REPRODUCTION
REVIEW

New insights into the role of centrosomes in mammalian fertilization and implications for ART

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Abstract

In non-rodent mammalian species, including humans, the oocyte and sperm both contribute centrosomal components that are most important for successful fertilization. Centrosome pathologies in sperm and the oocyte can be causes for infertility which may be overcome by assisted reproductive technologies based on proper diagnosis of specific centrosomal pathologies. However, we do not yet fully understand the cell and molecular mechanisms underlying centrosome functions in germ cells and in the developing embryo, which calls for directed specific investigations to identify centrosome-related pathologies that include components in sperm, egg, or centrosome regulation within the fertilized oocyte. The present review highlights cellular and molecular aspects of centrosomes and centrosome–nuclear interactions focused on nuclear mitotic apparatus protein during fertilization and proposes future directions in expanding therapeutic approaches related to centrosome pathologies that may play a role in still unexplained causes of infertility.
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Introduction

The discovery of centrosomes is credited to Theodore Boveri who brilliantly utilized sea urchin eggs to show the importance of centrosomes for fertilization and cell division (Boveri 1901). He further showed that fertilization with two sperm (dispermy) resulted in fertilization and division abnormalities with multipolar mitoses as hallmarks that he implicated to play a role in cancer cell division. These landmark observations served as foundation for many of our current studies on centrosomes in reproduction and cancer and generated an enormous resurgence in centrosome research and in centrosome pathologies underlying disease (reviewed in Badano *et al*. (2005)). The current review is focused on the role of centrosomes in non-rodent mammalian fertilization and on centrosome abnormalities that play a role in male and female factor infertility. Infertility is a major health problem in reproduction and affects about 10–15% of couples who now have the option to seek out assisted reproductive technologies (ART) as treatment to overcome infertility problems. Currently, in the Western world 1% of all babies are produced through ART (Giritharan *et al*. 2007), and it can be expected that this trend will increase as many women postpone having children until advanced stages in life. The percent of women undergoing IVF is already higher in Australia (2.7%) and in Denmark (3.9%) and the number may also be higher in other countries, as an increasing number of women suffer from low quality oocytes, decreasing the chances for fertilization success. However, ARTs are not yet perfected and it oftentimes takes multiple attempts before success is achieved which currently ranges between 70 and 80% in the most successful IVF clinics. To optimize the conditions for likely success it is important to understand the cell and molecular aspects that play a role in fertilization, cell division, and subsequent embryo development.

Successful fertilization significantly depends on oocyte quality that is acquired during a complex process of oocyte maturation (reviewed in Fan *et al*. (2003, 2009), Voronina & Wessel (2003), Brunet & Maro (2005), Liang *et al*. (2007), Ai *et al*. (2008*a*, 2008*b*, 2009), Jones (2008), Swain & Pool (2008), Gosden & Lee (2010) and Schatten & Sun (2011*b*)) as well as on sperm quality that includes the capacity to activate the oocyte and contribute components to the fertilized egg that are essential for cell division and subsequent embryo development. In most mammalian systems including humans the oocyte is fertilized at metaphase of meiosis II (MII) and completes meiosis when activated by sperm. The MII stage is generally described as the stage in which the oocyte is arrested before fertilization takes place; however, it is important to emphasize that this arrest is achieved by highly active molecular dynamics that are critical for maintaining MII spindle integrity and preventing spindle

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deterioration (reviewed in Schatten & Sun (2011*b*)) which may be compromised in aging oocytes or during IVF procedures (reviewed in Miao *et al*. (2009*b*)).

While progress has been made to determine oocyte quality with non-invasive methods (reviewed by Wang & Sun (2007)) we still do not yet have reliable indicators to adequately assess oocyte quality (reviewed in Gosden & Lee (2010)) and we also do not yet have adequate methods to assess sperm quality (reviewed in Talevi & Gualtieri (2004)). However, MII spindle integrity is one of the key indicators of oocyte quality and depends on centrosome integrity; live-cell imaging using PolScope microscopy based on polarized light optics (Wang *et al*. 2001) is frequently used for the evaluation of the MII spindle although this method does not allow for sufficient sensitivity to determine molecular defects including centrosomal abnormalities at the spindle poles. The assessment of MII spindle integrity is especially important, as this is the structure that contains the maternal genetic material and provides the molecular machinery that separates chromosomes to precisely extrude half of the chromosomes into the second polar body (PBII) while the other set remains in the oocyte to form the female pronucleus (reviewed in Miao *et al*. (2009*b*)).

The sperm, on the other hand, contributes the centriole–centrosome complex, an essential component in all non-rodent mammalian systems studied so far including humans. This complex had been reduced during spermatogenesis but it has retained the functional proximal centriole that is critical for sperm aster formation, for the zygote aster, and for the bipolar mitotic apparatus that forms after centriole duplication during the pronuclear stage (reviewed in Manandhar *et al*. (2005) and Schatten & Sun (2009*b*); see schematic diagram in Fig. 1). The sperm also contains critical centrosomal proteins surrounding the sperm centriole that attract additional centrosomal proteins from the oocyte's stockpile that had been retained within the oocyte after completing oogenesis.

An important aspect of sperm aster, zygote aster, and mitotic apparatus formation is the regulation of centrosomal components that become remodeled throughout the first and subsequent cell cycles for cell cycle-specific functions (reviewed in Schatten & Sun (2010, 2011*a*)). Dysfunctions of centrosomal components in sperm or egg will result in developmental abnormalities or dysfunctions. The following sections will discuss centrosomal components and abnormalities in sperm that may affect *in vivo* fertilization and may require specific *in vitro* procedures, summarized as ART (reviewed in Schatten & Sun (2009*b*)). Oocyte abnormalities include defective MII spindles and failure of centrosome regulation to form the sperm aster, zygote aster, and mitotic apparatus after fertilization.

The sperm centriole–centrosome complex and pathologies that are implicated in fertility problems

Factors that play a role in human male infertility have recently been reviewed in detail by Singh & Jaiswal (2011) who focused on chromosomal aberrations including genetic and environmental components although this review did not include centrosome pathologies. Specific sperm pathologies have been well reviewed by Chemes (2000) and Chemes & Rawe (2003) listing a large number of sperm defects with most of them related to centriole or centrosome abnormalities that are the result of genetic or environmental influences. Such defects are typically identified as abnormalities based on morphological criteria or on evaluation with molecular methods, using immunofluorescence microscopy to specific antigens or western blotting aimed at analyzing specific proteins that may be absent in sperm and manifested as pathologies. As we have become aware of such pathologies an increasing number of morphological and molecular defects in sperm have been related to centrosomal and/or centriole abnormalities (reviewed in Schatten & Sun (2009*b*) and references therein) that are increasingly being identified as causes for infertility. While research on sperm centrioles has yielded a wealth of new data primarily based on electron microscopy studies, research on sperm centrosomes is only at the beginning. We know relatively little about centrosomal components surrounding the sperm centriole and the requirements for centrosome growth after fertilization. We do know that the sperm centrosome complex

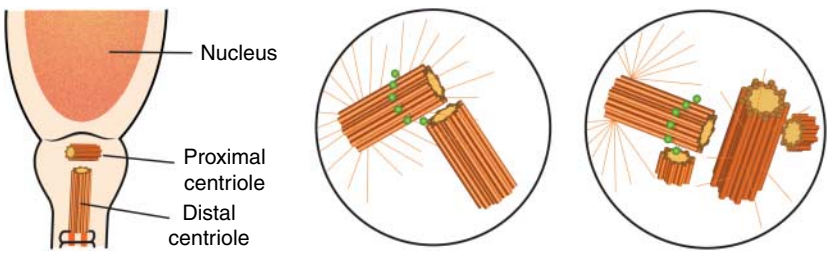

Figure 1 Schematic diagram of centriole complex in sperm and duplication after fertilization. The sperm's centriole complex primarily consisting of a prominent proximal centriole surrounded by sparse centrosomal material and a distal centriole that becomes degraded along with the sperm tail after fertilization (reviewed in Schatten & Sun (2009*a*, 2009*b*)). γ-Tubulin, pericentrin, and centrin are among the centrosomal proteins that have been identified around the proximal sperm centriole. Duplication of centrioles takes place at the pronuclear stage followed by separation of the duplicated centriole–centrosome complex and migration around the zygote nucleus to the opposite poles to form the centers of the mitotic spindle poles.

contains γ-tubulin that quantitatively increases after fertilization by recruiting additional γ-tubulin from the ooplasm, and we also know that it contains centrin as an important component for fertilization. Insufficient γ-tubulin and centrin have both been correlated with decreased ability to fertilize (Hinduja *et al*. 2010) and decrease in sperm aster formation after fertilization (Navara *et al*. 1996). Other proteins in the mature sperm are only incompletely understood although new studies have started to focus on this topic. Recently, Goto *et al*. (2010) reported that speriolin is present in sperm of human and mouse oocytes and is contributed to the oocyte after fertilization; it is detected at the mitotic poles in both systems despite the fact that in the mouse, the sperm centriole is destroyed and does not participate in the fertilization process (Schatten *et al*. 1985*a*). One complexity regarding sperm centrosomal components relates to the fact that many previous studies have used immunofluorescence microscopy without extensive control experiments to possibly unmask antigens that may not be detected because of inaccessibility to the antibody. In some cases western immunoblotting was able to identify the centrosomal proteins that had not been detected by immunofluorescence microscopy alone (reviewed in Manandhar *et al*. (2005)). However, a large body of evidence ascribes sperm pathologies to centrosomal dysfunctions that led to the novel approaches and suggestions to introduce donor centrosomal material along with ICSI procedures when IVF was performed. Such studies have been reported for the cat in which injection of testicular sperm into the oocyte resulted in short or absent sperm asters while ejaculated sperm was able to generate larger sperm asters (Comizzoli *et al*. 2006). Centrosome functions were restored by replacing the testicular sperm centrosome with a centrosome from ejaculated sperm. Such studies open up new possibilities for centrosomal male-factor therapies (reviewed in Schatten & Sun (2009*b*)).

The selection process for sperm used in ART is not yet perfected and still mainly relies on differential centrifugation or motility criteria employed for both IVF and ICSI, while during *in vivo* fertilization the selection process is more extensive and only one of the ∼300 million sperm will fertilize a single egg. Before fertilizing the egg in the oviduct (Fallopian tubes) the successful sperm has to enter the uterus, swim up the Fallopian tube and penetrate the zona pellucida before triggering egg activation and subsequent fertilization responses in the egg. As the sperm tail is not necessary for the fertilization process and ICSI will overcome sperm immotility problems if this is the underlying cause for infertility, we do not yet fully understand whether other factors play a role in the selection of the successful sperm during *in vivo* fertilization (reviewed in Talevi & Gualtieri (2004)).

New discoveries of a nuclear matrix in sperm have opened up further potential areas for sperm abnormalities that had not been addressed previously and relate to nuclear–centrosome interactions. Evidence has emerged for nuclear matrix proteins in the sperm nucleus that play a role in chromatin organization (reviewed in Johnson *et al*. (2011)) and may be important for sperm nuclear decondensation abnormalities after fertilization. A strong correlation between nuclear and centrosome proteins exist. Nuclear and centrosomal proteins are precisely regulated during cell cycle progression to coordinate spindle formation and chromosome segregation. One of the proteins that strongly link nuclear to centrosome functions is the nuclear mitotic apparatus protein (NUMA). NUMA is part of the nuclear matrix that becomes clearly detectable in the decondensing sperm nucleus after fertilization when immunofluorescence detection methods are employed (reviewed in Liu *et al*. (2006), Sun & Schatten (2006) and Alvarez Sedó *et al*. (2011)). Detailed direct studies on NUMA in the sperm nucleus before fertilization and in the decondensing pronucleus after fertilization are still needed to pursue important questions on the role of the nuclear matrix in sperm (reviewed by Johnson *et al*. (2011)) that may account for cases of infertility in which nuclear decondensation does not take place. Such studies may require more elaborate molecular or biochemical methods as well as specifically tailored ultrastructural methods to investigate nuclear matrix components in the nucleus (Degrouard *et al*. 2004, reviewed in Johnson *et al*. (2011)).

Oocyte centrosomes and centrosomal pathologies associated with oocyte dysfunctions

As mentioned in Introduction, centrosome components in the MII oocyte are critical for successful fertilization. The most prominent staining for centrosomal proteins is detected at the poles of the MI and MII spindles (see schematic diagram in Fig. 2) and these MI and MII spindle pole centrosomes are crucially important for accurate chromosome separation. The molecular mechanisms involved in chromosome separation in MI and MII oocytes have been well reviewed previously (Ai *et al*. 2008*a*, 2008*b*, Jones 2008, Holt & Jones 2009) while our understanding of centrosome dynamics and specific centrosome protein functions in MI and MII oocytes remains fragmented and complicated by the fact that most of the previous studies have been performed in the mouse model that we now know employs totally different mechanisms for meiotic spindle formation compared with non-rodent mammalian systems including humans (reviewed in Schatten & Sun (2010, 2011*b*)).

In meiosis I, half of the homologous chromosome set as well as one set of the spindle pole centrosomes is extruded into the first polar body (PBI). In MII, haploidy is restored in the sperm-activated oocyte when one set of chromatids is extruded in the second PBII along with one set of the spindle pole centrosomes while the female pronucleus is formed in the oocyte. However, we do not yet understand the nature and composition of the

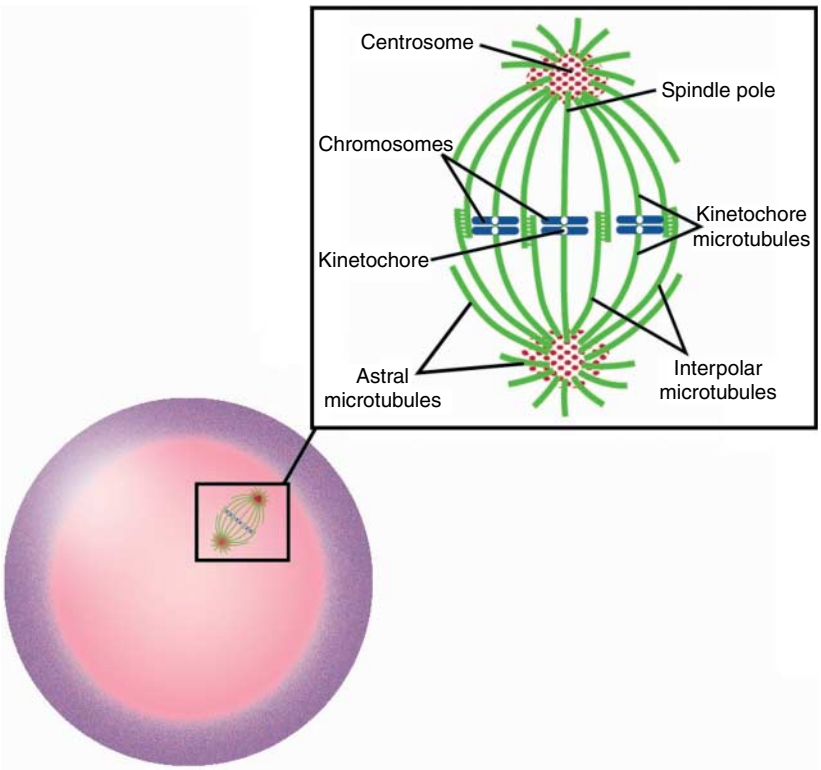

Figure 2 Schematic diagram of MII spindle in oocytes before fertilization. The MII spindle is organized by acentriolar centrosomes that nucleate kinetochore and pole-to-pole microtubules for separation of chromosomes.

extruded centrosomes and centrosome protein quantities that may play a role in the two consecutive highly asymmetric cell divisions during polar body formations. In somatic cells, centrosomes are key determinants for the establishment of cell divisions that includes establishment of asymmetry (reviewed in Schatten & Sun (2010)). We do not yet understand the fate of specific centrosome proteins during this critical stage of haploid oocyte formation. Understanding centrosome dynamics and functions during this process is highly important, as dysfunctional centrosomes may be among the underlying causes for the formation of multiple female pronuclei that indicates centrosome dysfunctions perhaps as a result of centrosome fragmentation resulting in unequal chromosome separation and subsequent formation of multiple pronuclei. To explain causes of aneuploidy, more detail on centrosome functions and dysfunctions during oocyte meiosis is needed. This topic is important, as therapies may be in reach and centrosome dysfunctions may be rescued and repaired with several agents that interact with centrosome signaling pathways and include caffeine, dithiothreitol, and nitric oxide, as well as others that are currently being explored (reviewed in Miao *et al*. (2009*b*)).

MI and MII spindle dynamics are complex and depend on precisely regulated centrosome, microtubule, and chromosome functions and on their structural and functional interactions. The molecular mechanisms that play important roles in chromosome–microtubule interactions have been well reviewed in previous papers (Ai *et al*. 2008*a*, 2008*b*, 2009, Jones 2008, Holt & Jones 2009) and are not detailed here while the role of centrosomes and their interactions with microtubules to form the functional meiotic spindle will be highlighted. We know little about molecular detail and mechanisms that play a role in the regulation of centrosome proteins in the MI and MII spindles while much of our knowledge about centrosome interactions with microtubules and chromosomes comes from mitotic spindles that do contain centrioles within the centrosome complex, unlike the acentriolar centrosomes in meiotic spindles. In all mammalian systems studied so far the MII spindle centrosomes do not contain centrioles; we know that centrioles had been destroyed during oogenesis (reviewed in Manandhar *et al*. (2005)). However, MI and MII spindles in mammalian oocytes do contain centrosomal components comparable to those described for mitotic spindles including the centrosomal components γ-tubulin, NUMA, and pericentrin although there are some variations in centrosome composition in different non-rodent mammalian species (reviewed in Schatten & Sun (2011*b*)) while the mouse employs totally different mechanisms regarding centrosome and cytoskeletal organization that are not addressed in this review (Maro *et al*. 1985, Schatten *et al*. 1985*a*, Lee *et al*. 2000, Schuh & Ellenberg 2007, reviewed in Schatten & Sun 2009*a*, 2009*b*, 2010, 2011*b*). Three centrosomal proteins, γ-tubulin, NUMA, and pericentrin are clearly associated with MI and MII spindle poles in non-rodent mammals in which centrioles are absent but the

formation of the acentriolar centrosomes has not yet been addressed sufficiently well in non-rodent mammalian systems (reviewed in Schatten & Sun (2011*b*)).

The centrosomes in the MI and MII spindles are important indicators of oocyte quality, as deteriorating centrosomal material indicates decrease in oocyte quality and problems for aneuploidy, decrease in developmental capacity, and developmental dysfunctions. The MI and MII spindles are highly susceptible to environmental influences (reviewed in Schatten & Sun (2009*a*)) including aging (Miao *et al*. 2009*a*, 2009*b*), cocaine (Combelles *et al*. 2000), bisphenol A (BPA; Can *et al*. 2005, Eichenlaub-Ritter *et al*. 2008, Pacchierotti *et al*. 2008), 2-methylestradiol (2-ME; Eichenlaub-Ritter *et al*. 2007), and perhaps numerous others including stress factors that have been implicated in causes for aneuploidy and developmental disorders (reviewed in Miao *et al*. (2009*b*)). It has generally been observed that in oocytes affected by aging or by environmental factors, centrosomes disintegrate from the MII spindle poles followed by loss of microtubule tension, loss of microtubule connections to the kinetochores, and MII spindle deterioration (reviewed in Miao *et al*. (2009*b*)). Studies are underway to determine mechanisms by which centrosome deterioration can be prevented and MII spindle integrity can be restored which includes supplementation of culture medium with caffeine as mentioned above although these studies have not yet been translated into clinical applications (reviewed in Miao *et al*. (2009*b*)).

Aside from the prominent meiotic spindle pole centrosomes it is important to emphasize that the oocyte contains centrosomal proteins in the ooplasm that are not easily detectable by immunofluorescence microscopy but do become detectable under activation conditions when centrosomal aggregates are formed within the ooplasm that can be induced by pH changes, calcium changes, or by microtubule stabilizing agents including heavy water (D_2O) or taxol (Schatten *et al*. 1992, Kim *et al*. 1996, reviewed in Schatten & Sun 2011*b*). While detailed studies on specific ooplasmic centrosomal components are still missing we do know from indirect studies in invertebrate systems that these centrosomal aggregates can undergo time-dependent changes that include aggregations of over a 100 small centrosomal foci into fewer but larger centrosomal aggregates. We also know that the sperm-derived centriole–centrosome complex when introduced in these activated oocytes exerts dominance over the ooplasmic centrosome clusters and aggregates the oocyte's centrosomal components toward the sperm's centriole–centrosome complex (Schatten *et al*. 1982, 1992, Schatten 1994). Such studies performed in sea urchin eggs have not yet been performed in non-rodent mammalian systems but may provide new insights into the oocyte's contribution of centrosomal components. These ooplasmic centrosomal proteins are important after fertilization to contribute to the formation of the sperm aster, the zygote aster, and the mitotic apparatus. Therefore, aside from the functions in the MI and MII spindles, centrosomal components in the MII ooplasm are very important for the egg's developmental potential. The oocyte further provides the reservoir for regulation of the sperm centrosome complex after fertilization, as will be discussed in the following section.

Post-insemination centrosome regulation

Centrosome integrity is important for successful fertilization and for all stages of subsequent embryo development. In most mammalian systems fertilization takes place in the MII oocyte in which sperm triggers oocyte activation and completion of MII resulting in the extrusion of the PBII and formation of the female pronucleus in the now haploid oocyte. As mentioned above, accurate centrosome functions are very important in this process for the accurate separation of chromosomes and prevention of aneuploidy or multinuclear formations within the oocyte.

In all non-rodent mammalian systems studied so far including humans the sperm contributes the centrioles as essential components for successful fertilization and cell division and therefore provides the precursor and most critical component for all developmental stages from fertilization to late-stage development (reviewed in Sathananthan (2009), Schatten & Sun (2009*a*, 2009*b*, 2010) and Alvarez Sedó *et al*. (2011)). All microtubule formations throughout development depend on sperm-derived centriolar integrity; a defective centriole–centrosome complex inherited by a human oocyte may lead to abnormal chromosome separation with subsequent genomic instability, therefore compromising embryonic development (Sathananthan 1991, 2009). Sperm centrosomal dysfunctions contribute to embryonic aneuploidy, polyploidy, and mosaicism (Munné 2006).

The sperm-derived proximal centriole in non-rodent mammalian systems serves as the seed for recruitment of critical centrosomal proteins from the oocyte to form the sperm aster, zygote aster, and mitotic apparatus during first and all subsequent cell divisions. In addition to providing centrosomal components for the formation of the sperm aster, zygote aster, and mitotic apparatus, the fertilized oocyte also serves as reservoir for sperm-derived centrosome regulation that depends on intracellular oocyte changes accomplished during oocyte activation. Sperm-triggered oocyte activation includes changes in pH and calcium that are both important for cytoskeletal and centrosome remodeling after fertilization. Both are instrumental for successful fertilization, as they directly impact accurate centrosome functions and microtubule formation that in turn are highly important for signal transduction cascades that utilize the centrosome–microtubule system for cellular communication. These functions may be impaired in cases of failed IVF,

especially when ICSI is employed, as critical oocyte activation may not have taken place in these cases. This topic is important and may impact IVF success that is supported by studies in which oocyte activation failures were rescued to obtain pregnancies (Eldar-Geva *et al*. 2003, Chi *et al*. 2004, Heindryckx *et al*. 2005) and other studies in which successful pregnancy was achieved after egg activation with calcium ionophore (Terada *et al*. 2009). More basic studies are still needed regarding the program of oocyte activation in non-rodent mammalian systems to improve ART technologies to build on existing data that have shown the importance of calcium oscillations after fertilization for successful subsequent embryo development (reviewed in Swain & Pool (2008)). Similarly, detailed studies on pH in non-rodent mammalian systems are still needed to build on previous studies that have shown the importance of pH for oocyte activation in several species (Schatten *et al*. 1985*b*, 1992, Epel 1988, Ruddock *et al*. 2001), and consequences for centrosome–microtubule functions during subsequent embryo development.

To mature into a division-competent centrosome complex after fertilization the sperm's centriole–centrosome complex needs to recruit additional centrosomal proteins and macromolecular complexes from the oocyte which includes recruitment of additional γ-tubulin to nucleate additional microtubules and form the zygote aster for the apposition of pronuclei and union of the paternal and maternal genomes and for the formation of the mitotic apparatus that separates chromosomes equally to the dividing daughter cells.

The importance of centrosomes for reproduction had been recognized in several previous key studies using sea urchin eggs (Boveri 1901) and the mouse model (Calarco-Gillam *et al*. 1983). With the advent of IVF we now have turned to the porcine and bovine systems as models for humans to allow reliable and repeatable experimentation that can be extrapolated to human reproduction (reviewed in Schatten & Sun (2009*a*, 2009*b*, 2010)). For centrosome and microtubule studies, the mouse has proven to be different and is not used as model for humans. Briefly, in the mouse, the mechanisms for the formation of the meiotic and mitotic spindles and fertilization as well as drug sensitivities are totally different from all other non-rodent mammalian species including humans. For example, during fertilization, in the mouse, the sperm centriole–centrosome complex is destroyed and cytoplasmic asters contribute to the formation of meiotic and mitotic spindles (reviewed in Schatten & Sun (2011*b*)). In all other mammalian species, the sperm centriole–centrosome complex is crucial for fertilization and provides the dominant material for the formation of the sperm aster and subsequent mitotic spindles. For these reasons, in recent years, the porcine and bovine models have been emphasized as most suitable model for humans. The fertilization mechanisms used by these species are similar to those used in human fertilization. Both systems depend on the sperm's centriole for successful fertilization and subsequent development, such as humans. For many studies related to humans the use of animal models is important, as it allows targeted and repeatable experimentation to analyze mechanisms that play critical roles in fertilization and uncover the underlying mechanisms for male or female factor infertility. For studies aimed at determining specific male and female factor infertility causes heterologous ICSI has been employed; such studies utilize bovine or porcine oocytes that are fertilized with human sperm to assess components contributing to male and female factor infertility and can be used to determine centrosomal dysfunctions (reviewed in Schatten & Sun (2009*b*)).

Once in the oocyte the sperm has to be modified into a male pronucleus that includes loss of sperm tail, changes in the nuclear envelope, and chromatin decondensation (reviewed in Swain & Pool (2008)). The role of MAPK for centrosome and microtubule functions and impact on subsequent cell signaling during fertilization and embryo development has been well addressed and the roles of several kinases and phosphatases in post-insemination events have been well reviewed in detail previously (Fan & Sun 2004) and will not be included in this section. In the present review, we will focus on centrosome–nuclear interactions and on interconnected cell signaling that will affect nuclear–centrosome relationships and centrosome–microtubule functions with focus on NUMA.

NUMA is essential for successful fertilization and embryo development; NUMA dysfunctions play a role in infertility and in developmental abnormalities. However, we do not yet understand the factors by which NUMA is regulated and we also do not yet understand the specific contributions by sperm and the oocyte to the fertilized embryo. New research on the sperm nuclear matrix allows the conclusion that NUMA is in part contributed by sperm and in part by the oocyte as essential component during the fertilization process and that NUMA regulation takes place by factors in the fertilized ooplasm.

As mentioned above, in unfertilized oocytes, NUMA is localized to the poles of the MI and MII spindles in non-rodent mammalian systems including humans. While we do not yet have detailed data on mechanisms by which NUMA becomes relocalized from the MI into the MII spindle and during MII (resulting in the extrusion of some NUMA from one spindle pole into the PBII and the formation of the female pronucleus in the oocyte), it is clear that NUMA becomes localized to the female pronucleus after completion of MII. In addition, as mentioned above, after fertilization, NUMA becomes detectable by immunofluorescence microscopy in the decondensing sperm pronucleus and disperses into the ooplasm after pronuclear apposition and nuclear envelope breakdown (NEBD). Failure in NUMA functions has recently been shown in human oocytes and in sperm that failed to decondense while displaying

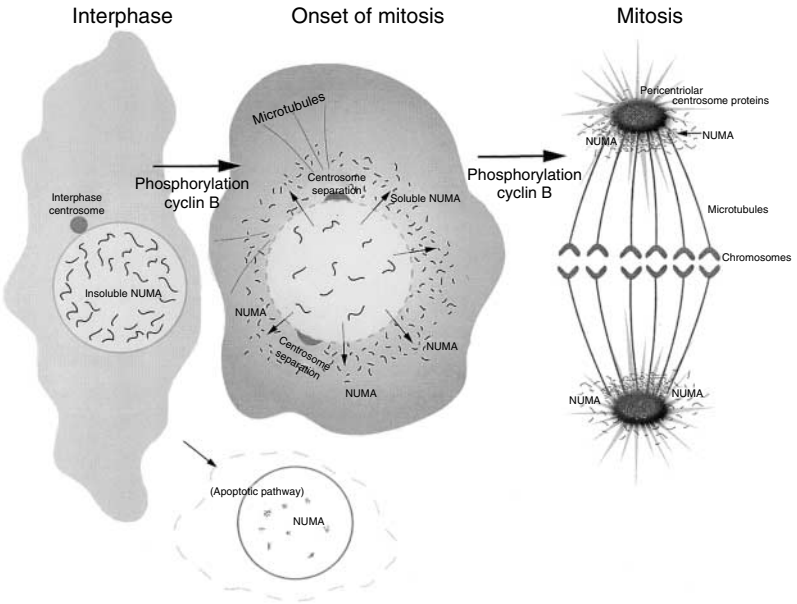

Figure 3 Schematic diagram of NUMA translocation, phosphorylation and regulation in somatic cell cycle. In interphase, NUMA is distributed as insoluble material in the nucleus where it serves as nuclear matrix protein but it is not associated with the interphase centrosome. During mitotic prophase after nuclear envelope breakdown NUMA is released into the cytoplasm, a process that requires stimulation by CDC2/cyclin kinase. NUMA becomes translocated along microtubules to the mitotic spindle poles aided by dynein/dynactin to form a stable crescent-shaped complex around the centrosome area facing chromosomes and tethers microtubules at their minus ends to form the spindle-shaped mitotic apparatus. Similar mechanisms may be employed for NUMA to move out of the zygote nucleus after nuclear envelope breakdown and associate with centrosomes during first mitosis in reproductive cells.

abnormal NUMA immunofluorescence staining patterns (Schatten *et al*. 2011, Alvarez Sedó *et al*. 2011). These results indicate that misregulation of NUMA may play a role in male and in female factor infertility problems, as regulation of NUMA in the decondensing sperm pronucleus and the female pronucleus involves factors that are provided by the fertilized oocyte. However, so far, research on NUMA regulation has only been reported for somatic cells in which cyclin B plays a major role (reviewed in Sun & Schatten (2006); see schematic diagram in Fig. 3) but not yet for embryonic cells that may employ regulation mechanisms that are different from somatic cells.

In somatic cells, NUMA is an intriguing multifunctional protein with roles as nuclear matrix protein involved in DNA organization in the nucleus that becomes a significant centrosome-associated protein during mitosis (reviewed in Sun & Schatten (2006)). In embryo cells, NUMA is critically important for accurate mitosis and cell divisions throughout embryogenesis. The sperm nucleus likely contains NUMA (although not detectable by immunofluorescence methods because of the sperm's nuclear density) which becomes dispersed after fertilization in the decondensing sperm pronucleus and commences its functions in the oocyte directly after fertilization which we have shown in the porcine model (Liu *et al*. 2006) and recently in human oocytes (Schatten *et al*. 2011, Alvarez Sedó *et al*. 2011). NUMA moves out of the nucleus during NEBD to play a critical role in the formation of the mitotic apparatus (Alvarez Sedó *et al*. 2011). Therefore, NUMA directly links nuclear functions with mitotic centrosome functions. Failure in NUMA's association with the mitotic centrosome core structure has been implicated in embryonic division dysfunctions. NUMA is not associated with the interphase centrosome and it is not associated with the sperm's centriole complex.

Most of the studies on NUMA in reproductive cells employed immunofluorescence microscopy in fertilized eggs and cloned embryos (Zhong *et al*. 2005) and NUMA dysfunctions have been reported for several mammalian systems but none of the previous studies have addressed NUMA regulation. It has been proposed that NUMA is *de novo* synthesized in the oocyte based on indirect data on nuclear transfer experiments (reviewed in Schatten & Sun (2009*a*, 2009*b*, 2010, 2011*b*)) that displayed a delay in NUMA immunofluorescence staining patterns in the transferred somatic cell nucleus (Liu *et al*. 2006). However, increasing evidence suggests that this may not be accurate, as new evidence has emerged for nuclear matrix proteins in the sperm nucleus that plays a role in chromatin organization (reviewed in Johnson *et al*. (2011)) which provokes the idea that NUMA may be part of the nuclear matrix in the sperm nucleus that becomes detectable in the decondensing sperm nucleus after fertilization. No direct studies on NUMA in the sperm nucleus before fertilization and in the decondensing pronucleus after fertilization are presently available but new methods and approaches have made it possible to pursue this important question. The NUMA-related abnormalities that we found in human oocytes (Schatten *et al*. 2011, Alvarez Sedó *et al*. 2011) may have been part of an inability of NUMA to be regulated by the fertilized ooplasm. This idea finds support by studies that provide evidence for sperm nuclear matrix instability influencing male factor fertility (reviewed in Johnson *et al*. (2011)).

Future directions

Compared with centrosome biology in somatic cells, our understanding of centrosomes in germ cells and embryos lags far behind despite the fact that centrosomes had

been discovered in germ cells. To understand and design therapies for centrosome-related infertility problems basic research is still needed in suitable animal models that include but are not limited to the following. 1) Analysis of centrosomal components contributed by sperm to the fertilized oocyte; 2) investigation of the composition and regulation of centrosomes in the unfertilized meiotic spindle and in the unfertilized ooplasm; 3) analysis of the regulation of centrosomes within the fertilized oocyte; 4) investigations on the relationships of nuclear–centrosome interactions; 5) analysis of centrosome-associated proteins; and 6) characterization of centrosome components in all stages of fertilization. These studies require cell and molecular methods as well as live-cell imaging and ultrastructural methods that are now readily available for even single cell studies. We further need to develop reliable non-invasive methods to determine oocyte quality for selection of optimal oocytes for developmental potential resulting in healthy offspring.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the review.

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