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COMMENTARY

Minor poles, major impact: Insights into spindle bipolarization and female fertility

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Oocyte maturation is a complex process that ensures only euploid egg cells—those with the correct number of chromosomes—proceed to fertilization and subsequent embryonic development.[1,2] In this process, an immature oocyte extrudes the first polar body to become a mature egg through the first asymmetric meiotic division (meiosis I), setting the stage for embryogenesis upon fertilization. During meiosis I, homologous chromosomes separate, with one set extruded into the polar body and the other retained within the egg.[1,3] This precise chromosome segregation is directed by the meiotic spindle; however, errors can result in aneuploid eggs, which are linked to miscarriage and infertility in humans. To ensure accurate separation of chromosomes, the meiotic spindle must establish a bipolar structure that partitions chromosomes into two distinct sets, one for the polar body and one for the egg.

While spindle bipolarization is a universal requirement for accurate chromosome segregation, its mechanisms vary between mitosis and meiosis. In mitosis, spindle bipolarization unfolds through a series of sequential steps. First, duplicated centrosomes begin to separate during prophase, prior to nuclear envelope breakdown (NEBD). This centrosome separation, supported by increased microtubule polymerization, naturally defines the spindle poles. Following NEBD, microtubules from each centrosome engage with chromosomes and connect with one another. Additionally, microtubules extending outward from the centrosomes are anchored by cortical factors at the cell cortex. These different microtubule populations, along with their associated proteins, collectively regulate mitotic spindle bipolarization.

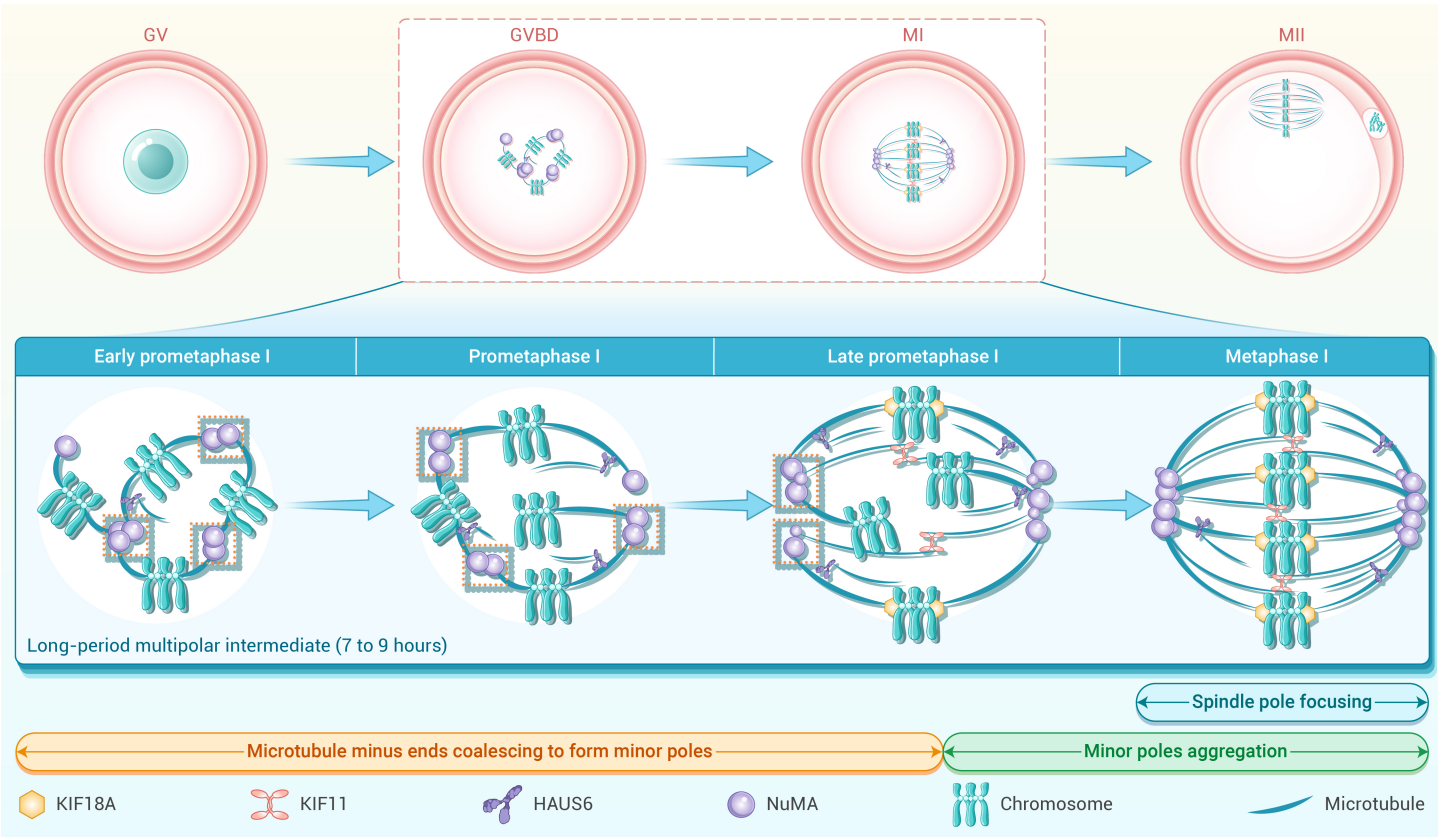

Figure 1. Sequential spindle bipolarization and key protein regulators in human oocyte maturation Spindle bipolarization in human oocytes progresses gradually through distinct stages. During early prometaphase I, microtubule minus ends from kinetochore clusters coalesce, forming multiple minor poles and generating a transient multipolar spindle configuration that persists for 7 to 9 hours. Subsequently, these minor poles aggregate into two main poles, establishing spindle bipolarity, which is maintained through metaphase I. Throughout this process, HAUS6 is involved in microtubule amplification, KIF11 supports spindle elongation, and KIF18A ensures spindle stability. Black boxes highlight the minor poles.

Unlike mitotic cells, mammalian oocytes eliminate centrosomes during oogenesis, leaving them without natural opposing spindle poles. Studies have shown that in mouse oocytes, centrosomes are functionally replaced by acentriolar microtubule-organizing centers (aMTOCs).[4] During meiosis I, these aMTOCs stretch, fragment, and eventually cluster into two spindle poles. In human oocytes, however, both centrosomes and aMTOCs are absent from meiotic spindle poles, indicating a unique mechanism of spindle bipolarization.[5] Understanding this distinctive process is critical for identifying factors that contribute to chromosomal stability, insights that may ultimately lead to improved fertility treatments.

In a recent study published in *Science*, Wu and colleagues[5] used immunofluorescence and live-cell imaging to monitor spindle bipolarization in more than 1800 human oocytes. The full progression of spindle bipolarization was initially visualized, including early and late prometaphase I (ProMI) and metaphase I (MI). To observe spindle bipolarization, the nuclear mitotic apparatus (NuMA) was used as a precise marker of spindle poles in human

oocytes. After spindle microtubule polymerization, multiple nascent spindle poles, referred to as "minor poles," were detected (Figure. 1). These minor poles represent coalescences of microtubule minus ends and originate from kinetochore clusters that drive microtubule polymerization in human oocytes. Due to the random distribution of kinetochore clusters, the minor poles form at various positions, creating multipolar acentrosomal spindles. The organization of minor poles and formation of multipolar intermediates were further observed through live-cell time-lapse imaging, showing that these multipolar intermediates persist for 7 to 9 hours before ultimately resolving into a bipolar spindle at MI. During bipolarization, the minor poles coalesce into two major poles, which then organize and focus to form the final spindle poles. With these focused spindle poles, the bipolar spindle structure is established, enabling accurate chromosome segregation.

To identify key factors in spindle bipolarization, the authors selected 21 proteins related to spindle formation and examined their presence on MI spindles in human oocytes. They observed that only nine proteins (HAUS6, KIF11, KIF18A, HAUS3, KIF16A, KNSTRN, DLGAP5, KIF2A, and KIF2C) were present on the MI spindle. To assess the biological function of these proteins, the team used the Trim-Away technique to deplete each from human oocytes. Depletion of HAUS3, KIF16A, and KNSTRN resulted in defocused spindle poles without disrupting the bipolar configuration, whereas loss of HAUS6, KIF11, and KIF18A led to spindle bipolarization failure. Thus, HAUS6, KIF11, and KIF18A were identified as key factors for spindle bipolarization in human oocytes (Figure 1).

Spindle bipolarization in human oocytes progresses through three main stages: microtubule polymerization, sorting and sliding of antiparallel microtubules, and regulation of microtubule dynamics. Wu et al. reveal critical roles for HAUS6 and KIF11 in ensuring microtubule amplification and spindle elongation. HAUS6, localized initially at the human oocyte microtubule organizing center (huoMTOC) and later relocated to spindle microtubules, is essential for amplifying microtubules within the spindle. Acute depletion of HAUS6 resulted in a progressive loss of intraspindle microtubules, destabilizing spindle structure and leading to multipolarity. Further analysis demonstrated that the N-terminal domain of HAUS6 is indispensable for its function, as its deletion caused spindle multipolarity, underscoring its critical role in spindle bipolarization in human oocytes. KIF11, another vital factor, promotes spindle elongation through its role in antiparallel microtubule cross-linking and sliding. Using the KIF11 inhibitor monastrol, Wu et al. observed dose-dependent effects on spindle morphology, suggesting KIF11's dose-sensitive function in elongation. Interestingly, KIF11's C-terminal domain was found essential for microtubule attachment, indicating a dual-domain mechanism that stabilizes spindle elongation and helps achieve bipolar configuration.

In the final phase of spindle bipolarization, KIF18A was identified as a stabilizing agent, critical for maintaining spindle bipolarity through dynamic microtubule growth control. Initially observed near chromatin in germinal vesicle (GV) oocytes, KIF18A localizes to spindle microtubules after nuclear envelope breakdown and progressively accumulates until MI. Its role becomes particularly pronounced at the plus ends of kinetochore microtubules, where KIF18A manages microtubule length, preventing destabilization of the bipolar spindle structure. KIF18A depletion led to excessive microtubule growth and disrupted bipolarity, while its overexpression limited microtubule length, achieving a stable spindle architecture. The study further underscores the importance of KIF18A's kinesin motor domain, as its deletion induced multipolarity, revealing that KIF18A's regulatory activity is essential for preserving the delicate balance required for correct chromosome segregation. Together, these findings present HAUS6, KIF11, and KIF18A as vital components in human oocyte spindle assembly, offering insights that may influence future research on female infertility linked to spindle defects.

Building on these mechanistic insights, Wu et al. extended their research to explore the impact of genetic mutations in *HAUS6*, *KIF11*, and *KIF18A*, finding that disruptions in these key spindle regulators contribute to female infertility. By screening 3,627 infertile patients with unexplained abnormalities, the authors found mutations in these three genes across 11 patients. These mutations presented a spectrum of phenotypes, including oocyte maturation defects, fertilization failures, and developmental arrest. Notably, mutations in *HAUS6* were linked to oocyte MI arrest and degeneration, while *KIF11* mutations caused spindle multipolarity, leading to the extrusion of multiple first polar bodies. Additionally, certain *KIF11* mutations resulted in aneuploid embryos, further highlighting the critical role of spindle integrity in reproductive success.

To confirm the pathogenicity of these mutations, the team introduced patient-derived mutant *HAUS6*, *KIF11*, and *KIF18A* mRNAs into normal human oocytes, which led to disrupted spindle bipolarization. Interestingly, *KIF11* mutations were especially potent, demonstrating dominant-negative or gain-of-function effects that produced multipolar spindles. Attempts to rescue this spindle defect with wild-type *KIFC1* mRNA proved unsuccessful, indicating that these mutations may induce irreversible functional changes within the spindle assembly pathway. These findings emphasize the essential roles of HAUS6, KIF11, and KIF18A in maintaining spindle bipolarity, with their mutations posing significant barriers to successful meiosis and fertility. This study not only advances our understanding of genetic factors underlying infertility but also suggests new diagnostic and therapeutic strategies for patients with spindle-related reproductive disorders.

In summary, this work represents a significant step forward in our understanding of spindle bipolarization and its impact on female fertility, shedding light on the distinct, minor pole-mediated mechanisms governing human oocyte spindle assembly and stability. By identifying HAUS6, KIF11, and KIF18A as pivotal regulators in microtubule amplification, spindle elongation, and stability, these findings elucidate the comprehensive physiological process underlying spindle bipolarization in human oocytes. Importantly, this study links these mechanisms to clinical relevance, enhancing our understanding of the pathological processes associated with impaired oocyte maturation, fertilization, and early embryonic development in infertile patients. This research not only advances our molecular understanding of human reproductive health, but also suggests new avenues for diagnostics and therapeutic strategies in treating infertility. Future interdisciplinary work might build on these findings by exploring targeted interventions to restore spindle function in affected individuals, exemplifying how foundational cellular research can directly inform and improve clinical outcomes.

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AUTHOR CONTRIBUTIONS

All authors conceived the original idea, wrote and reviewed the manuscript.

DECLARATION OF INTERESTS

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