Before Fertilization

In non-rodent mammalian systems, the sperm contains one proximal and one distal centriole that are organized perpendicular to each other (Fig. 4.1) and located within the sperm's connecting piece between the midpiece and the sperm's nucleus. Only the proximal centriole is associated with the sperm nucleus and surrounded by a small amount of centrosomal proteins including γ-tubulin and centriole-associated centrin as well as other newly discovered centrosomal components (Goto et al. 2010) for which functions remain to be determined (reviewed in Schatten and Sun 2011a, 2011b, 2011c). The distal centriole is associated with the sperm tail and its functions primarily include organization and assembly of sperm tail microtubules. The close association and functional relationships of the proximal centriole with the sperm nucleus has been assessed on structural levels before fertilization and it becomes clearly apparent after fertilization when the

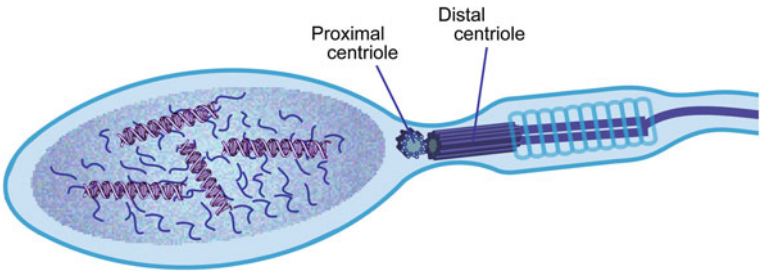

Fig. 4.1 *Schematic representation of non-rodent sperm*: showing sperm head with DNA and nuclear matrix proteins and the centriole complex. The sperm's centriole complex consists of two perpendicularly oriented centrioles. The centriole close to the nucleus is termed the proximal centriole and contains sparse centrosomal material that will become important for microtubule nucleation of sperm aster, zygote aster, and mitotic apparatus in the fertilized oocyte while the centriole associated with the sperm tail (distal centriole; basal body) will not participate in the embryo's microtubule formations and will be subjected to degeneration along with the sperm tail after fertilization

sperm head decondenses and the centriole–centrosome complex matures within the zygote to become a division-competent centrosome that forms the mitotic spindle poles and separates chromosomes equally to the dividing daughter cells, as detailed in Sect. 3 of this chapter.

Excellent electron micrographs are available on the centriole complex in human sperm that have been presented in several original papers and review articles (Chemes 2000; Chemes and Rawe 2003; Mitchell et al. 2006; Rawe et al. 2002; Rawe and Chemes 2009; Sathananthan et al. 1991, 1996, 2001; Sathananthan 1997, Sathananthan 2009) displaying clear structural associations between the sperm nucleus and the proximal centriole. Examples are presented in Chaps. 2 and 5 of this book (by Hector E. Chemes and A. Henry Sathananthan, respectively), providing remarkable detail of normal structure and structural abnormalities in the proximal centriole-sperm nucleus associations that impact fertilization. Structural abnormalities and dysfunctions associated with male factor infertility have been documented and it has also been shown that in some cases of infertility centriole–nuclear detachment sites are impaired (Liska et al. 2009; Kierszenbaum et al. 2011). While morphological abnormalities have clearly been identified as underlying causes for sperm centriole–centrosomal dysfunctions, molecular abnormalities have been determined by using a variety of molecular methods including immunoblotting techniques (Bohring and Krause 2003; reviewed in Schatten and Sun 2009a, 2009b). Several studies have been performed on human sperm that correlate decreased γ-tubulin and decreased centrin to lower fertilizability in humans (Hinduja et al. 2008; 2010) and correlated below-normal quantities to decreased sperm aster formation and developmental capacity, as also discussed by Comizzoli and Wildt in Chap. 3 of this book.

While recent research has focused on the sperm's centrosome complex as causes of infertility, studies related to nuclear components within the sperm

nucleus have been sparse which may in part be related to the density of the sperm nucleus that has made experimental and analytical approaches difficult (reviewed in Johnson et al. 2011). The mature sperm nucleus is distinguished from other nuclei by its extreme chromatin condensation state which is achieved during spermiogenesis when the majority of histones are replaced by protamines, small basic proteins that are bound to sperm DNA and become cross-linked through the formation of disulfide bridges when spermatozoa transit through the epididymis (reviewed in Delbés et al. 2010). Proper chromatin compaction is important for male factor fertility in which accurate protamine, histone, and nuclear matrix component functions are essential. Only recently has it been possible to dissect structural aspects within the sperm nucleus and it has clearly been shown that nuclear matrix components are present in sperm (reviewed in Johnson et al. 2011) which opens up speculations that the nuclear mitotic apparatus (NuMA) protein may exist within the sperm nucleus. The multifunctional protein NuMA has been best studied in somatic cells (reviewed in Sun and Schatten 2006) and it has been shown that it plays important roles as nuclear matrix protein in interphase and as centrosome-associated protein during meiosis and mitosis (reviewed in Sun and Schatten 2006). We do not yet know when NuMA functions become important for sperm nuclear functions and for nuclear–centrosome relationships but we know for certain that it plays an important role in the decondensing sperm pronucleus after fertilization (reviewed in Sun and Schatten 2006; Alvarez-Sedo et al. 2011; Schatten et al. 2012) which will be addressed in Sect. 2 of this chapter. To better understand yet unexplained causes of male factor infertility it will be important to determine new methods to better analyze nuclear components in sperm that play a role in nuclear–centrosome synchrony after fertilization.

4.3 Centrosome–Nuclear Relationships During Pronuclear/ Zygote Stage, Cell Division, and Embryo Development

A schematic representation of the nuclear and centriole–centrosome cycle within the first embryonic cell cycle is shown in Fig. 4.2a–d and described in the figure legend. Significant changes in sperm chromatin structure begin immediately after fertilization when protamines are replaced by histones and several epigenetic modifications take place (Chao et al. 2012). Sperm chromatin becomes decondensed and the sperm nucleus matures into the male pronucleus while the sperm-derived centriole–centrosome complex matures by recruiting and accumulating centrosomal components from the sperm-activated oocyte (reviewed in Schatten and Sun 2010, 2011a, 2011c).

Intimate structural and functional relationships between nuclear and centrosome proteins are important to fulfill cell cycle-specific functions including microtubule organization, chromosome alignment, and chromosome separation during the embryo's first cell cycle (reviewed in Schatten 2008; Schatten and Sun

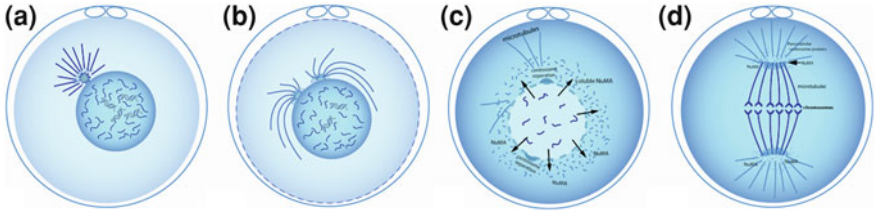

Fig. 4.2 **a–d**: schematic representation of the nuclear and centriole–centrosome cycle within the first embryonic cell cycle. **a** Sperm aster formation from the sperm-derived proximal centriole–centrosome complex; DNA and the nuclear matrix protein NuMA are localized in the nucleus; **b** duplication of centrioles at pronuclear stage; **c** duplicated centriole–centrosome complex separates and migrates around the zygote nucleus, relocating to opposite poles to form the centers of the mitotic spindle poles; NuMA becomes a centrosome-associated protein and participates in the formation of the spindle poles by forming a crescent around the centrosome area facing chromosomes; **d** mitosis of the first cell cycle

2011a, 2011c); synchronized centrosome and nuclear maturation within the fertilized oocyte is critical for all subsequent cell divisions within the developing embryo. Nuclear–centrosome cell cycle synchronization has been studied in detail for the S phase in somatic cells which correlates to the pronuclear stage in the zygote embryo cell and to the S phases of all cells in the developing embryo when synchronized DNA and centrosome duplication takes place. In somatic cells, critical regulatory processes have been identified including activation of CDK2-cyclin E (Okuda et al. 2000; Tokuyama et al. 2001; Ferguson and Maller 2010) and other cell cycle-specific proteins (reviewed in Chap. 8 of this book by Fisk and in Chap 11 of this book by Boutros). In addition, centrosome-related protein degradation becomes important, as detailed in Chap. 8 of this book by Fisk and in Chap. 9 of this book by Posser and Fry.

In normal cell cycles synchronization is tightly controlled through cell cycle checkpoints, coordinated signal transduction cascades, and several other regulatory processes that, while well-studied in somatic cells, remain only partly explored in embryo cells during preimplantation development. We do know that the maturation promoting factor (MPF) and mitogen-activated protein kinase (MAPK) are important for nuclear maturation and both are important for regulation of several cell cycle events during oocyte maturation, in the fertilized embryo cell, and in the embryo's first cell cycle (reviewed in Fan and Sun 2004; Snook et al. 2011) but we do not know details on cell cycle checkpoints and regulatory processes that drive centrosome maturation and dispersion of nuclear proteins into the cytoplasm for subsequent specific functions during cell cycle progression of the first and subsequent cell cycles in embryonic cells. It is clear that synchronized signaling of nuclear and centrosome dynamics is important to ensure accurate participation in the mitotic process during first and subsequent cell divisions. Studies have begun to investigate the regulation of NuMA as one of the essential nuclear and centrosome-associated proteins important for successful fertilization and embryonic cell divisions.

NuMA is a multifunctional 236 kDa protein that in interphase is a component of the nuclear matrix, a proteinaceous network that plays a role in DNA organization. NuMA's specific roles in the nucleus are only partly understood (reviewed in Sun and Schatten 2006) while significantly more studies are available on NuMA's functions as centrosome-associated protein in mitosis (reviewed in Sun and Schatten 2006). We do not yet know whether NuMA plays a role in sperm DNA organization and nuclear decompaction in the zygote embryo but we know that the nuclear matrix is important for DNA replication in the zygote (reviewed by Yamauchi et al. 2011; Johnson et al. 2011). Research on the sperm's nuclear matrix has accelerated in recent years, and it has been proposed that non-genetic male factor infertility problems may be related to nuclear matrix instability (reviewed in Johnson et al. 2011), contributing to transgenerational non-genetic instability. It has further been shown that chronic exposure of sperm to low doses of specific toxins is correlated with an altered nuclear matrix protein profile and includes abnormal chromatin condensation (Codrington et al. 2007a, 2007b) which affects fertilization and embryo development and may also affect nuclear matrix-centrosome interactions and nuclear–centrosome synchronization. This and other examples indicate the effects of non-genetic components on the nuclear matrix that may affect synergistic interactions with centrosomes and may have implications in male factor infertility.

NuMA is an important link in synchronizing nuclear and centrosome maturation events after fertilization; studies in somatic cells have shown that NuMA requires precise regulation including regulation by cyclin B to move out of the nucleus into the cytoplasm during prophase and associate with mitotic centrosomes to stabilize centrosome–microtubule interactions for the formation of the mitotic apparatus. NuMA is not associated with the interphase centrosome; it strictly serves as nuclear protein in interphase and becomes a centrosome-associated protein only in mitosis (reviewed in Sun and Schatten 2006). NuMA is highly insoluble in the nucleus but at the time of nuclear envelope breakdown NuMA becomes hyperphosphorylated by $p34^{cdc2}$ which allows dispersion of NuMA into the cytoplasm and subsequent translocation to the spindle poles in a dynein-mediated process. NuMA remains associated with the spindle poles until anaphase; it dissociates from spindle poles after dephosphorylation as a result of Cdk1 inactivation and loss of cyclin B that occurs by proteasome-mediated degradation (Gehmlich et al. 2004).

Most of the studies on NuMA have been performed in somatic cells while only a few detailed studies are available for embryonic cells. Our recent studies in human oocytes showed a requirement for dynein to mediate NuMA translocation to the spindle poles during first mitosis (Alvarez Sedó et al. 2011; Schatten et al. 2012). In the MI and MII meiotic spindles NuMA was localized to the meiotic spindle poles and displayed abnormalities in aged oocytes. It also displayed abnormalities in fertilized oocytes in which sperm decondensation failed which coincided with abnormal NuMA immunofluorescence staining patterns and suggests that NuMA abnormalities are associated with fertilization failures. Dispersed NuMA fluorescence staining patterns were seen in male and female pronuclei in

normal fertilization (Liu et al. 2006; Alvarez-Sedó et al. 2011; Schatten et al. 2012).

Detailed studies on NuMA before and after fertilization will be important to determine whether NuMA dysfunctions play a role in male-factor infertility, embryo abnormalities, and whether NuMA dysfunctions at this early stage of an embryo's development will result in adulthood diseases, as misregulation of NuMA can result in the formation of multipolar mitoses which are hallmark features for cancer development and progression (Kammerer et al. 2005; reviewed in Sun and Schatten 2006). In this context it is also worth noting that NuMA becomes extensively modified after herpes simplex virus (HSV) infection which induces solubilization and relocalization of NuMA (Yamauchi et al. 2008); it may affect subsequent NuMA dynamics that may play a role in mitotic abnormalities underlying diseases such as cancer.

Close attachment between the centrosome and the nucleus is important for coordinated pronuclear migration and apposition of male and female pronuclei and formation of the zygote aster that evolves into the mitotic apparatus to separate the parental genomes equally to the dividing daughter cells (reviewed in Schatten and Sun 2009a, 2009b, 2010, 2011a, 2011c). The structural associations of centrosomes with the nucleus have been described in somatic cells (Meyer et al. 2011) and studies in *C. elegans* have explored the mechanisms of centrosome–nuclear relationships but only sparse information is available on such structural and functional relationships in mammalian embryo cells. In *C. elegans*, ZYG-12, SUN-1, and LIS-1 interact with dynein to contribute to the attachment of centrosomes to the nucleus in early development and it was proposed that recruitment of dynein to the cytoplasmic surface of the nuclear envelope is critical for the attachment of centrosomes (Malone et al. 2003; Meyerzon et al. 2009); two genes, zyg-12, and sun-1 were shown to be essential for centrosome attachment and embryonic development in this system (Fridkin et al. 2004; Malone et al. 2003). It was further proposed that the inner nuclear membrane and nuclear lamina proteins are involved in centrosome–nucleus attachment (Askjaer et al. 2002; Galy et al. 2006). In *C. elegans*, Meyerzon et al. (2009) described a novel role for nuclear lamina proteins in centrosome attachment to the nuclear envelope. The mechanisms described for nuclear–centrosomal attachment in *C. elegans* have been explored for the first few cell divisions in early embryogenesis but may be different during later development, as ZYG-12 is not required for later stages of embryogenesis. These studies in *C. elegans* provide important steps toward understanding embryonic centrosome–nuclear attachment mechanisms while we still know only little about such molecular mechanisms for mammalian embryos.

In fibroblast cells, it was shown that lamin and the integral inner nuclear membrane protein emerin are involved in centrosome attachment, as fibroblasts from emerin-defective human patients or lamin A/C mutant mice displayed centrosome detachment phenotypes (Lee et al. 2007; Salpingidou et al. 2007). Emerin is present at both the inner and outer nuclear membrane and it was determined that emerin interacts with tubulin which led to the proposed model that emerin on the

outer nuclear envelope directly interacts with microtubules to attach the centrosome to the nuclear envelope (Salpingidou et al. 2007).

Another nuclear protein of interest regarding centrosome–nuclear relationships is the multifunctional structural protein 4.1R that localizes within nuclei, at the nuclear envelope, and in the cytoplasm. It has recently been shown that 4.1R, the nuclear envelope protein emerin and the intermediate filament protein lamin A/C co-immunoprecipitate in human cells and that its depletion affects the distribution of NuMA as well as other nuclear proteins (Meyer et al. 2011). These studies extend on the previous findings in different cell systems reporting that emerin couples centrosomes to the nuclear envelope (Markiewicz et al. 2006; Salpingidou et al. 2007). Meyer et al. showed that the functional effects of 4.1R deficiency included disruption of its association with emerin and A-type lamins and an increase in nucleus–centrosome distances, affecting centrosome–nuclear envelope association (Meyer et al. 2011). Previous reports had shown that 4.1 binds NuMA and contributes to the organization of the nucleoskeleton and nuclear membrane proteins; it plays a role in the attachment of centrosomes to the nucleus in *C. elegans* (Meyerzon et al. 2009; Simon and Wilson 2011). While we do not yet have a complete understanding of the processes and molecular interactions involved in nucleus–centrosome attachments, the role of nuclear proteins in centrosome attachment to the nucleus is beginning to emerge although our current still fragmented knowledge has been derived from different cell systems and different mechanisms may be employed in different cell systems. Because protein 4.1R is also integral to mitotic spindle and centrosome assembly and structure (Krauss et al. 2004, 2008) it may further allow us to generate new insights into nuclear–mitotic centrosome relationships as an important step toward understanding cell cycle-specific nuclear–centrosome synchronization.

As mentioned above, the synchronized distribution of centrosomal and genetic material to the dividing daughter cells is facilitated by the tight association of centrosomes with the nucleus; this interaction is important for all cells in the developing embryo and the close association also allows synchronized distribution of critical cellular components to the dividing daughter cells. The role of centrosomes during embryo development includes gathering and distribution of cellular components including critical cell fate determinants to the dividing daughter cells. Such cell fate determinants differ in different systems (reviewed in Knoblich 2010) but substantial evidence exists that they are transported along microtubules during interphase and distributed to the differentiating blastomeres during subsequent cell divisions. It includes cytoplasmic factors such as transcripts of developmental genes, eventually resulting in different gene activity; the inheritance of mRNA localized at centrosomes has been described for *Ilyanassa* (Lambert and Nagy 2002; Kingsly et al. 2007).

Transport along microtubules to centrosomes is also utilized for protein degradation which has been described for embryonic stem cell divisions, allowing asymmetric inheritance of proteins destined for different degradation in the dividing daughter cells (reviewed in Chap. 8 of this book by Fisk).

4.4 Centrosome–Nuclear Interactions and Reprogramming Abnormalities in Somatic Cell Nuclear Transfer Embryos

The tight structural connections of the centrosome with the nucleus are also apparent in nuclear isolations in which the centrosome is typically co-isolated along with the nucleus unless specific centrosome–nucleus separation methods are employed. This close connection becomes important for SCNT embryos in which an oocyte is enucleated and genomic material is replaced with a somatic cell nucleus that also contributes the nucleus-associated centriole–centrosome complex, thereby providing the centrosomal core structure that is normally contributed by sperm during fertilization. Like the somatic cell nucleus, the somatic cell's centriole–centrosome complex needs to be reprogrammed in SCNT to fulfill functions that are normally carried out by the blended sperm–oocyte centrosomal complex (reviewed in Schatten et al. 2009a, 2009b) which includes formation of the mitotic apparatus during cell division. Reprogramming of the somatic cell's centrosome complex depends on regulation by the enucleated oocyte to provide components that are important for embryonic centrosome cell cycles. While reconstructed SCNT eggs provide an ideal analysis system for centrosome regulation very few studies have been performed so far on centrosome regulation in the SCNT embryo system. However, studying the complexities between requirements for embryonic cells compared to somatic cells may bring about further insights into centrosome biology and correlations between nuclear–centrosome interactions.

Live births resulting from SCNT reconstructed embryos have been obtained for most animal species; however, in most cases the success rate is limited to 1–5 % which indicates incompatibilities of the oocyte to reprogram the somatic cell nucleus and its associated centrosomal complex. Indeed, our studies in porcine SCNT reconstructed eggs revealed that 39.4 % of reconstructed eggs displayed centrosomal abnormalities during the first cell cycle (Zhong et al. 2007) as determined by γ-tubulin and/or centrin-2 and correlated microtubule staining patterns. These centrosomal mitotic abnormalities may result in developmental abnormalities or contribute to cellular pathologies that may be manifested as adulthood diseases later in life. Reprogramming of somatic cell centrosomes begins shortly after SCNT within the first embryonic cell cycle which spans ca. 24 h and is a very short time for centrosomal reprogramming considering that somatic cell centrosomes are different from reproductive cell centrosomes, perhaps containing different centrosomal compositions and different capabilities to perform cell cycle-specific functions that are precisely provided by regulatory factors in the somatic cell cytoplasm for somatic cell cycles; centrosome functions may require different regulation in embryonic cells.

Centrosomal abnormalities may account for abnormal cell divisions during different stages of development and may be part of the cellular incompatibilities that allows only 1–5 % of reconstructed embryos to develop to full term resulting in healthy offspring. Details and thoughts on the possible reasons underlying

centrosomal incompatibilities are provided in our more detailed previous review on SCNT (Schatten and Sun 2009a).

The case may be made that in the 10 times smaller somatic cell the requirements for somatic (donor) cell centrosomes are likely to be different compared to those in the huge reconstructed egg of about 100 μm. We know from somatic cell studies that microtubule lengths and numbers are regulated by changes in γ-tubulin recruitment to the centrosome (reviewed in Schatten 2008). As the sperm contributes only a small amount of γ-tubulin to the regular fertilization process most of the γ-tubulin components come from the oocyte after fertilization. We do not yet have detailed information on the recruitment of γ-tubulin from the oocyte to the somatic cell centrosome complex which may differ from recruitment to the sperm centrosome during normal fertilization resulting in abnormal microtubule formations in SCNT reconstructed eggs.

While we do not yet know the underlying causes for centrosomal abnormalities in SCNT reconstructed embryos, our knowledge of nuclear reprogramming has increased in recent years (reviewed in Prather 2000; Sun and Schatten 2007; Schatten and Sun 2009a) and we now have indications that nuclear matrix dysfunctions may play a role in the low success rate following SCNT, as nuclear matrix dysfunctions impacts DNA replication (reviewed by Yamauchi 2011) and it may also impact centrosome functions. As NuMA is part of the nuclear matrix, NuMA-derived spindle abnormalities have been reported for cancer cells (Kammerer et al. 2005); NuMA dysfunctions may be among the reasons for the multipolar spindle pole formations that we find in SCNT porcine embryo cells (Zhong et al. 2007).

To study the contributions of spindle pole centrosomal components in SCNT eggs, Zhong et al. (2005) used intraspecies and interspecies SCNT reconstructed eggs to determine specific centrosomal components that are contributed by the donor cell centrosome complex and the donor cell nucleus. This study used mouse MII oocytes as recipients, mouse fibroblasts, rat fibroblasts, or porcine granulosa cells as donors to produce intraspecies and interspecies nuclear transfer embryos. Specifically, to study NuMA dynamics in SCNT reconstructed eggs, a specific NuMA antibody was employed that did not recognize NuMA protein of mouse oocytes but recognized NuMA in porcine granulosa cells, thereby being able to distinguish NuMA contributed by the oocyte and donor. The results clearly showed that NuMA was localized to the donor cell nucleus and was translocated out of the nucleus into the cytoplasm followed by translocation to the mitotic spindle poles where donor cell NuMA participated in spindle pole formation during first mitosis in SCNT eggs. Further analysis of NuMA translocation out of the nucleus in porcine SCNT eggs (Liu et al. 2006) revealed that NuMA was contributed by fetal fibroblast donor cells to reconstructed porcine eggs and it took about 6 h after nuclear transfer before NuMA could be visualized with immunofluorescence microscopy, indicating a lag period for NuMA reprogramming by the reconstructed egg which is significantly longer than NuMA detection in decondensing sperm nuclei that takes place within minutes after fertilization (Liu et al. 2006). This study concluded that cytoplasmic factors in the recipient porcine

oocyte were able to remodel the donor cell's NuMA although it took 6 h for the remodeling to take place.

While we do not yet know the exact molecular composition of the zygotic centrosome and we also do not yet know how it compares to the interphase somatic cell centrosome and to the centrosome in reconstructed eggs we know that precise regulation is important for centrosome functions. Phosphorylation plays a significant role in centrosome functions and we know from cancer cell centrosomes that abnormal increases in phosphorylation results in increased microtubule nucleation with consequences for abnormal spindle formation (Lingle et al. 1998). In somatic cells, NuMA is in part regulated by cyclin B (reviewed in Sun and Schatten 2006) but the regulation of NuMA is still unknown for mammalian embryonic cells. It is possible that the NuMA-related abnormalities that we found in human oocytes (Alvarez-Sedó et al. 2011; Schatten et al. 2012) may have been the result of inaccurate regulation by the fertilized ooplasm. It is also possible that NuMA may be part of the sperm's nuclear matrix that may play a role in nuclear matrix instability and dysfunctions (reviewed by Johnson et al. 2011).

Taken together, while we have started to analyze nuclear–centrosome relationships and centrosome-nuclear reprogramming more detailed studies are needed to determine how the somatic cell centrosome becomes remodeled to fulfill the functions of the embryo's blended centrosome that contains precise amounts and compositions of centrosomal proteins that are precisely regulated by the fertilized oocyte to serve embryo-specific functions including symmetric and asymmetric cell divisions during embryo differentiation and development.

4.5 Conclusion and Future Perspectives

Numerous lines of evidence have established the importance of centrosome–nuclear interactions and synchronized cell cycle progression to ensure accurate fertilization, zygote formation, and cell divisions during embryogenesis. However, most of our knowledge of centrosome–nuclear regulation comes from somatic cells and more research is needed to study abnormalities underlying male and female factor infertility problems and developmental abnormalities related to dysfunctions in centrosome–nuclear interactions.

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