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Review Article

The Functional Significance of Centrosomes in Mammalian Meiosis, Fertilization, Development, Nuclear Transfer, and Stem Cell Differentiation

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Centrosomes had been discovered in germ cells and germ cells continue to provide excellent but also challenging material in which to study complex centrosomal dynamics. The present review highlights the importance of centrosomes for meiotic spindle integrity and the susceptibility of meiotic spindle centrosomes to aging and drugs or toxic agents which may be associated with female infertility, aneuploidy, and developmental abnormalities. We discuss cell and molecular aspects of centrosomes during fertilization, a critical stage in which centrosomes play crucial roles in precisely organizing the sperm aster that allows apposition of male and female genomes followed by formation of the zygote aster that is important for the formation of the bipolar spindle apparatus during cell division. Development of an embryo involves sequential cell divisions in which centrosomes play a critical role in establishing asymmetry that allows differentiation of cells and targeted signal transductions for the developing embryo. Asymmetric centrosome dynamics are also critical for stem cell division to maintain one daughter cell as a stem cell while the other daughter cell undergoes centrosome growth in preparation for differentiation. This review also discusses the complex interactions of somatic cell centrosomes with the recipient oocyte in reconstructed (cloned) embryos in which centrosome remodeling is crucial to fulfill functions that are carried out by the zygote centrosome in fertilized eggs. We close our discussion with a look at centrosome dysfunctions and implications for male fertility and assisted reproduction. Environ. Mol. Mutagen. 50:620–636, 2009.

Key words: centrosomes; meiosis; fertilization; cell division; signal transduction; nuclear cloning; cytoskeleton

INTRODUCTION

Centrosomes in Reproduction

Reproductive cell systems have been the first in which centrosomes had been discovered; research on eggs from *Ascaris* and sea urchins has resulted in profound insights into the importance of centrosomes during fertilization and embryogenesis [Flemming 1875; Van Beneden, 1876; Boveri, 1887a,b; Boveri 1901]. Above all, Theodore Boveri's remarkable discoveries on centrosomes paved the path for all subsequent research on centrosomes well into our present time and reproductive cell systems continue to be excellent and most suitable resources to study centrosome composition, dynamics, molecular components and abnormalities that are implicated in infertility and disease. As early as 1914, Boveri brilliantly recognized the role of centrosomes in cancer [Boveri, 1914] which was entirely based on his studies on fertilized sea urchin eggs, and his remarkable interpretations based on his discovery that dispermic or multispermic fertilization resulted in abnormal mitosis and subsequent unequal cell divisions with abnormal chromosome distribution. Boveri's studies revealed that sperm contributes dominant centrosomal material to the fertilized egg that is crucial for the establishment of the mitotic poles during cell division. These studies also provided much of the foundation for modern cancer research and since then, an enormous amount of new data has yielded a new appreciation for the fundamental role(s) of centrosomes in multiple cellular functions and dysfunctions associated with infertility and disease.

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Germ cells are unique in providing advantages and challenges for research on centrosomes. Naturally synchronized sperm and egg-cells can be obtained in abundance from invertebrateswhich has allowed us to gain insights into molecular details that could not have been obtained readily in mammalian cell systems. Data obtained on invertebrate germ cells provided the foundation that facilitated further studies in mammalian reproductive cells. Germ cells are extreme in size. Sperm cells are about 1 μm in diameter, while oocyte cells are large (100–1,000 μm) and mammalian oocytes oftentimes require specific handling that may include carrying individual cells through numerous procedural steps to the final analysis while only few cells can be analyzed for a given time point. Reproductive cells were also the first to allow new information on centrosomes when Calarco-Gillam et al. [1983] discovered that an autoimmune serum (5051) from a patient with CREST syndrome (an autoimmune disease; see list of abbreviations and definitions) recognized centrosomes in mouse oocytes which pioneered new research into centrosomes of various mammalian species and paved the path for a great number of immuno-electron and immunofluorescence microscopy studies on mouse oocytes and other mammalian species [Schatten et al., 1986, 1987; reviewed in Sun and Schatten, 2007; Schatten, 2008]. Since then, numerous monoclonal antibodies have been produced that allowed us to gain detailed insights into specific centrosome proteins and has opened fascinating new avenues to study centrosome protein functions in reproductive and somatic cells.

In recent years, a wealth of new information on centrosome composition and functions has been accumulated and we now not only appreciate the centrosome as major microtubule organizing center (MTOC) but also as most important platform for multiple signaling factors that play significant roles in cell cycle regulation [reviewed in Schatten, 2008], symmetric and asymmetric cell divisions, and embryo development. New functions for centrosomes in stem cell differentiation have recently been discovered [Yamashita et al., 2007] and ascribe a central role to centrosomes as determinants for one daughter cell to remain a stem cell while the other undergoes differentiation. It was elegantly shown that one daughter cell maintains stem cell characteristics keeping the mother centriole/centrosome complex while the other daughter cell remodels the daughter centriole/centrosome complex and undergoes differentiation [Yamashita et al., 2007]. Recent new findings uncovered additional roles for centrosomes in communicating signals from the primary cilium to the cell body to elicit responses for adaptation to different environmental changes [reviewed in Satir and Christensen, 2008]. Centrosome and centrosome signaling dysfunctions have been implicated in various diseases and may also play a determining role in developmental abnormalities [Zhong et al., 2007].

The requirements for centrosomes in reproductive cell systems are complex. Despite the fact that centrosomes had been discovered in germ cells, and germ cells had provided abundantly rich material for centrosome studies, we know much less about centrosome composition and regulation in germ cells than in somatic cells, as more recent research had been focused on somatic cells and relatively little centrosome research has been devoted to germ cells. However, crucial and significant information has come from studying germ cells and we are again in a new phase of discovering the important roles of centrosomes in symmetric and asymmetric cell divisions including those that play a role in embryo differentiation and development.

In the present review we address various aspects of centrosome regulation in mammalian gametogenesis, MII oocyte maintenance prior to fertilization, the role of centrosomes in oocyte aging and chromosomal instability, the effects of drugs on MII oocytes, fertilization, nuclear transfer, cell differentiation, and embryo development.

Definition of Centrosomes in Reproductive Cells

The centrosome is generally decribed as the cell's main MTOC, which we now know includes multiple important functions and ascribes a significant role to centrosomes as central station for signal transduction, translocation of cell organelles, macromolecular complexes and others that are detailed below. As a subcellular nonmembrane bound semiconservative organelle of ~1 μm in size, the centrosome consists of a large number of centrosomal proteins that typically surround a pair of perpendicularly oriented cylindrical centrioles (Fig. 1a), and is therefore also referred to as pericentriolar material (PCM). However, centrioles are not always present in centrosomes and some investigators refer to centrosomes without centrioles as ''acentriolar centrosomes'' while others do not make this distinction. In mouse oocytes, for example, MII spindles do display centrosomal material at both meiotic poles that is formed by assembly of multiple small asters but centrioles are not present. Nevertheless, they do perform functions that are typical for MTOCs as described for mitotic spindles and therefore ''qualify'' as centrosomes. Because the MII spindle takes a special place in reproductive biology, below we will dedicate a special section to MII spindle centrosomes (see section Centrosome Dynamics During Meiosis and the Importance of Accurate Centrosome Organization in MI and MII Meiotic Spindles).

The centrosome's three-dimensional architecture is primarily maintained through specific protein-protein interactions and microtubules are anchored with their minus ends to the centrosome core structure [Bornens, 2002]. Microtubule growth is regulated by distal plus-end addition of tubulin subunits [McIntosh and Euteneuer, 1984] and microtubule numbers and lengths are regulated

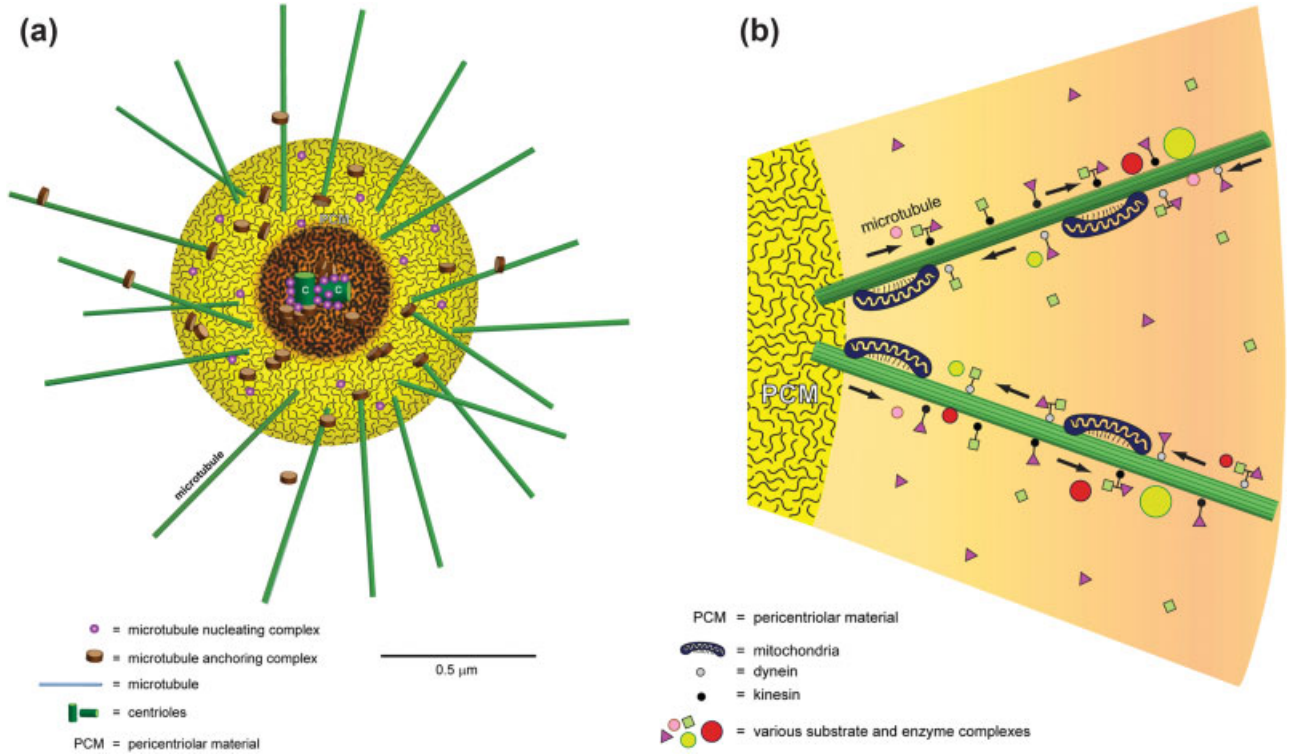

Fig. 1. (**a**) Schematic diagram of a typical mammalian centrosome composed of two centrioles surrounded by a meshwork of pericentriolar material (PCM). Microtubules are nucleated by the microtubule nucleating complex and anchored by the microtubule anchoring complex. Modified after Schatten, 2008 (**b**) Schematic diagram of the centrosome as major central station that mediates translocation of substrate and enzyme activities and organelles such as mitochondria along microtubules towards the minus ends driven by dynein (white circles) and toward the plus ends driven by kinesin (black circles). Modified after Schatten, 2008.

throughout the cell cycle in a highly dynamic process. The PCM or core centrosomal material consists of a fibrous scaffolding lattice with a large amount of coiled-coil centrosome proteins that may undergo shape changes such as required in the rapidly developing sea urchin zygote in which nuclear apposition occurs within minutes after fertilization [reviewed in Schatten et al., 2000]. The highly dynamic centrosomes undergo cell cycle-specific changes that are particularly rapid during the first cell cycle in reproductive cells to accommodate sperm and egg nuclear migrations that are important for union of maternal and paternal genomes. As in somatic cells, major reorganizations of centrosomal proteins and microtubule nucleation takes place at the transition from G2/M to form the division-competent centers of the mitotic poles.

While some centrosome proteins are permanently associated with the centrosome core structure such as γ-tubulin, the γ-tubulin ring complex (γ-TuRC), and centrin (addressed below), several other centrosomal proteins are required for cell cycle-specific functions and include the Nuclear Mitotic Apparatus (NuMA) protein that is crucial for tethering mitotic microtubules into the mitotic apparatus. These cell cycle-specific centrosome proteins should not be confused with regulatory proteins that also associate with centrosomes but utilize centrosomes as platform for signaling and include proteins including some enzymes (kinases, phosphatases and others) that may be colocalized with centrosomes but are not centrosome or centrosome-associated proteins. As mentioned above, the centrosome takes a special place among cellular organelles in that it does not have a defined membrane boundary. This may already signify that the centrosome needs to be readily accessible for its multiple functions as major platform for signal transduction that can rapidly direct cell cycle-specific functions and respond to altered environmental conditions. Rapid reorganization of centrosomal material is important to allow rapid changes in microtubule formations that interact closely with other cellular components. Because of the nature of centrosomes without clear boundaries, it is difficult to define the exact composition of centrosome proteins and very few studies have been performed to produce a qualitative and quantitate analysis [reviewed in Wilkinson et al., 2004]. The somatic cell's compact interphase centrosome has been studied more closely than mitotic centrosomes and about 500 different proteins have been associated with the centrosome as rescaled by mass spectroscopy [Andersen et al., 2003] although not all of these proteins may be centrosome proteins but some may be colocalized and coisolated as molecules that use the centrosome as docking

station. Centrosomal proteins in reproductive cells that have been studied more closely include γ-tubulin, centrin, pericentrin, and NuMA, as will be addressed below.

Taken together, the centrosome's special role as non-membrane-bound organelle allows it to function as unique signaling platform to freely recruit and distribute enzymes for cell cycle specific and adaptive regulations. Because of its role as main microtubule organizing center, these functions can precisely be performed by nucleating and organizing microtubules for recruitment and dispersion of enzyme-containing vesicles, cell organelles, macromolecular complexes and numerous other molecular components that are critical for specific cellular functions (Fig. 1b). The present time has moved us again into an exciting new phase in which we recognize the centrosome as a remarkably important organelle that Flemming once called as important as the nucleus (Flemming, 1891). We now know that nuclear and centrosomal functions are tightly coupled to ensure development of a fertilized egg into an entire well-functioning organism.

Centrosome Dynamics During Meiosis and the Importance of Accurate Centrosome Organization in MI and MII Meiotic Spindles

The importance and faithful segregation of chromosomes into daughter cells has recently been reviewed in excellent detail by Yin et al. [2008] which highlights the complex mechanisms that play a role in cell cycle coordination. In this section, we will focus on the role of centrosomes as partners in chromosome segregation and maintenance of meiotic spindle integrity.

Mammalian oocytes are arrested in diakinesis of prophase I at birth and reside within the primordial follicle pool. Following follicle growth, a relatively large nucleus (germinal vesicle; GV) is observed and fully-grown oocytes are still arrested at the prophase stage of the first meiotic cell cycle. Stimulation by gonadotrophins induces GV breakdown (GVBD) and meiotic resumption. The first meiotic cell cycle ensues with stages of prometaphase I, metaphase I, anaphase I, and telophase I that results in chromosome separation and extrusion of the first polar body (PBI) followed by the second meiotic cell cycle to the metaphase II stage when the oocyte becomes arrested until fertilization or parthenogenetic activation takes place. The reorganization of the microtubule network during these stages of meiosis is displayed in Figure 2 that shows the importance of accurate centrosome formation at the meiotic poles for accurate microtubule organization and chromosome segregation. Asymmetric cytokinesis takes place in oocytes after first and second meiotic metaphase when the first and second polar bodies are extruded, respectively, to remove half of the chromosome numbers and excess centrosome material in mammalian cells. While we have learned excellent details about extrusion of the centriole-centrosome complex in starfish oocytes in which reproductive capacity is associated with some but not all centrioles [Sluder et al., 1989; Kato et al., 1990; Shirato et al., 2005], relatively little is known about centrosome extrusion into polar bodies in mammalian oocytes [Barrett and Albertini, 2007].

Centrosomes at the two meiotic spindle poles play significant roles in microtubule stabilization and in maintenance of functional meiotic spindles. Numerous centrosome proteins are involved in maintaining spindle integrity including γ-tubulin, pericentrin, centrin, and NuMA, aided by microtubule motor proteins and regulatory kinases [reviewed in Schatten, 2008]. In most mammalian cells, except for the mouse and perhaps other rodents (see below), NuMA associates with the meiotic centrosome structure to form a crescent that stabilizes and bundles microtubules precisely into the meiotic apparatus [reviewed in Sun and Schatten, 2006, 2007]. NuMA itself depends on interactions with dynein for its association with the minus ends of microtubules [Saredi et al., 1997; Merdes and Cleveland, 1998; Gehmlich et al., 2004; [reviewed in Sun and Schatten, 2006, 2007]. Centrosome stability at the meiotic poles is crucial for maintaining spindle integrity to ensure accurate microtubule attachment to chromosomes and accurate chromosome segregation. Meiotic centrosome dysfunctions are implicated in chromosomal mis-segregation that plays a significant role in female infertility and developmental abnormalities.

Species-Specific Differences in Centrosome and Microtubule Organization

A number of studies on centrosomes have been performed in the mouse system, which historically has been favored for numerous genetic studies. The mouse system has been useful to explore the role of centrosomes in oocytes and eggs and it has been the first to gain insights into mammalian oocyte centrosomes using an autoimmune antibody that recognizes centrosomal proteins [Calarco-Gillam et al., 1983]. However, importantly, it is now clear that centrosome and cytoskeletal organization in the mouse are significantly different compared to most other mammalian species investigated for this and therefore the mouse is not a good animal model representing centrosome organization and mechanisms in other mammalian species including humans [Sathananthan, 1992; Neuber and Powers, 2000 (reviewed in Sun and Schatten, 2007; Schatten, 2008)] as will be detailed below. Because centrosome biology in the mouse and perhaps other rodents differs from other mammalian species studied so far [reviewed in Sun and Schatten, 2006], the porcine system has been highlighted in recent years as the system that optimally represents human centrosome and microtubule organization in meiosis and fertilization. The porcine system has numerous similarities with humans and is extensively used

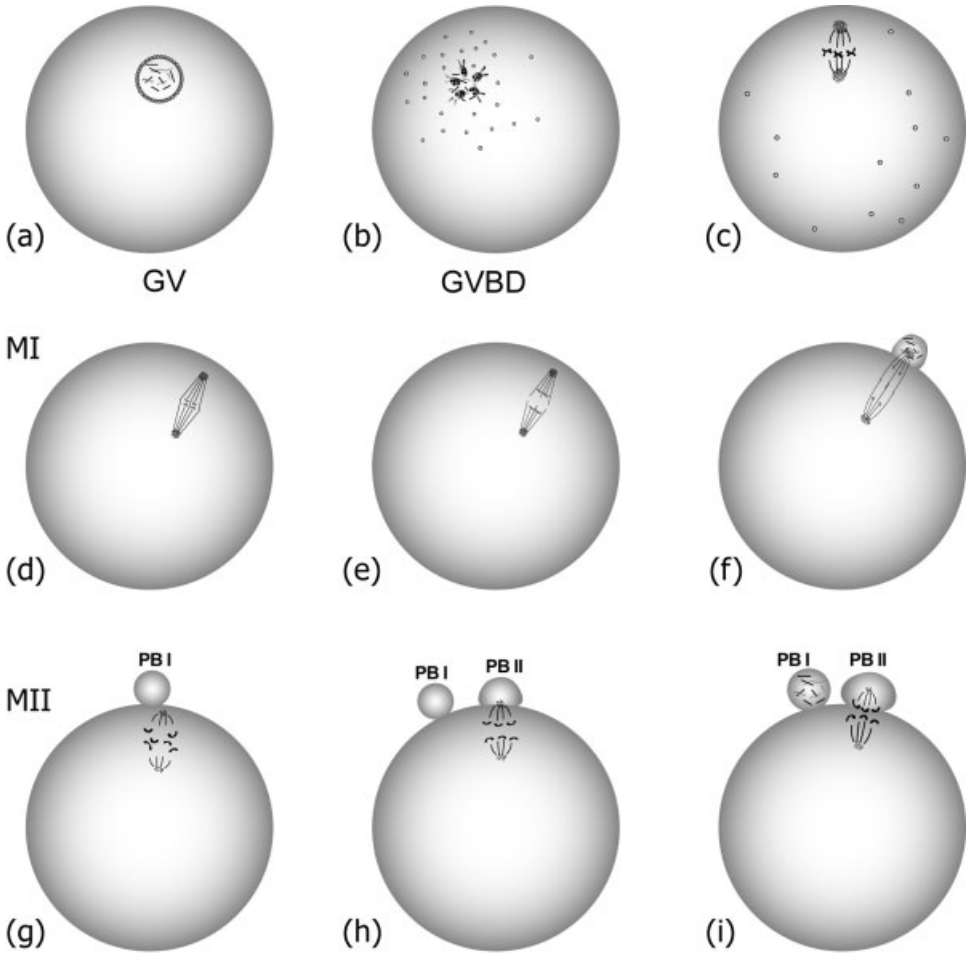

Fig. 2. Schematic diagram of oocyte cycle stages before fertilization: Top row: Germinal vesicle (GV) stage (**a**), Germinal Vesicle Breakdown (GVBD) stage (**b**), formation of the first meiotic spindle (**c**), center row: Meiotic phase I (MI) with MI spindle at metaphase (**d**), anaphase I (**e**), telophase I (**f**), with first polar body being extruded. Bottom row: Metaphase II (MII) with MII spindle (**g**), anaphase II (**h**), and telophase II (**i**) with second polar body being extruded.

as model to study human disease [reviewed by Prather, 2007; Prather et al., 2003]. Fertilization mechanisms in porcine oocytes are similar to those in humans and therefore porcine oocytes are increasingly being utilized as model for human meiosis and fertilization; most notably, the mouse ooplasm does not tolerate centrioles and sperm centrioles become destroyed after sperm incorporation [Schatten et al., 1985] while in all other mammalian systems studied so far, the sperm centriole serves as major nucleating center for the sperm aster during fertilization. Excellent data have been produced by Lee et al. [2000] who compared MI/MII spindle dynamics in the mouse and the pig and revealed substantial species-specific differences [see diagram in Lee et al., 2000]. The following highlights the differences between centrosome dynamics in mouse and pig oocytes.

In pig, during the first meiotic cell cycle from G2 to the end of MI phase, γ-tubulin and NuMA are initially detected in uniform distribution in the nucleus. NuMA then changes from an amorphous to a highly aggregated form in proximity to chromosomes in late diakinesis while γ-tubulin remains amorphous at the chromosome location. NuMA then translocates from the condensed chromosomes to the opposite poles of the metaphase I spindle while γ-tubulin is localized along the spindle microtubules but not to spindle poles, perhaps playing a role in microtubule stabilization without microtubule association with spindle poles. In anaphase I and telophase I of pig oocytes, NuMA and γ-tubulin become localized to the spindle midzone. During the subsequent second meiotic cell cycle, porcine MII spindles are predominantly organized by NuMA and γ-tubulin is dispersed along microtubules.

In contrast, in mouse MII oocytes, several centrosomal foci are associated with the meiotic spindle poles, and additional centrosomal foci are localized to the cytoplasm [Maro et al., 1985; Schatten et al., 1985, 1986, 1988a] which is not the case in all other mammalian oocytes studied so far. The mouse is an exception in that these small γ-tubulin-containing centrosomal foci organize small microtubule-asters within the cytoplasm [Schatten

et al., 1985, 1986, 1988a (reviewed in Manandhar et al., 2005; Sun and Schatten, 2006, 2007)]. In contrast to the mouse, metaphase II-arrested oocytes of the pig [Kim et al., 1996], sheep [Le Guen and Crozet, 1989], and cow [Long et al., 1993] are representative of other mammalian species and do not display cytoplasmic MTOCs. Furthermore, pig and mouse meiotic spindles have different origins in that the pig meiotic spindle is formed through bundling of microtubules by NuMA whereas the mouse meiotic spindle is formed through gathering MTOCs from the cytoplasm and mainly contain γ-tubulin while NuMA is localized along spindle microtubules. Drug resistance is different for pig and mouse meiotic centrosomes; in mouse oocytes, centrosomes of the meiotic spindle poles, composed of several γ-tubulin foci, are resistant to microtubule inhibitors such as nocodazole [Gueth-Hallonet et al., 1993; Palacios et al., 1993; Lee et al., 2000]. In contrast to the mouse, porcine meiotic spindle poles are sensitive to microtubule inhibitors; NuMA staining at the poles as well as γ-tubulin along microtubules disappears after nocodazole treatment. This clearly shows species-specific differences in meiotic spindle organization and dynamics in the mouse compared to the porcine model that represents most other mammalian species studied for this so far. Morphologically, unlike pigs and humans, the mouse MII spindle is oriented parallel to the oocyte surface [Schatten et al., 1985, 1986, 1988a] and does not contain comparable centrosome and microtubule organizations as seen in most other animal species. Oocytes of most other species, including the pig, contain a vertically oriented MII spindle and do not display cytoplasmic asters.

As will be further detailed below, the mechanism of fertilization is also significantly different in the mouse; during gametogenesis, the mouse sperm centriole is completely degraded, as the mouse oocyte does not tolerate centrioles [Szollosi et al. 1972] while in all other systems studied so far sperm contributes centrioles to the fertilized egg (see chapter below on fertilization). Based on these well established findings, human sperm fertility tests are now being performed in rabbits [Terada et al., 2004] or bovine [Yoshimoto-Kakoi et al., 2008] rather than rodent oocytes because of the closer similarities of these systems with human fertilization; such studies have resulted in remarkable advances in human infertility tests in which centrosomes play a significant role [reviewed by Hewitson, 2004].

The Meiotic Spindle in Young and Aging Oocytes

The MII spindle takes a special place in the reproductive cell cycle as this is the stage at which fertilization takes place in most mammalian species. The MII spindle represents a particularly sensitive and highly dynamic structure whose integrity has to be maintained until fertilization occurs. Maintenance of centrosome and microtubule integrity is important for fertilization to be successful

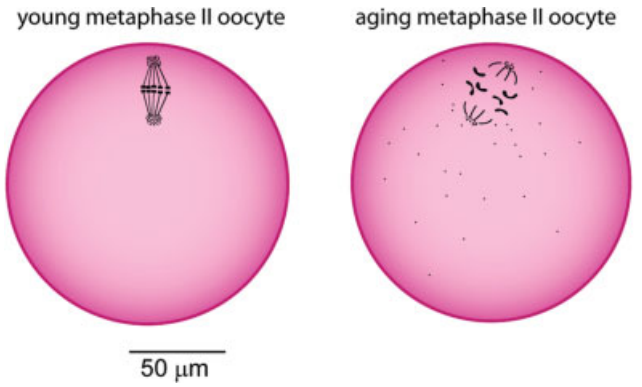

Fig. 3. Schematic diagram of young oocyte (left) and aging oocyte (right) in which centrosomal material becomes dispersed into the ooplasm and chromosomes become misaligned.

and many cases of infertility and developmental abnormalities are directly related to loss of MII spindle integrity (reviewed by [Miao et al., in press].

The MII spindle is different from the mitotic spindle in most animal species in that mammalian oocytes lack centrioles which are only present until the pachytene stage of oogenesis [Szollosi et al., 1972 (reviewed in Manandhar et al., 2005)]. In mammals, oocytes arrested at the MII stage are normally fertilized soon after ovulation which is less than 24 hr in humans [Austin, 1974]. If fertilization does not occur in time, unfertilized oocytes remain in the oviduct and undergo a time-dependent deterioration in oocyte quality resulting in aged oocytes which represents serious problems for fertilization and embryo development (addressed below). In ageing and aged oocytes, destabilization of centrosomes and the microtubule network severely affects microtubule organization, microtubule attachment to chromosomes, and subsequent chromosomal segregation. Spindle abnormalities play a major role in aneuploidy that is increased in women past age 35. It can result in trisomy 21 or other defects of chromosomal separation [reviewed in Eichenlaub-Ritter, 1998; Jones, 2008]. It is estimated that in humans about 15–20% of oocytes undergo segregation errors resulting in chromosomal abnormalities [Pellestor et al., 2005] and that 5% of all pregnancies are aneuploid resulting from such errors which is increased with maternal age [Hassold and Hunt, 2001]. Other reports estimate aneuploidy to be as high as 40–60% [Kuliev et al., 2005; Rosenbusch and Schneider, 2006; Pachierotti et al., 2008]; many of these oocytes may deteriorate and not undergo fertilization. In aged oocytes, spindles become elongated and/or smaller compared to fresh oocytes and become displaced from the oocyte's cortex to the center of the oocyte as shown in Figure 3; furthermore, the centrosome structure disintegrates and cytoplasmic centrosomal foci are observed that organize smaller cytoplasmic microtubule asters [Eichenlaub-Ritter et al., 1986, 1988; Goud et al., 2004; Segers

et al., 2008]. We do not yet fully understand the mechanisms underlying oocyte aging which may involve a complex cascade of cytoskeletal destabilization including centrosome and microtubule instability [Schatten et al., 1999].

Two major regulatory components (the maturation promoting factor MPF and the mitogen-activated protein kinase MAPK) are important for MII spindle dynamics and both are decreased in aged oocytes. MAPK plays a critical role as cytostatic factor to maintain MII spindle arrest. We have previously shown that MAPK is clearly associated with centrosomes [Sun et al., 2002] and that MAPK may be involved in microtubule stabilization at the minus ends of microtubules facing centrosomes.

Susceptibility of the MII Spindle to Drugs and Toxic Agents

As highlighted above for aging, meiotic spindles are also highly susceptible to drugs and toxic agents which is particularly important, as fertilization and embryo development may be affected. Recent attention has been focused on bisphenol- A (BPA), an alkylphenol and a widely used environmental estrogen-like chemical with weak estrogenic activity that affects centrosome and spindle integrity in MI and MII spindles of mice [Can et al., 2005]. These studies showed that during formation of the MII spindle, when oocytes were exposed to 10 μM BPA, 61% of oocytes reached the MII spindle stage while 41% of cells after treatment with 30 μM BPA reached the MII spindle stage. No chromosomes were detected in the first polar body while BPA caused loosening and elongation of meiotic spindles and compaction and dispersion of PCM that was associated with spindle abnormalities. The authors concluded that BPA causes a time- and dose-dependent delay in cell cycle progression, primarily by interfering with centrosomal proteins and it was proposed that BPA may cause degradation of centrosomal proteins which adds to previous studies that had shown an effect on microtubules in mitotic cells or cell-free extracts [Pfeiffer et al., 1997; Hunt et al., 2003]. BPA may act as a reproductive toxin, as it is more toxic for meiotic cells than mitotic cells. Another study by Pacchierotti et al. [2008] reported a significant increase of MII oocytes with prematurely separated chromatids after chronic exposure to BPA which was the only detectable effect in these studies. In another study, Eichenlaub-Ritter et al. [2008] observed effects of high concentrations of BPA on spindle formation, distribution of PCM, and chromosome alignment but no increase in hyperploidy at MII. Taken together, all studies indicate severe effects of BPA on centrosomes in MII spindles.

Other examples on different drugs confirm the susceptibility of meiotic spindles to drugs or toxic compounds. Drugs with specific effects on the MII spindle include 2-methyestradiol (2-ME) [Eichenlaub-Ritter et al., 2007] and cocaine [Combelles et al., 2000]. 2-ME caused dramatic dose-dependent increases in hyperploidy of metaphase II mouse oocytes, perturbed spindle integrity and misalignmenet of pericentrin-positive centrosomes that was associated with multipolarity. Cocaine exposure caused striking changes in meiotic spindle structure and cytoplasmic centrosome organization in mouse oocytes. Spindle length was reduced and associated with loss of nonacetylated microtubules and spindle pole centrosome fragmentation.

We previously determined an effect of the tranquillizers chloral hydrate and diazepam on centrosome structure and function in reproductive cell cycles of sea urchin eggs [Schatten and Chakrabarti, 1998] and effects of diazepam were also shown on mouse meiotic spindles by Yin et al. [1998]. These studies raise awareness of agents that could possibly cause infertility and developmental abnormalities, as they directly affect centrosomes and chromosome segregation of meiotic spindles.

Egg Activation and Parthenogenesis

Egg activation and parthenogenesis studies have clearly shown that unfertilized oocytes and eggs contain centrosomal material. In most species, except for the mouse, centrosomes are undetectable in unfertilized ooplasm and egg cytoplasm and are only detected after specific activation procedures which includes activation with the Ca^{2+} ionophore A23187, ammonia to change internal pH, heavy water (50% D_2O), and others [Schatten et al., 1992 (reviewed in Schatten et al., 2000)]. In mammalian oocytes, different activation procedures have been employed to mimic activation by sperm which is most important for activation of oocytes to induce development after nuclear transfer (see below) although it does not produce the exact activation patterns as that seen after sperm activation [reviewed in Malcuit and Fissore, 2007; Sun and Schatten, 2007; Schatten, 2008]. In mammals, exit from MII arrest and meiotic resumption is typically achieved by the fertilizing sperm which evokes in the egg a cascade of calcium signaling that results in an increase in concentration of intracellular free calcium ($[Ca^{2+}]_i$) and initiation of embryonic development by inactivation of MPF and MAPK activities [reviewed by Malcuit and Fissore, 2007]. To mimic activation by sperm, compounds or agents such as 7% ethanol (an optimal concentration for most species; Yi and Park, 2005], the Ca^{2+} ionophore A23187 or ionomycin are used to study egg activation. $SrCl_2$ is a compound that is mostly used for activation of mouse oocytes to achieve development after somatic cell nuclear transfer (SCNT). Electrical DC pulses are typically used for activation of porcine oocytes to achieve development after SCNT, as well as 6-DMAP (6-dimethylaminopurine), a ser/thr kinase inhibitor [reviewed in detail in Malcuit and Fissore, 2007].

In the mouse, parthenogenesis has been studied by activating unfertilized oocytes in 7% ethanol to produce haploid parthenotes; diploid parthenotes were produced by incubating oocytes in 10 μm cytochalasin B to block second polar body formation after activation [Schatten et al., 1991]. These eggs displayed centrosome and microtubule organizations similar to those found in fertilized mouse oocytes [Schatten et al., 1985]. Haploid as well as diploid parthenotes divided to form two blastomeres. Parthenogenetic activation and development of porcine oocytes has been achieved by Machaty et al. [1995] after treatment with guanosine-5′−0-(3′-thiotriphosphate), by Yi and Park [2004] after treatment with ethanol, cycloheximide, cytochalasin B and 6-DMAP, and by Ito et al. [2003] after activation with calcium ionophore. Such experiments are aimed at precisely determinining the molecular cascades that are triggered to mimic fertilization by sperm and so far, these experiments have shown centrosome and cytoskeletal activation [Kim et al., 1996] but further experiments are needed to achieve a precise qualitative and quantitative analysis. Oftentimes, parthenogenetic activation leads to 2-cell cleavage stages [Szollosi and Ozil, 1991; Prather, 2001; Cibelli et al., 2002; Vrana et al., 2003] and may pursue development to specific stages before developmental arrest; it oftentimes results in blastocyst stages and further development has been possible by experimental manipulation although the centriole/centrosome complex has only been studied in a few cases. *De novo* centriole formation after artificial activation has been reported in invertebrates [Kallenbach, 1982], and to a limited extend in mammalian species. In rabbits, *de novo* centriole formation is seen in parthenogenetic blastocysts [Szollosi and Ozil, 1991; Szollosi et al., 1972; Magnusen and Epstein, 1984]. Recently, parthenogenetic activation to create embryonic stem cells has gained much attention and will provide a most suitable alternative to embryonic stem cells that are produced from fertilized embryos [Kim et al., 2007].

The Central Role of Centrosomes in Fertilization, Cell Differentiaton, Embryo Development, and in Stem Cells

Fertilization releases the oocyte from the MII arrest to resume the second meiotic cell cycle in mammalian species; it triggers cascades of egg activations that result in reorganizations of centrosome and cytoskeletal components to accommodate the requirements of the developing embryo. For completion of the second meiosis after fertilization, a second polar body is formed that contains nuclear and centrosomal material while the oocyte retains a haploid DNA content as well as most of the centrosomal material. For fertilization to be successful, sperm and egg centrosomal components need to combine accurately to form a functional centrosome that is able to nucleate and organize the sperm aster that unites male and female genomes and it needs to have reproductive capacity to duplicate its material during the pronuclear stage (or S), separate at the beginning of mitosis, and form the opposite mitotic poles that precisely nucleate and organize microtubules of the bipolar mitotic apparatus for equal chromosome segregation during first cell division [reviewed in Sun and Schatten, 2006; Schatten, 2008].

Stages of fertilization and development are shown in Figure 4. During fertilization, in most mammalian species studied so far except for the mouse and perhaps other rodents, centrosomal material that has been retained in sperm and egg after centrosomal reduction during gametogenesis [reviewed in Manandhar et al., 2005] becomes united to form a functional centrosome that is competent to generate embryo-specific microtubule formations for nuclear apposition, cell division, cell differentiation, and embryo development. The precise composition and functional capacities of the zygotic centrosome is therefore a crucial requirement that will affect all subsequent developmental stages of an embryo. As reviewed by Manandhar et al. [2005] and Sun and Schatten [2007], the mature sperm contains its proximal centriole while most of the PCM is lost. The oocyte on the other hand does not contain centrioles while containing centrosomal proteins that had been retained after gametogenesis. In most species (except for the mouse) the sperm centriole-centrosome complex is the dominant structure that exerts recruiting power over egg centrosomal material although the precise mechanism and nature of the dominant factor(s) have not yet been determined. We do know that in most species, except for the mouse, the sperm's centriolar complex recruits γ-tubulin, centrin, pericentrin, and NuMA from the oocyte's cytoplasm shortly after sperm incorporation. The significant accumulation of γ-tubulin around the decondensing male pronucleus is able to nucleate the sperm aster that pushes the sperm head from the egg cortex and grows towards the female pronucleus allowing nuclear apposition. The zygotic centrosome then organizes a radial aster that becomes organized around the appositioned (or fused) male and female pronuclei (zygote nucleus). As mentioned above, the centriole-centrosome complex duplicates during the S phase and separation follows at the beginning of mitosis. The stages of centriole and centrosome duplication have been described in previous reviews [Sluder, 2004; Sun and Schatten, 2007; Schatten, 2008, Salisbury, 2008] and include centriole replication in which a daughter centriole grows laterally from the mother centriole while centrosomal proteins accumulate around it. We still lack complete knowledge on how centrosomal material becomes duplicated but we know that it is under cytoplasmic control and driven by cyclin-dependent kinase 2 (Cdk2) complexed with cyclin E or cyclin A [reviewed by Sluder, 2004].

The intriguing dominance of sperm centrosomes has been studied in the invertebrate sea urchin system that,

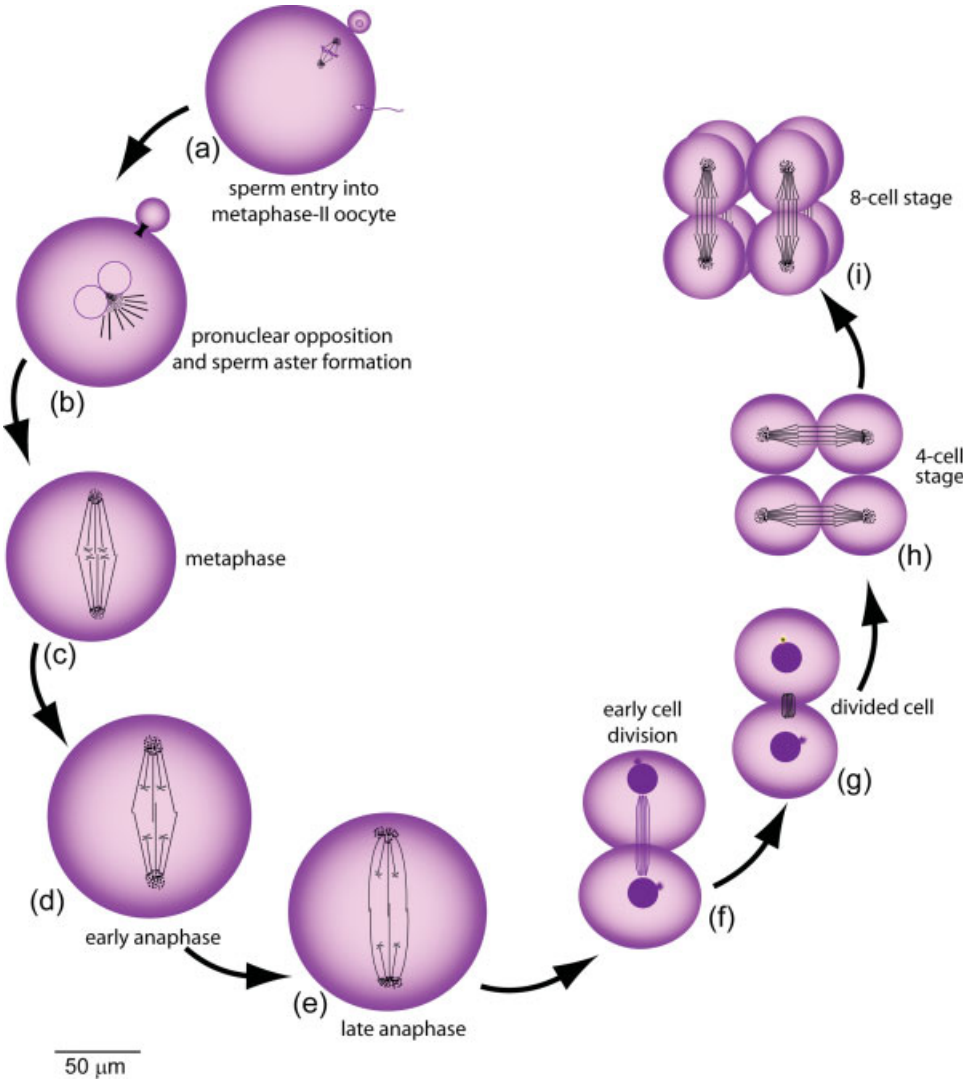

Fig. 4. Schematic diagram of cell cycle stages after fertilization. Sperm enters the MII-stage oocyte (**a**), followed by pronuclear apposition (**b**), metaphase (**c**), early anaphase (**d**), late anaphase (**e**), early cell division (**f**), divided cell stage (**g**), 4 cell stages (**h**), and 8-cell stages (**i**). Modified after Sun and Schatten 2006.

compared to the rodent model, in many aspects provides a more suitable model for mammalian fertilization. This system had been used for Boveri's remarkable discoveries on centrosomes [Boveri, 1901] and it has also been used by various labs, including ours to study the effects of drugs on centrosome and microtubule dynamics. We have utilized the sea urchin to study the effects of dithiothreitol (DTT) [Schatten, 1994] and found that membrane fusion was inhibited by 1–5 mM DTT, while microtubule and centrosome dynamics continued although with some modifications. When DTT was added before pronuclear apposition, each of the pronuclei initially displayed detectable centrosomal material. During cell cycle progression, centrosomal material associated with the female pronucleus diminished while the male's centrosomal material progressed to form a bipolar, although diminished mitotic apparatus. Remarkably, an additional half spindle became organized from one of the poles to contact the female chromatin allowing its migration toward the pole from which the half aster had originated. These experiments clearly showed the dominance of the male's centrosomal contributions which was also shown in other experiments in which the microtubule inhibitor griseofulvin was applied to prevent pronuclear apposition [Schatten et al., 1982]. In this case, male centrosomal material displayed dominance over female centrosomal material after recovery from griseofulvin treatment.

The formation of the bipolar mitotic apparatus is a crucial event for cell division. Cell division ensures that the resulting daughter cells receive equal amounts of DNA and up until recently it was also thought that they receive equal amounts of centrosome material. However, this

assumption has recently been challenged [Fuentealba et al., 2008]. New research has shown that centrosome distribution during cell division may not always be equal and differences in centrosome distribution to the dividing daughter cells may set up the pattern for differentiation. While studies on the role of centrosomes in asymmetric cell division are still largely unexplored recent research on asymmetric stem cell division in embryos has revealed fascinating new aspects that will undoubtedly lead to further establish an important role for centrosomes as essential determinant for cell differentiation and development in which centrosome dominance and activation/inactivation of centrosomal proteins may play a role. It was shown in somatic cells that a pattern for unequal distribution is already set during first division. Fuentealba et al. [2008] showed that proteins specifically targeted for proteasomal degradation are inherited preferentially by one of the two daugther cells during somatic cell division. Experiments on dividing human stem cells clearly documented asymmetric protein distribution and preferential association of an antibody specific for Mad (a member of the Smad family) phosphorylated by MAPK with one of two daughter centrosomes in blastoderm stage embryos of *Drosophila* [Fuentealba et al., 2008]. Different experiments on embryonic *Drosophila* stem cells revealed unequal centrosome formation in the daughter stem cell and the differentiating daughter cell which provided fascinating insights into centrosome maturation in daughter cells that undergo differentiation [Yamashita et al., 2007]. Such experiments have not yet been performed in mammalian germ cells but similar mechanisms may also account for cell divisions and cell differentiation in mammalian embryos. We still know little about centrosome distribution to the blastocyst cells and it will be important to perform detailed analyses on the precise centrosome organization during cell division and embryogenesis. New studies are clearly needed to investigate the role of centrosomes in these processes and in developmental abnormalities. As diseases related to centrosome abnormalities and dysfunctions are numerous [reviewed by Badano et al., 2005] abnormal centrosome formation may also be associated with developmental abnormalities.

Remodeling of Somatic Cell Centrosomes in Somatic Cell Nuclear Transfer (SCNT) Eggs

A most exciting area in reproductive biology began with the successful cloning of sheep Dolly [Wilmut et al., 1997] that opened entirely new possibilities for genetic modifications of animals for agricultural purposes and for biomedical research [Prather et al., 1993; [reviewed in Prather, 2007; Sun and Schatten, 2007; Schatten, 2008]. However, cloning efficiency in mammalian species is still low (ca. 1–5%) and current research is aimed at determining the reasons for the low cloning success. Much research is focused on remodeling of the somatic cell nucleus [reviewed in Prather, 2007] but remodeling of the somatic cell's centrosome within the ooplasm is increasingly gaining attention because of the importance of centrosomes for accurate microtubule organization, chromosome separation, cell cycle-specific signaling functions [Sun and Schatten, 2007; Zhong et al., 2007; reviewed in Schatten, 2008] as well as its significance in symmetric and asymmetric cell divisions during cell differentiation and embryo development.

Nuclear transfer cloning is achieved by removing the MII meiotic spindle (containing the oocyte's genomic DNA) from the unfertilized oocyte and transferring a somatic cell (or isolated somatic cell nucleus and associated centrosome) into the enucleated oocyte followed by electrical activation [reviewed in Prather, 2007; Schatten et al., 2005, Sun and Schatten, 2007; Schatten, 2008]. As the somatic cell components become incorporated into the enucleated oocyte to produce an embryo that now contains DNA of the somatic donor cell (Fig. 5) the reconstructed oocyte now also contains somatic cell centrosomes that substitute for the zygotic centrosomes and need to be remodeled to perform all functions that are normally carried out by the blended centrosomal material of sperm and oocyte. While accurate blending of oocyte and sperm centrosomal proteins is an absolute requirement for accurate cell division and development, we do not yet know the composition of the donor cell centrosome within the recipient oocyte and we do not yet know whether recipient oocyte centrosomal material is also recruited to the donor cell centrosome. We further do not know whether the donor cell centrosome undergoes similar cell cycle-dependent regulations as the zygotic centrosome. Very few detailed studies have been performed on centrosome remodeling in the reconstructed egg and it will be important to study how specifically the somatic cell centrosome becomes remodeled to serve embryo-specific functions that include symmetric and asymmetric cell divisions during embryo differentiation and development. We know that centrosomes undergo centrosome maturation in somatic cells at the G2/M stage in preparation for mitosis in which γ-tubulin becomes enriched around centrosomes to nucleate the microtubule-rich mitotic apparatus. Centrosome maturation in the reconstructed egg has not yet been studied in detail but recent studies indicate that abnormalities exist [Zhong et al., 2007]. In pig oocytes, we recenty determined that 39.4% of reconstructed eggs displayed centrosomal abnormalities during the first cell cycle [Zhong et al., 2007] which was analyzed by determining γ-tubulin and/or centrin2 staining patterns that were correlated to microtubule staining patterns. Live incorporation of GFP-centrin2-labeled donor cells were analyzed at various times after nuclear transfer. Abnormal spindles with irregular microtubule organizations included tripolar and multipolar organiza-

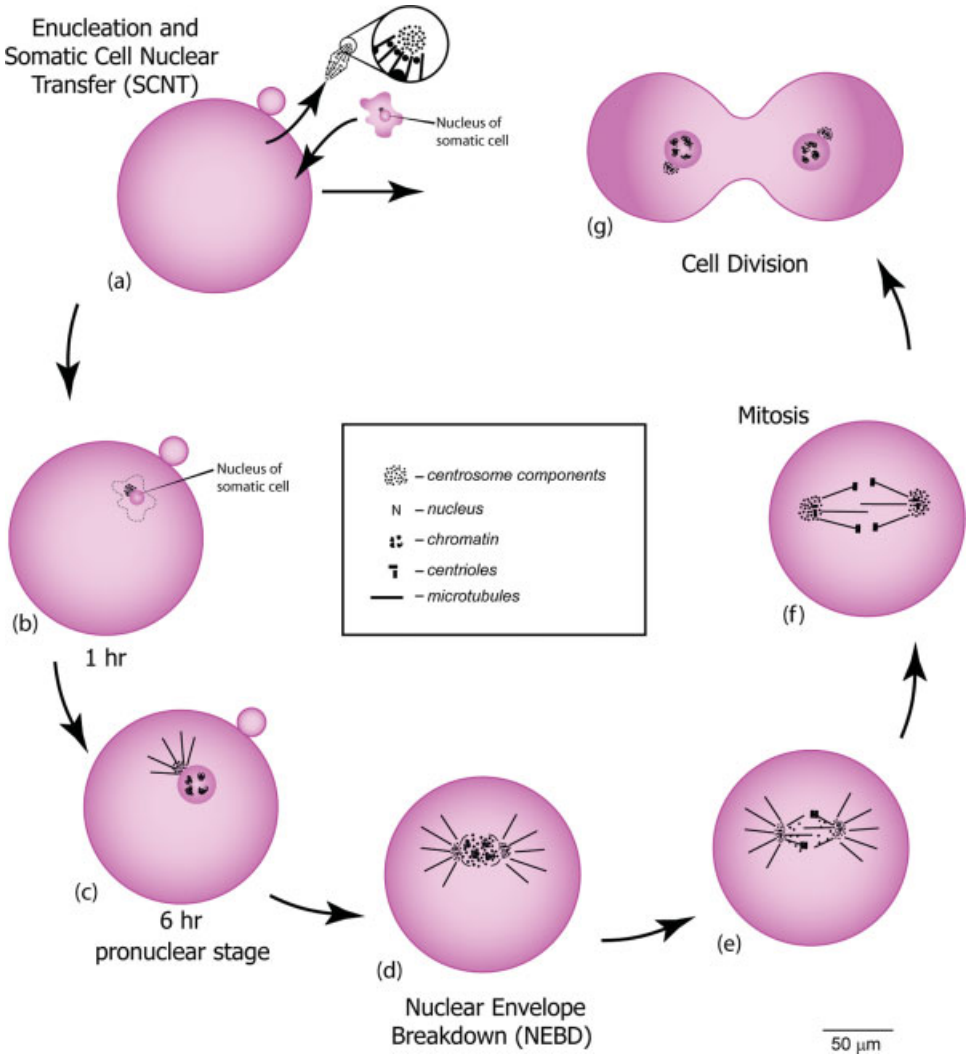

Fig. 5. Schematic diagram of centrosome organization after nuclear transfer which includes removal of the meiotic spindle (**a**) and transfer of a somatic cell containing nucleus and somatic cell centrosome. Donor cell centrosomes disperse around the donor cell nucleus (**b,c**), separate to the opposite poles (**d**) and form the mitotic spindles (**e,f**) that divides the egg into two blastomere cells (**g**). Modified after Sun and Schatten 2007. The cell cycle is subdivided into four stages. G1 refers to gap phase 1 (or growth phase 1); S refers to DNA synthesis or duplication phase; G2 refers to gap phase 2 during which the cell checks for completion of DNA replication; protein synthesis occurs in the G2 phase, following DNA replication in the S phase. This is the time when the cell stockpiles on cytoplasmic contents, before mitosis and cytokinesis occurs. M refers to mitosis phase during which chromosomes condense and attach to the mitotic spindle that separates chromosomes in preparation for cell division. G2/M refers to progression from the G2 phase to mitosis, the interval and transition from completion of DNA synthesis to the beginning of mitosis. Mitosis involves prophase, prometaphase, metaphase, anaphase, and telophase of the mitotic cell cycle.

tions, all of which will result in abnormal cell divisions and unequal distribution of chromosomes to the dividing cells (numerical chromosomal instability). It is easy to understand that unequal numbers of chromosomes in different cells will either produce embryos with abnormalities or not allow development and result in embryo loss.

The high percentage of centrosome abnormalities in reconstructed eggs is not surprizing given that remodeling of the somatic cell centrosome has to take place within a very short time after SCNT to accommodate oocyte-specific functions. Somatic cell centrosomes are different from reproductive cell centrosomes and contain 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. As only 1–5% of reconstructed embryos develop to full term and result in healthy offspring, centrosomal abnormalities may play a significant role in abnormal cell divisions during different stages of development. Whether or not the donor cell centrosome serves as the sole complex for microtubule nucleation and organization or whether additional centrosomal proteins such as γ-tubulin are recruited from the reconstructed oocyte is currently unknown. As presented above,

precise composition of centrosomes that are competent to assure accurate cell division, cell differentiation, and development is crucial and over-recruitment of γ-tubulin results in abnormal microtubule formations that will inaccurately attach to chromosomes during cell division and produce unequal blastomere cells in which certain genes may be lacking that are important for development while others may be increased and cause further unregulated cell divisions. Cells with mitotic abnormalities may be removed by mitotic cell death or apoptosis [reviewed in Schatten, 2008] which has been reported to be more frequent in reconstructed embryos compared to IVF embryos [Hao et al., 2003]. The resulting imbalances in inner cell mass (ICM) cells and trophectoderm (TE) cells will negatively impact implantation and lead to embryo loss.

As mentioned above, the causes for centrosome and mitotic abnormalities in reconstructed eggs have not yet been fully determined. It may be related to inaccurate composition of centrosome proteins as a result of insufficient remodeling of the donor cell centrosome and/or inaccurate regulation by factors in the reconstructed egg's cytoplasm. Consequences include the possibility that the somatic cell's centrosome is not able to nucleate and organize sufficiently long microtubule asters that are able to elongate and allow accurate translocations of organelles or macromolecular complexes from the egg cortex to the perinuclear area [Katayama et al., 2006; Zhong et al., 2008]. In the 10 times smaller somatic cell the requirements for donor cell centrosomes are different than those in the huge reconstructed egg of about 100 μm and it is possible that accurate remodeling might not take place under the current culture conditions.

We know from somatic cell studies that microtubule length and numbers are regulated by changes in γ-tubulin recruitment to the centrosome in cell cycle-specific patterns [reviewed in Schatten, 2008]. The somatic cell centrosome recruits γ-tubulin to the maturing centrosome structure that takes place at the transition from G2 to mitosis and during centrosome reconfiguration from prophase to metaphase. The metaphase centrosome contains the highest amount of γ-tubulin. We do not yet know the precise mechanisms for γ-tubulin recruitment but we know that in somatic cells the centrosomal proteins pericentrin [Doxsey et al., 1994] and ninein [Mogensen et al., 2000] are needed. This area of reproductive biology research on centrosomes is new and has not been studied as thoroughly as in somatic cells. Very few molecular and biochemical studies have been performed on centrosomes in SCNT eggs and embryos. If we understand the conditions needed for somatic cell centrosomes to function in the reconstructed oocyte, it will be possible to add factors for regulation along with the somatic cell nucleus during transfer and improve cloning efficiency.

Questions that have been asked but little investigated so far include: First, which components of the MII spindle are removed for enucleation before nuclear transfer (NT)? Several investigators have started to address this topic and it has been proposed in nonhuman primates that centrosome proteins as well as microtubule motor proteins are removed that are important for centrosome functions within the reconstructed oocyte [Simerly et al., 2003]. Our own lab has studied reconstructed pig oocytes in which the meiotic spindle was retained and found a significant increase in embryo development to the blastocyst stages compared to reconstructed oocytes whithout the MII spindle as routinely required for SCNT [Katayama et al., 2006]. In mouse oocytes, Van Thuan et al. [2006] did not find significant centrosomal deficiencies affecting development in reconstructed mouse embryos produced with or without the MII spindle which highlights again the differences of the mouse system compared to other mammalian reproductive systems. The second question that also has not yet been addressed is the centrosome content in the enucleated oocyte. We know that γ-tubulin is present in the enucleated oocyte which has been shown by artificially activating oocytes and analyzing γ-tubulin foci as well as microtubule asters that are nucleated from γ-tubulin foci. We do not yet know whether other centrosomal proteins are localized in the unfertilized enucleated oocyte that may associate with the somatic donor cell centrosome after SCNT. The third question is: how does the somatic cell centrosome in the reconstructed oocyte differ from the zygotic centrosome? As presented above, the zygotic centrosome is composed of blended centrosomal components contributed by sperm and egg. It is not known if the somatic cell centrosome can perform all functions that are typically performed by the zygotic centrosome and whether or not the somatic cell centrosome recruits the correct or additional centrosomal components from the oocyte (like sperm does during fertilization). Gamma-tubulin, the centrosomal protein that nucleates microtubules, is present in sperm and oocyte cytoplasm and becomes blended at fertilization to result in the optimal quantity of γ-tubulin that nucleates the optimal number and length of microtubules. If excess γ-tubulin is present in the NT centrosome (compared to zygotic centrosome), an excess number of microtubules become nucleated that will separate chromosomes unevenly and result in genomic instability (developmental abnormalities). If centrosomal proteins and microtubule nucleation in fertilized eggs compared to NT eggs differs, it is likely that abnormalities in cell division are the result as had been shown for reconstructed pig oocytes [Zhong et al., 2007]. The fourth question addresses regulation of the somatic cell centrosome by the recipient oocyte and includes pH, cyclins, Ca^{2+} and perhaps several others for which only fragmented data exist so far [reviewed by Malcuit and Fissore, 2007]. Similarly, while centrosome duplication has been studied well in somatic cells we do not yet know which mechanisms are required for centro-

some duplication in reconstructed eggs. Cyclins play a critical role in centrosome duplication but different cyclins are required for different systems [reviewed in Sluder, 2004]. Any imbalances in regulation can lead to centrosome dysfunctions.

We know that the ooplasm actively provides regulatory components. In the mouse, donor cell centrioles are degraded by the oocyte while centrosomal proteins from both donor cell and recipient oocytes contribute to centrosome formation in mitotic poles of the reconstructed egg [Zhong et al., 2005]. Intolerance for centrioles in the mouse oocyte had previously been shown by Szollosi et al. [1972] who fused thymocytes with oocytes and clearly showed that thymocyte centrioles were degraded. In reconstructed embryos, few studies are available on specific centrosome proteins in reconstructed embryos and only limited data are available for γ-tubulin, centrin2, and NuMA. No data have been published so far on centrosome regulation by the reconstructed oocyte's cytoplasm and factors that play a role in centrosome remodeling. All studies using γ-tubulin to detect centrosomes associated with the donor cell nucleus revealed microtubule organization from the donor cell centrosome that sometimes has been referred to as monaster, reflecting the interphase aster as typical for the interphase centrosome in somatic cells [reviewed in Sun and Schatten, 2006]. However, quantitative studies on γ-tubulin and exact determination of microtubule length and numbers as nucleated from the donor cell centrosome have not yet been performed. Such studies are important to compare the reconstructed egg's centrosome with that seen after physiological fertilization or IVF to determine differences that may indicate abnormalities.

To study centrosome protein contributions by the oocyte and the donor cell centrosome, Zhong et al. [2005] used mouse MII oocytes as recipients, mouse fibroblasts, rat fibroblasts, or porcine granulosa cells as donors to produce intraspecies and interspecies nuclear transfer embryos. Centrin was observed in centrosomes of donor cells before nuclear transfer but it was not detected at the mitotic poles during first division. These results supported earlier studies by Manandhar et al. [2005] who found in SCNT pig embryos that centrin is degraded during early stages and appeared only during blastocyst stages which is also the case in IVF pig embryos. Different results were obtained for NuMA. To study NuMA dynamics in reconstructed eggs, Zhong et al. [2005] utilized NuMA antibody that did not recognize NuMA protein of mouse oocytes but clearly recognized NuMA in porcine granulosa cells to distinguish NuMA contributed by the oocyte and donor. The donor nucleus clearly contained NuMA that was translocated out of the nucleus to the cytoplasm and participated in spindle pole formation during first mitosis of the reconstructed egg. Liu et al. [2006] had shown earlier in porcine oocytes that NuMA was contributed by fetal fibroblast donor cells to reconstructed porcine eggs. These results also showed that it took about 6 hr after nuclear transfer before NuMA could be visualized with immunofluorescence microscopy which indicates a lag time compared to sperm nuclei that display NuMA immunofluorescence shortly after nuclear decondensation. It was concluded that cytoplasmic factors in the recipient porcine oocyte were able to remodel the donor cell's NuMA but in a delayed reaction. In nonhuman primates, NuMA depletion after removal of the MII spindle during enucleation was implicated in abnormal spindle formation [Simerly et al., 2003] although the contribution of NuMA from the donor cell nucleus to spindle formation was not addressed. By using different methods the authors reported in a subsequent study that ineffective targeting of NuMA to spindle poles is a main factor in abnormal spindle formation [Simerly et al., 2004]. In the mouse, depletion of NuMA by enucleation does not play a significant role in spindle pole formation [Van Thuan et al., 2006] which may further highlight the differences between the mouse and most other mammalian systems studied for this thus far. Further experiments are clearly needed to study factors that play a role in NuMA regulation in reconstructed embryos, as NuMA is a key protein for accurate organization of the mitotic apparatus and it may play an important role in asymmetric cell division in which spindle size varies as a crucial determinant for cell differentiation and development. In somatic cells, cdc2/cyclin B regulates NuMA translocation from the nucleus to the cytoplasm and the mitotic poles while inactivation of cdc2/cyclin B kinase is needed for dissociation of NuMA from the mitotic poles and relocation to the nucleus [Saredi et al., 1997; Merdes and Cleveland, 1998; Gehmlich et al., 2004]. Failure to relocate to the nucleus results in cytoplasmic NuMA foci that are able to form cytoplasmic asters [reviewed in Sun and Schatten, 2007].

NuMA is an important protein for another reason in that it bridges nuclear remodeling with centrosome remodeling, as it plays a significant role as nuclear matrix protein during interphase and it also is one of the first key targets during the process of apoptosis [reviewed in Sun and Schatten, 2006]. Therefore, NuMA provides a link between nuclear and cytoplasmic interactions that can support mitosis or apoptosis which comprises a highly critical aspect in nucleo-centrosomal remodeling.

Taken together, the regulation of centrosomes within reconstructed eggs has not yet been properly investigated and it is not clear if and how the oocyte remodels somatic cell centrosomes for embryo-specific functions. As detailed above, blended centrosomal material from egg and sperm is precisely regulated to produce a zygotic centrosome. We do not yet know the exact molecular composition of the zygotic centrosome and we do not yet know how it compares to the interphase somatic cell centrosome and the centrosome in reconstructed eggs. Furthermore,

does the somatic cell centrosome acquire centrosomal proteins that are stored within the enucleated oocyte? If so, does this result in increased centrosomal material compared to zygotic centrosomal components? We know that pathological increases in γ-tubulin as seen in cancer cells [Lingle et al., 1998] results in increased microtubule nucleation with consequences for abnormal cell divisions and numerical chromosomal abnormalities in the dividing daughter cells. The possibility exists that the recipient oocyte may recruit centrosomal proteins that produce abnormal microtubule patterns with consequences for subsequent cellular abnormalities. For example, how does the enucleated oocyte regulate centrosomal proteins using its cytoplasmic components? Are regulatory enzymes such as MAPK recruited to centrosomes in similar ways as they are to centrosomes in fertilized eggs? Our knowledge regarding these aspects is limited and fragmented and new investigations will undoubtedly be necessary to explore centrosome regulation and their role in the low cloning percentage. The pig provides a most suitable model for these studies for a number of different reasons, most of all because porcine centrosome and microtubule organization is similar to most mammalian species including humans.

Centrosome Dysfunctions and Implications for Fertility and Assisted Reproduction

Numerous diseases have been linked to centrosome dysfunctions [reviewed in Badano et al., 2005] and centrosome dysfunctions can also be caused by environmental and genotoxic stresses [reviewed in Schatten, 2008]. In reproduction, centrosome dysfunctions have severe implications and can lead to aneuploidy in female germ cells (oocytes) or infertility in male germ cells (sperm). Several reports have linked male infertility to centrosomal defects [Sutovsky and Schatten, 2000; Sathananthan et al., 2001] and restoring centrosomal functions in such cases of infertility is the goal of current investigations although this research is still in an early stage of investigations [Nakamura et al., 2002, 2005]. As our knowledge of centrosome functions and dysfunctions has increased enormously during the past decade as a result of advanced molecular methods and methodologies including centrosome isolations, forward and reverse genetics, RNA-mediated interference, mass-spectrometry-based proteomics, live cell imaging and laser microsurgery, it is likely that future applications will be able to repair centrosome pathologies and restore the affected functions.

NOMENCLATURE

Aneuploidy, gain or loss of chromosomes, resulting in abnormal number of chromosomes; CREST syndrome, abbreviation for Calcinosis, Raynaud phenomenon, Esophageal dysmotility, Sclerodactyly, and Telangiestasia, a disorder affecting connective tissue. It is an autoimmune disease characterized by thickening, hardening, tightening of the skin, and involvement of the esophagus, intestine, lungs, heart and kidneys in more severe cases; Diakinesis, The last stage of meiotic prophase, in which the nucleolus and nuclear envelope disappear, spindle fibers form, and the chromosomes shorten in preparation for anaphase; GV, Germinal Vesicle; In almost all animal species, immature oocytes are arrested at first meiotic prophase. GV refers to the large nucleus of the primary oocyte before meiosis is completed and before polar bodies are formed; GVBD, Germinal Vesicle Breakdown Meiotic reinitiation of oocytes is characterized by entry into metaphase I, beginning with germinal vesicle breakdown; G2/M, The cell cycle is subdivided into four stages. G1 refers to gap phase 1 (or growth phase 1); S refers to DNA synthesis or duplication phase; G2 refers to gap phase 2 during which the cell checks for completion of DNA replication; protein synthesis occurs in the G2 phase, following DNA replication in the S phase. This is the time when the cell stockpiles on cytoplasmic contents, before mitosis and cytokinesis occurs. M refers to mitosis phase during which chromosomes condense and attach to the mitotic spindle that separates chromosomes in preparation for cell division. G2/M refers to progression from the G2 phase to mitosis, the interval and transition from completion of DNA synthesis to the beginning of mitosis. Mitosis involves prophase, prometaphase, metaphase, anaphase, and telophase of the mitotic cell cycle; Hyperploidy, Chromosome number greater than but not an exact multiple of the normal euploid number; Mad: a member of the Smad family, a novel group of proteins which mediate signals by the TGF-β superfamily; MII oocyte maintenance, MII refers to metaphase of second meiosis following meiosis I. Meiotic divisions are necessary for chromosome reduction to one set of chromosomes (1n). In most mammalian species, fertilization takes place in MII, restoring the diploid (2n) condition; Ninein, a coiled-coil protein of the centrosome. The protein is important for positioning and anchoring the microtubule minus-ends in epithelial cells; Pachytene, The stage of prophase I of meiosis in which chromatids are first distinctly visible; PBI, first polar body, a minute cell produced and later discarded in the development of an oocyte. Polar bodies contain only a small amount of cytoplasm but contain one of the nuclei derived from the first or second meiotic division; Pericentrin, Conserved protein (200–220 kD) of the pericentriolar region involved in the organization of microtubules during meiosis and mitosis, concentration highest at metaphase, lowest at telophase; Proteasomes; Proteasomal degradation, Proteasomes are part of a major mechanism by which cells regulate the concentration of particular proteins and degrade misfolded proteins; Semiconservative, Semiconservative refers to mode of centriole

duplication. After separation of the two centrioles a new centriole grows adjacent to each to form a new set of centrioles; Trisomy 21 or Down's syndrome: a chromosomal disorder caused by the presence of all or part of an extra 21st chromosome; Trophectoderm cells, trophectoderm cells give rise to extra-embryonic structures such as the placenta, of the blastocyst.

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