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Review
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Review

Meiotic spindle formation in mammalian oocytes: implications for human infertility†

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Abstract

In the final stage of oogenesis, mammalian oocytes generate a meiotic spindle and undergo chromosome segregation to yield an egg that is ready for fertilization. Herein, we describe the recent advances in understanding the mechanisms controlling formation of the meiotic spindle in metaphase I (MI) and metaphase II (MII) in mammalian oocytes, and focus on the differences between mouse and human oocytes. Unlike mitotic cells, mammalian oocytes lack typical centrosomes that consist of two centrioles and the surrounding pericentriolar matrix proteins, which serve as microtubule-organizing centers (MTOCs) in most somatic cells. Instead, oocytes rely on different mechanisms for the formation of microtubules in MI spindles. Two different mechanisms have been described for MI spindle formation in mammalian oocytes. Chromosome-mediated microtubule formation, including RAN-mediated spindle formation and chromosomal passenger complex-mediated spindle elongation, controls the growth of microtubules from chromatin, while acentriolar MTOC-mediated microtubule formation contributes to spindle formation. Mouse oocytes utilize both chromatin- and MTOC-mediated pathways for microtubule formation. The existence of both pathways may provide a fail-safe mechanism to ensure high fidelity of chromosome segregation during meiosis. Unlike mouse oocytes, human oocytes considered unsuitable for clinical in vitro fertilization procedures, lack MTOCs; this may explain why meiosis in human oocytes is often error-prone. Understanding the mechanisms of MI/MII spindle formation, spindle assembly checkpoint, and chromosome segregation, in mammalian oocytes, will provide valuable insights into the molecular mechanisms of human infertility.

Summary Sentence

Recent finding in meiotic spindle formations of mammalian oocyte uncover new insights into the molecular mechanism of human infertility.

Key words: meiosis, spindle, microtubule-organization centers, aneuploidy, oocyte maturation.

Introduction

In the final stage of mammalian oocyte maturation, a meiotic spindle is formed within the oocyte, followed by segregation of bivalent chromosomes and extrusion of the first polar body [1]. Error-free segregation of chromosomes during oocyte maturation is essential for the normal development of mammalian embryos after fertilization [2]. Aneuploidy, caused by aberrant segregation of chromosomes, can lead to miscarriage or birth defects, such as trisomy 21 (Down syndrome) [3], trisomy 18 (Edwards syndrome) [4], and trisomy 13 (Patau syndrome) [5].

The mechanisms of age-related aneuploidy have been studied extensively using the mouse oocyte as a model system [6]. Recent studies have shown that the mechanisms for spindle formation and

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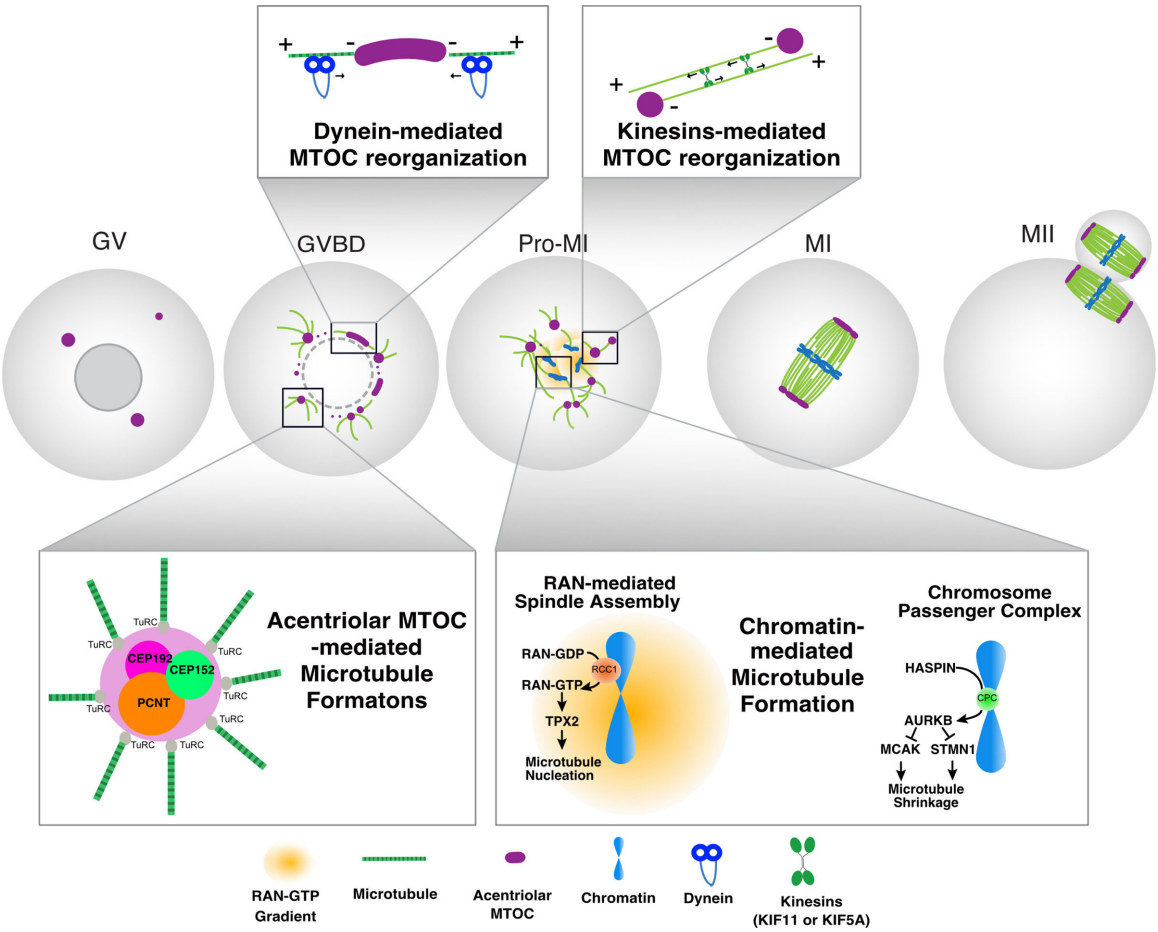

Figure 1. Mechanisms of acentriolar spindle formation in mouse oocytes. During mouse oocyte maturation, acentriolar MTOCs undergo dynamic remodeling. In GV stage oocytes, MTOCs exist as large clusters, but are fragmented after GVBD. Bipolar spindles are reorganized by microtubule-based motors including plus-to-minus directed motor dynein and minus-to-plus directed kinesins. Mouse oocytes can generate microtubules from acentriolar MTOCs or chromatin. Acentriolar MTOCs contain several components in the pericentriolar material of somatic cells including PCNT, CEP192, CEP152, NEDD1, and TuRC. Several kinases that regulate the cell cycle, including PLK1 and CDK1, are involved in the regulation of acentriolar MTOCs (details in Figure 2). RAN-specific GTP exchange factor, RCC1, binds to chromatin and converts RAN-GTP to RAN-GDP gradient towards chromatin. RAN-GTP activates TPX2, which, in turn, recruits the AURKA to the spindle. AURKA activates NEDD1 and TuRC, and initiates microtubule nucleation. The chromosome passenger complex, Aurora B/C-mediated phosphorylation of MCAK, or STMN1, inhibits the shrinkage of microtubules, thereby promoting their growth (adapted from [47]).

chromosome segregation, in human oocytes, are distinct from those in mouse oocytes [7, 8]. Understanding the mechanisms of meiotic spindle formation in mammalian oocytes, particularly in humans, will provide valuable information for the development of diagnostic and therapeutic strategies for aneuploidy-caused infertility.

In this review, we examine the mechanisms of meiotic spindle formation in mammalian oocytes and focus on the differences between these mechanisms in mouse and human oocytes. Additionally, we review the mechanistic aspects of meiotic spindle formation and examine the factors implicated in the development of spindle abnormalities and erroneous chromosome segregation. In later sections, we describe the roles of spindle assembly checkpoint (SAC) and cohesin in regulating the fidelity of segregation and show that abnormalities in quality control mechanisms of chromosome segregation can result in aneuploidy. Finally, we address the importance of methods used to detect aneuploidy in oocytes, and those used to increase the success rate of assisted reproductive techniques such as in vitro fertilization (IVF) and intracytoplasmic sperm injection.

Acentriolar spindle formation in mammalian oocytes

In contrast to somatic cells, mouse and human oocytes do not contain typical centrosomes. A centrosome, which consists of two centrioles and the surrounding layers of pericentriolar matrix (PCM) [9–14], serves as the main microtubule-organizing center (MTOC) during spindle formation in mitotic cells [15]. Instead, mammalian oocytes rely on several different mechanisms for acentriolar spindle formation [1, 16], as illustrated in Figure 1.

Chromatin-mediated formation of microtubules in the mitotic spindle has been well characterized by studies using *Xenopus* oocyte and human mitotic cell extracts as model systems [17–20]. The small GTPase RAN (Ras-related nuclear protein) is involved in nuclear transport and regulation of nucleus-related biochemical processes [21]. RAN exists in the GTP- or GDP-bound form; RAN-GDP is converted to RAN-GTP by the RAN guanine nucleotide exchange factor called regulator of chromosome condensation 1 (RCC1) [17], which is bound to chromatin. This GDP/GTP exchange activity of RCC1 generates a RAN-GTP gradient from chromatin

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[22]. The RAN-GTP gradient induces activation of spindle assembly factors [23] including TPX2 [24]. TPX2 targets the Aurora-A kinase (AURKA) to the spindle and recruits the tubulin ring complex (TuRC), which initiates microtubule nucleation at the spindle poles.

The role of chromatin-based RAN-GTP gradient-dependent formation of microtubules has been intensively studied in mouse and human oocytes [7, 25]. In mouse oocytes, the inactivation of the RAN-GTP gradient, by the expression of dominant-negative or constitutively active RAN mutants, does not inhibit the formation of the meiotic spindle [25], indicating that RAN-mediated nucleation of microtubules is not essential for meiotic spindle formation. Instead, RAN-mediated generation of microtubules is involved in the later stages of microtubule formation, after the initial formation of microtubules from acentriolar MTOCs [26]. This suggests that TPX2, which is a RAN-controlled spindle assembly factor, is also involved in meiotic spindle formation in mice [27], indicating that RAN-mediated assembly of the spindle occurs in mouse oocytes. Recently, mouse oocytes lacking functional MTOCs have been shown to generate microtubules. However, these MTOC-devoid oocytes show differences in spindle assembly and erroneous chromosome attachment associated with increased aneuploidy and female subfertility [28]. This indicates that acentriolar MTOC-mediated microtubule assembly is required for normal spindle formation. However, RAN-mediated formation of microtubules can compensate for the absence of MTOC-mediated assembly of microtubules to a certain extent. Therefore, the existence of both acentriolar MTOC-mediated and RAN-mediated pathways for microtubule assembly in mouse oocytes may provide a fail-safe mechanism to ensure fidelity of chromosome segregation during female meiosis.

In contrast to mouse oocytes, human oocytes utilize RAN-GTP-mediated microtubule formation as an essential pathway for meiotic metaphase I (MI) spindle assembly. Expression of a dominant-negative RAN mutant protein (T24N) prevents spindle assembly in human oocytes, which reportedly lack acentriolar MTOC-associated proteins (discussed in the later sections) [7].

Another mechanism for chromatin-mediated microtubule nucleation involves the chromosomal passenger complex (CPC) [29], which consists of scaffolding protein inner centromere protein (INCENP), Aurora B protein kinase (AURKB), and centromere targeting proteins baculoviral IAP repeat-containing protein 5 (BIRC5, also known as survivin) and Cell division cycle-associated protein 8 (CDCA8, also known as Borealin). CPC induces a gradient of AURKB activity emanating from chromatin [30]. The pool of activated AURKB phosphorylates and inactivates microtubule-destabilizing proteins including mitotic centromere-associated kinesin (MCAK) [31] and stathmin (STMN1) [32]. Consequently, CPC can induce microtubule nucleation at the kinetochore [23]. Depletion of INCENP in mouse oocytes results in chromosome misalignment, which demonstrates the importance of CPC in error-free chromosome segregation [33]. In mouse oocytes, MCAK is localized at the centromere. A loss-of-function study on MCAK showed an arrest at the MI stage and defects in chromosome congression and spindle bipolarity, which indicates that MCAK is involved in the formation of the MI spindle [34].

Perturbation of AURKB and aurora C protein kinase (AURKC) impairs meiotic spindle assembly [35]. AURKC is regulated by the kinase HASPIN. Inhibition of HASPIN activity impairs clustering of MTOCs at the ends of the bipolar spindle; overexpression of AURKC corrects defects in the clustering of MTOCs mediated by HASPIN [36].

Few studies tested the role of CPC in development of aneuploidy in human oocytes. A recent study linked single-nucleotide polymorphisms in *AURKB* and *AURKC* with human aneuploidy [37].

Acentriolar microtubule-organizing centers and spindle formation in mammalian oocytes

The acentriolar MTOCs were discovered in mouse oocytes in the 1970s [9–14]. However, the exact roles of MTOCs in meiotic spindle formation and acentriolar MTOC composition remain unclear.

In mitotic cells, a pair of centrioles is surrounded by various pericentriolar proteins including pericentrin (PCNT), neural precursor cell expressed developmentally down-regulated protein 1 (NEDD1), centrosomal protein of 120 kDa (CEP120), centrosomal protein of 152 kDa (CEP152), CDK5 regulatory subunit-associated protein 2 (CDK5RAP2), and centrosomal protein of 192 kDa [38]. The components of the PCM are essential for the recruitment of TuRC, which initiates the formation of new microtubule filaments [39].

The molecular composition and organization of acentriolar MTOCs in oocytes are not completely understood. Many PCM proteins, such as PCNT [40], gamma-tubulin (TUBG1) [14], NEDD1 [41], centrin-1 (CENT1) [42], nuclear mitotic apparatus protein (NuMA) [43], cancerous inhibitor of protein phosphatase 2A protein (CIP2A) [44], CEP192, and CEP152 [45], are common to the mitotic centrosome and meiotic MTOCs. However, their mechanistic roles in the formation of microtubules and MTOCs are unknown. During maturation of mouse oocytes, acentriolar MTOCs undergo spatial reorganization. In mouse oocytes that are at the germinal vesicle (GV) stage, MTOCs exist as large clusters surrounding the GV [26, 46, 47]. Just before GV breakdown (GVBD), MTOCs extend along the nuclear membrane of oocytes. After GVBD, MTOCs are fragmented into small foci, which are followed by microtubule nucleation from MTOCs. During maturation of mouse oocytes, a network of approximately 80 MTOCs self-organizes to both ends of the spindle, forming a bipolar spindle, by the combined actions of microtubule motor proteins like kinesin heavy chain isoform 5A (KIF5A) and dynein (discussed in a later section), as illustrated in Figure 2 [26, 46, 47].

CEP192 is one of the core PCM proteins [48–51], which serves as a scaffold protein and recruits several other PCM proteins including polo-like kinase 4 (PLK4), polo-like kinase 1 (PLK1), CEP152, and centrosome protein 63 kDa (CEP63) [52, 53]. In mouse oocytes, PCNT and CEP192 are localized to the MTOCs during all stages of oocyte maturation. Depletion of CEP192 by RNAi impairs MTOC formation, delays microtubule spindle assembly, and causes chromosome misalignment. However, it does not completely inhibit formation of meiotic spindle [45]. Similar events are observed in mouse oocytes with depleted PCNT [28], indicating that MTOC-mediated microtubule formation is necessary for maintaining high fidelity of chromosome segregation.

During oocyte maturation, acentriolar MTOCs undergo dynamic remodeling, which involves stretching along the nuclear membrane at the GV stage, fragmentation after GVBD, and self-organization at the spindle poles [26, 47]. PCNT and CEP192 are persistently localized to MTOCs during all stages of oocyte development; however, the localization of CEP152is restricted to the GV stage. CEP152 is rapidly delocalized from MTOCs after GVBD [45], which is CEP152 linked to subsequent MTOC fragmentation. For example, inhibition of cyclin-dependent kinase 1 (CDK1) activity causes retention of CEP152 on MTOCs even after GVBD, indicating that CDK1 activity is linked to CEP152 displacement. The knockdown of CEP152

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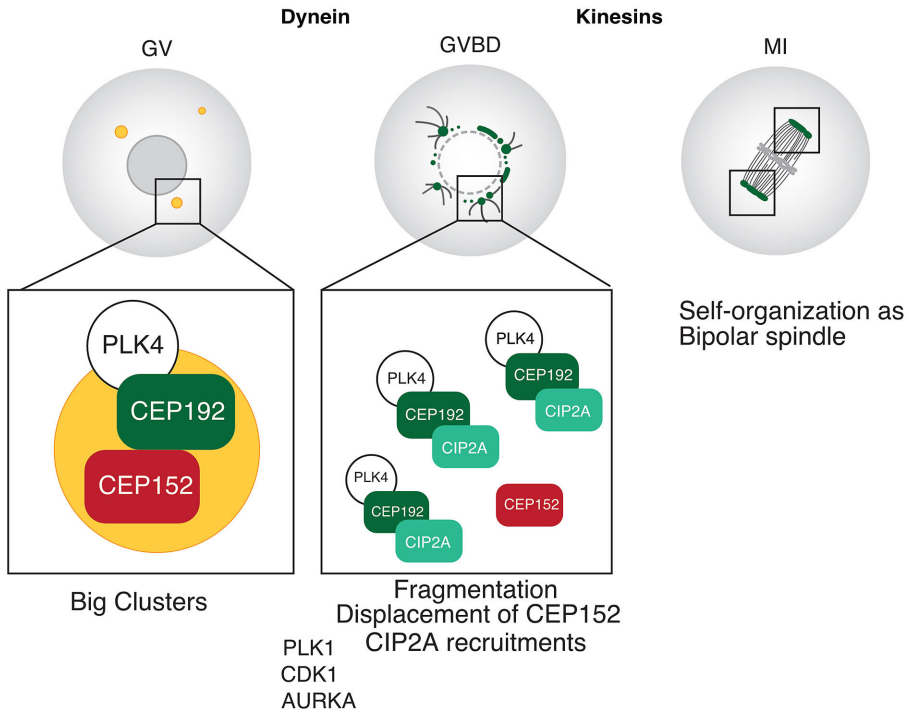

Figure 2. PCM materials and kinases involved in remodeling of acentriolar MTOCs. During maturation of mouse oocytes, acentriolar MTOCs undergo several remodeling steps. In the GV stages, MTOCs exist as a cluster; however, the cluster is extended and fragmented after GVBD. MTOCs mediate the formation of microtubules, and dynein is involved in the remodeling of MTOCs. CEP192 (marked in green) is present in MTOCs at all stages of oocyte maturation, while CEP152 is only present in MTOCs in the GV stage [45]. CIP2A, a component of MTOCs, is present in MTOCs after GVBD [44]. During spindle formation, MTOCs are self-organized to both ends of the spindles by the action of kinesins, which results in the formation of bipolar spindles. Several other protein kinases are involved in the control of these processes, including PLK1, AURKA, and CDK1 (adapted from [45]).

causes premature fragmentation of MTOCs, which indicates that CEP152 is involved in the clustering and fragmentation of MTOCs. In somatic cells, the recruitment of CEP152 to MTOCs depends on PLK4. However, in mouse oocytes, the expression of CEP152, lacking the N-terminal PLK4 binding site, does not alter the localization of CEP152. This indicates that CEP152 localization at the GV stage is independent of PLK4 and demonstrates that acentriolar MTOCs and PCM differ in somatic cells wherein PLK4 is critical in centriole duplication.

In addition to CEP192 and CEP152, CIP2A has been identified as a new component of acentriolar MTOCs in oocytes [44]. In contrast to previous reports showing that CIP2A is an inhibitor of protein phosphatase 2A (PP2A) [54, 55], overexpression of CIP2A did not inhibit PP2A activity [44], which is essential for the meiotic maturation of oocytes [56]. Instead, CIP2A is localized to MTOCs. Interestingly, CIP2A does not localize to MTOCs in the GV stage, but rather after GVBD. This localization differs from that of CEP152, which is localized at MTOC during the GV stage, but extruded from MTOC after GVBD [45]. Similar to the results for CEP192, the knockdown of CIP2A causes depletion of MTOCs, which indicates that CIP2A is essential for maintaining MTOCs. The sequential recruitment of CEP152 and CIP2A to acentriolar MTOCs indicates that the two proteins may compete with each other to bind to CEP192. The detailed mechanistic roles of CIP2A, in the formation of acentriolar MTOCs, require further analysis.

Several kinases have been implicated in controlling the spatial reorganization of MTOCs and recruiting various PCM proteins in oocytes. For example, PLK1 plays several roles in cell-cycle control and spindle formation in oocytes, participating in meiotic resumption, anaphase transition, and remodeling of acentriolar MTOC [45, 47, 57]. Chemical inhibition of PLK1 activity, in mouse oocytes, impairs fragmentation of MTOCs after GVBD [47] and eventually causes the depletion of MTOCs [45, 57], demonstrating the importance of PLK1 in oocyte maturation. In mitotic cells, PLK1 phosphorylates CEP192, which is a key component of the PCM layer and acentriolar MTOCs [53]. In addition to CEP192 phosphorylation, PLK1 is implicated in the phosphorylation of CIP2A [44]. PLK1 and AURKA reciprocally facilitate their localization on MTOC and recruit TuRC to CEP192 [52]. Aside from recruiting AURKA and TuRC, the exact mechanistic role of PLK1 in spatial reorganization of acentriolar MTOCs in oocytes remains unclear. A recent study on in vitro reconstitution using PCM proteins from *Caenorhabditis elegans* embryos showed that centriolar proteins can form condensed, phase-separated spherical structures under conditions of macromolecular crowding [58]. This indicates that PLK1 facilitates the formation of a phase-separated structure and can facilitate microtubule formation [58]. A similar phase-separated formation of CEP192 and pericentriolar material may occur during the dynamic remodeling of MTOCs in mammalian oocytes, particularly during self-organization of the PCM to both ends of the meiotic spindles. However, detailed mechanism of this process and PCM composition require further analyses.

In addition to kinases, several microtubule motor proteins have been implicated in spatial reorganization of MTOCs to spindle poles. For example, a minus-end directed microtubule motor dynein [59] is involved in the initial fragmentation of MTOCs at the onset of GVBD [47, 60, 61]. Inhibition of activity of another motor protein, KIF5A, impairs clustering and bipolarization of MTOCs [26, 62]

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indicates that microtubule motor proteins play important role in meiotic spindle formation.

In summary, formation of meiotic spindle in mouse oocytes represents a robust mechanism for accurate chromosome segregation.

Do human oocytes lack microtubule-organizing center-mediated spindle formation?

Several important differences in MI spindle formation between mouse and human oocytes were observed in a recent study [7].

As illustrated in Figure 3, human oocytes considered unsuitable for clinical IVF procedures lack detectable acentriolar MTOC markers including PCNT and gamma-tubulin. Instead of utilizing the acentriolar MTOC-mediated microtubule formation pathway, spindle formation in human oocytes relies entirely on RAN-GTP mediated and chromatin-mediated formation of microtubules. The expression of dominant-negative RAN mutants in oocytes completely inhibits formation of the meiotic spindle. The opposite holds true in mouse oocytes in which RAN-mediated nucleation of microtubules is not essential for the formation of MI spindle [25, 26]. Thus, the expression of dominant-negative RAN mutants only delays microtubule formation in its later stage. Notably, the completion of MI spindle formation (16 h) in human oocytes requires considerably longer time compared to mouse oocytes (3–5 h). Prolonged MI spindle formation in human oocytes can be mimicked by mouse oocytes by depleting MTOCs via knocking down PCNT [28] or CEP192 [45]. Acentriolar MTOC-mediated spindle formation is required for the robust and error-free chromosomes segregation in mouse oocytes. SAC fails to detect spindle abnormalities in mammalian oocytes and gives rise to the development of aneuploidy. This is described in detail in the subsequent section.

One of the limitations in the Holubcova et al study is that the collected human oocytes were ovulated, but did not resume meiosis nor did they undergo GVBD or progress to the MII stage even after hormonal stimulation for IVF [7]. Hence, it is possible that such oocytes may not fully represent mechanisms of meiotic spindle formation in healthy human oocytes. Therefore, it is necessary to compare oocytes collected from healthy donors in order to confirm the absence of MTOCs in human oocytes.

The mechanism of spindle formation in oocytes and preimplantation embryos is evolutionarily diverse among mammals [10, 43, 63]. Acentriolar MTOCs have not been observed in human and porcine oocytes [7, 43]. In bovine oocytes, gamma-tubulin is observed at spindle poles and in the cytoplasm, which is consistent with the localization in mouse oocytes [63] and implies the presence of acentriolar MTOCs in bovine oocytes. Similar to oocytes, early mouse embryos until the blastocyst stage also utilize acentriolar MTOCs for mitotic spindle assembly [64, 65]. However, in human embryos as well as in most other mammals, a centriole from the sperm is responsible for the mitotic spindle formation of [66]. This demonstrates the diversity of acentriolar MTOC-mediated spindle formation in mammalian oocytes and early embryos. Collectively, microtubule nucleation pathway using MTOCs is important in maintaining the high fidelity of chromosome segregation during MI.

Spindle assembly checkpoint in oocytes

SAC prevents premature progression of the cell cycle until the kinetochores are attached to the mitotic spindle and are under tension [67, 68]. In the mitotic cell, the mitotic checkpoint complex (MCC) consists of mitotic arrest deficient protein 2 (MAD2), mitotic checkpoint serine/threonine-protein kinase BUB1 beta (BUB1B), and mitotic checkpoint protein BUB3 (BUB3) in complex with cell division cycle protein 20 (CDC20) [69]. When kinetochores are not attached to the mitotic spindle, the MCC inhibits the activity of the anaphase-promoting complex/cyclosome (APC/C), which is a ubiquitin ligase, and prevents the degradation of cyclin-B1 (CCNB1) and Pituitary tumor-transforming gene 1 (PTTG1, also known as securin). The MCC is assembled by the outer kinetochore KMN network [70]. Kinetochores that are not attached to microtubules act as "catalytic platforms" to generate the MCC. The MCC inhibits the activity of APC/C, and cell-cycle progression is suspended until the

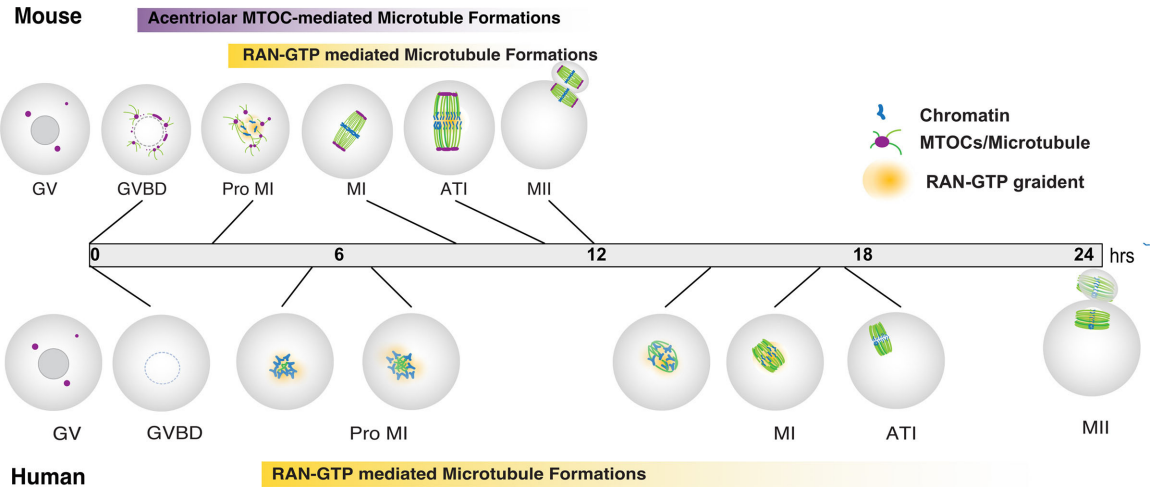

Figure 3. Comparing the time course of meiotic spindle formation in human and mouse oocytes. Considerable differences are found in the spindle formation of mouse and human oocytes. The human oocytes were obtained from patients with infertility [7]; therefore, they may not represent the healthy status of the human oocyte. Under conditions of in vitro maturation, the maturation of mouse oocytes is typically completed in 12 h, and mature MII oocytes are formed. In humans, the maturation of MII oocytes requires more than 24 h. A major difference in oocyte maturation, between the two organisms, is the time required to generate the meiotic spindles. In mouse oocytes, microtubule nucleation can occur via MTOC- and RAN-mediated pathways, while only RAN-GTP-mediated pathway is involved in microtubule formation in human oocytes. Loss-of-function studies, examining MTOC components of mouse oocytes, show reduced chromosome segregation, indicating that the pathway of microtubule formation is essential for the robust formation of meiotic spindles in mouse oocytes. Stages for microtubule formation by MTOC are indicated in violet and Ran-mediated pathway is indicated in yellow (adapted from [1, 7]).

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kinetochores are attached [71]. The lack of tension, caused by merotelic or syntelic attachment, activates AURKB and induces SAC activation [72]. After kinetochores are correctly attached to the mitotic spindle, MCC dissociates from APC/C, which then mediates degradation of PTTG. This results in activation of extra spindle poles-like 1 (ESPL1, also known as seperase) that degrades cohesin and eventually initiates chromosome segregation [73].

In mouse oocytes, the presence of the different components of SAC, including BUB1R [74], MAD2 [75], mitotic spindle assembly checkpoint protein MAD1 (MAD1) [76], dual specificity protein kinase TTK (also known as MPS1) [77], kinetochore protein SPC25 (SPC25) [78], and BUB3 [79], has been confirmed. This indicates the presence of SAC activity and prevention of premature anaphase transition before the attachment of kinetochores to the spindle. However, several studies on mouse oocytes have shown that reduced SAC activity tolerates unattached chromosomes. For example, mouse oocytes, lacking a functional NuMA protein, generate highly disorganized spindles and show defects in chromosome alignment. However, the SAC is not silenced and anaphase progresses even in the presence of one or few unattached chromosomes [80]. Similarly, oocytes with depleted PCNT or CEP192 [28, 45], or those treated with a PLK1 inhibitor BI2536 [57], show defects in chromosome alignment, aneuploidy, or fertility. These results indicate that SAC in mammalian oocytes is not robust enough to prevent all chromosome segregation errors. Why do mammalian oocytes have inherently reduced SAC activity compared with mitotic cells? A recent study shows that a particularly large volume of mammalian oocytes (mouse and human oocytes with a diameter of 80 and 120 μm, respectively) is directly responsible for reduced SAC activity [81]. The amount of MCC components localized in the germinal vesicle may be thus the limiting factor in SAC activity [82] as is the case in the *C. elegans* embryos [83].

Age-related decrease in the SAC activity of human oocytes may explain the increased incidence of age-related aneuploidy. Messenger RNA levels of certain SAC genes in human oocytes decrease with age [84]. An age-dependent decrease in levels of BUB1 and BUBR1 in the kinetochores of human oocytes has been reported [85]. It suggests that SAC activity decreases with advanced age and contributes to increased incidence of aneuploidy. However, the rate of premature anaphase transition in oocytes obtained from aged mice does not increase considerably despite the increased incidence of aneuploidy [86]. This suggests that SAC activity is not related to increased incidence of aneuploidy in mouse oocytes. These discrepancies in age-dependent SAC activity in mouse and human oocytes may shed light on the differences between human and rodent oocytes. Further investigation of SAC activity in the human oocyte will help understand the exact role of the limited SAC activity in preventing human aneuploidy.

Cohesins and chromosome segregation errors

After the alignment of chromosomes and movement of the spindle towards the cortex are completed, the anaphase–telophase transition proceeds and the first polar body is extruded. For anaphase I transition, the cohesin complex, which forms the complex between sister chromatids and homologous chromosomes, undergoes degradation by ESPL1 in chromosome arm, facilitating separation of the homologous chromosomes [87]. The centromeric cohesin links sister chromatids and is protected from degradation by shugoshin (SGO2) to maintain the diploidy of chromosomes after meiosis I [88, 89]. The mechanisms of PTTG1/ESPL1 activity and their relationship with APC/C and maturation promoting factor have been reviewed previously [87]. Mutations in the meiosis-specific cohesin subunit gene, cohesin *Smb1*, lead to infertility in male and female mice [90, 91]. The levels of centromere cohesin on chromosomes at MI stage oocyte are reduced by depletion of centromere cohesion protection protein shugoshin 2 (SGO2) [92–94] in oocytes of aged mice, indicating a strong correlation between age-dependent decrease in cohesin levels and increased incidence of aneuploidy.

Depletion of SGO2 in aged mice causes premature separation of sister chromatids [92]. The levels of cohesin in oocytes obtained from aged human donors are considerably lower than those in oocytes from young donors [85, 95], indicating a relationship between reduced levels of cohesin and age-related aneuploidy. However, whether the loss of cohesin is directly related to increased incidence of aneuploidy in human oocytes remains unclear [96].

More than 60% of sister kinetochores in oocytes obtained from young human donors are physically separated from each other, indicating compromised centromere cohesion [8]. This contrasts with mouse oocytes in which each pair of sister kinetochores is properly attached [93]. The fraction of separated kinetochores in human oocytes increases with age so that 87% kinetochores in human oocytes from donors over 35 years are separated. This suggests that increased incidence of aneuploidy is caused by merotelic kinetochore attachments [8]. Considering that the oocytes used in this study were immature and obtained from infertile patients, the observed differences may not conclusively reflect the differences between human and mouse oocytes.

Clinical implications of spindle abnormalities in human oocytes

As described above, aberrant spindle formation during meiosis I may be the direct cause of aneuploidy. The analysis of MII spindle in human oocytes will provide valuable information on the developmental competence of oocytes, which will improve the success rate of IVF in clinical practice.

Because of its invasive nature and requirements for fixation and staining, immunofluorescence confocal microscopy, which is used to characterize the structure of the meiotic MII spindle, cannot be used for oocyte selection in clinical IVF procedures. This highlights the need for noninvasive methods for observing the structure of the MII spindle in live human oocytes. One such method is polarized light microscopy (poloscope) [97], which utilizes birefringence generated by spindle microtubules. However, studies comparing confocal microscopy and the use of the poloscope showed that current poloscope-based methods provide limited information on spindle abnormalities that may lead to developmental problems [98–100]. A newer, noninvasive microscopic technique called holotomography utilizes refractive index for intrinsic image contrast [101] and may potentially be used to observe MII spindle.

Another clinical consideration comprises cryopreservation and thawing of human oocytes during IVF procedures. These procedures are usually conducted to preserve female fertility before the rapid decline of fecundity by the age 30 and above [102, 103]. Since cold treatment depolymerizes microtubules [104], oocyte cryopreservation and thawing can lead to meiotic spindle damage [105]. A recent study showed that slow freezing (vitrification) of oocytes results in abnormal spindle morphology [106], which highlights the importance of noninvasive diagnostic methods for spindle morphology analyses in IVF procedures.

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Conclusions

Recent studies on meiotic spindle formation in mammalian oocytes have provided a wealth of information on the molecular mechanisms driving the increased incidence of aneuploidy in human females of advanced maternal age. However, many of these studies used model organisms, particularly mice, which limits their clinical relevance. Studies of spindle assembly mechanisms have revealed potential differences between mouse and human oocytes, including the lack of acentriolar MTOC formation in human oocytes of limited quality. This indicates the importance of conducting comparative studies using human and mouse oocytes.

In conclusion, understanding the mechanisms of spindle formation in mammalian oocytes and their links to aneuploidy would improve diagnosis and treatment of aneuploidy-related infertility.

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