Unique geometry of sister kinetochores in human oocytes during meiosis I may explain maternal age-associated increases in chromosomal abnormalities

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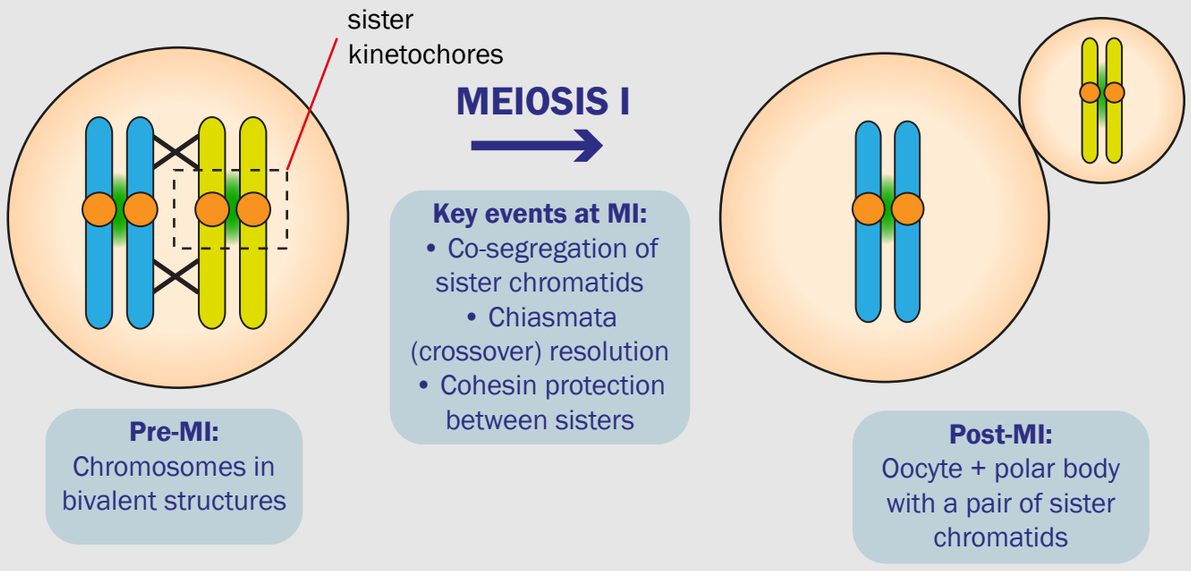
We studied sister kinetochores in fixed meiosis I-stage human oocytes to understand their geometry prior to the first meiotic division. We found that kinetochores in meiosis I are distinctly separate and are capable of acting as individual attachment sites for microtubule fibres from the meiotic spindle. These unique features may help us to understand why the first meiotic division in human oocytes is so error-prone.

Background

The first meiotic division (MI) is highly error-prone in human oocytes. This division entails segregation of homologous chromosomes (unlike mitosis/MII).



Our aim was to understand how kinetochores (protein structures that assemble on centromeres) on sister chromatids are arranged to facillitate this division.

Methods

- Human oocytes donated by women undergoing IVF/ICSI
- Oocytes fixed whole in paraformaldehyde
- Immunofluorescence to stain kinetochores
- Acquisition of high-resolution image stacks (100x)
- Image stacks analysed in Fiji and Imaris software

Kinetochores are separated during MI

In MI oocytes, sister kinetochores form two distinct spots rather than a single spot, indicating that they are not fused. Kinetochores in MI oocytes were classified into: distinct pairs, overlapping pairs, unpaired or unclear. Most sister kinetochores appeared distinct pairs, suggesting that separated sisters are intrinsic feature of human oocytes.

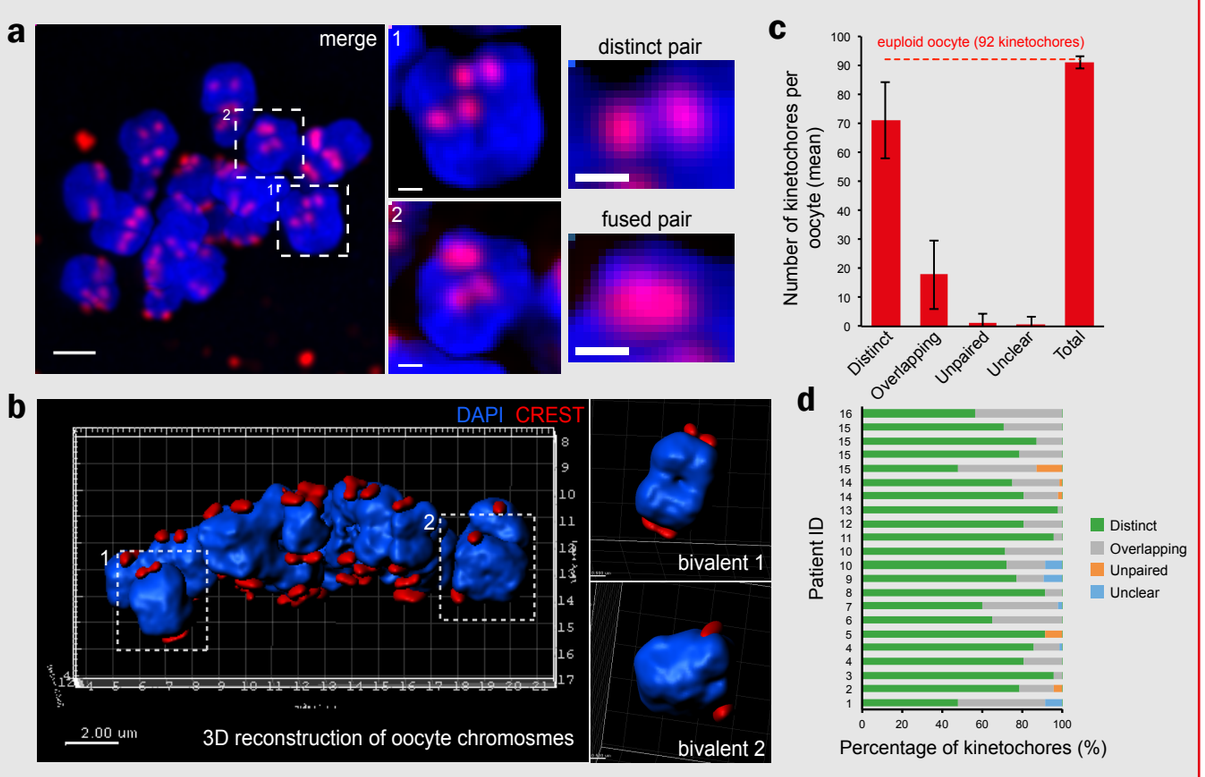


Figure 1: (a) 100x maximal projection of a MI oocyte from a 30.2 year-old woman. (b) 3D Imaris reconstruction of oocyte chromosomes and kinetochores. (c) Number of kinetochores per category (average per oocyte across 19 oocytes) and (d) per individual oocyte.

Meiotic kinetochore architecture

Oocytes were stained with markers for the inner (CREST), outer (Bub1) and fibrous corona (CENP-E) regions of the kinetochore. These regions were also distinct, indicating that the entire kinetochore structure is separated in MI.

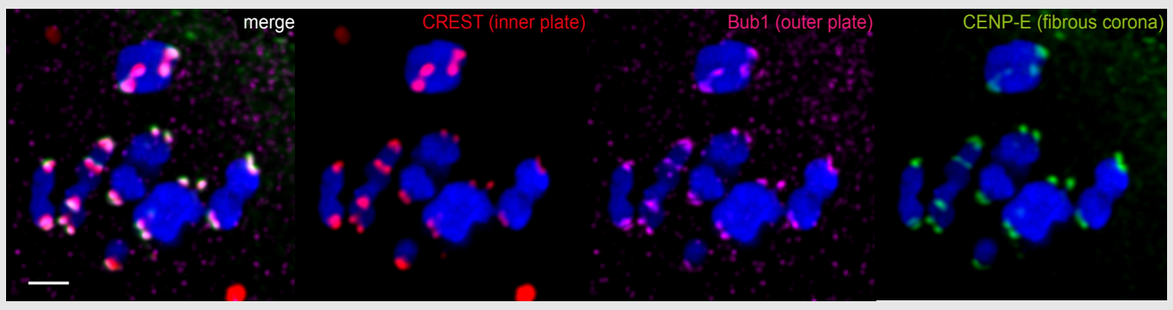


Figure 2: Section from an oocyte stained with CREST, anti-Bub1 and anti-CENP-E antibodies to label different regions of the kinetochore.

Dual kinetochore-fibre attachments

To determine how separated sisters form microtubule attachments, we used cold shock treatment to visualise individual kinetochore-fibre attachments. This revealed that individual kinetochores within a pair could act as separate attachment sites.

Figure 3: (a) Two MI human oocyte spindles stained for microtubules (anti-alpha-tubulin) and kinetochores (CREST). Scale bar = 2 µm. (b) Enlarged z-projections of kinetochore pairs with dual and monotelic attachments as indicated.

Interkinetochore distance inceases with age

We observed an increase in interkinetochore distance with age. This increase in interkinetochore distance may reflect a loss of cohesin, causing sister kinetochores to gradually come apart.

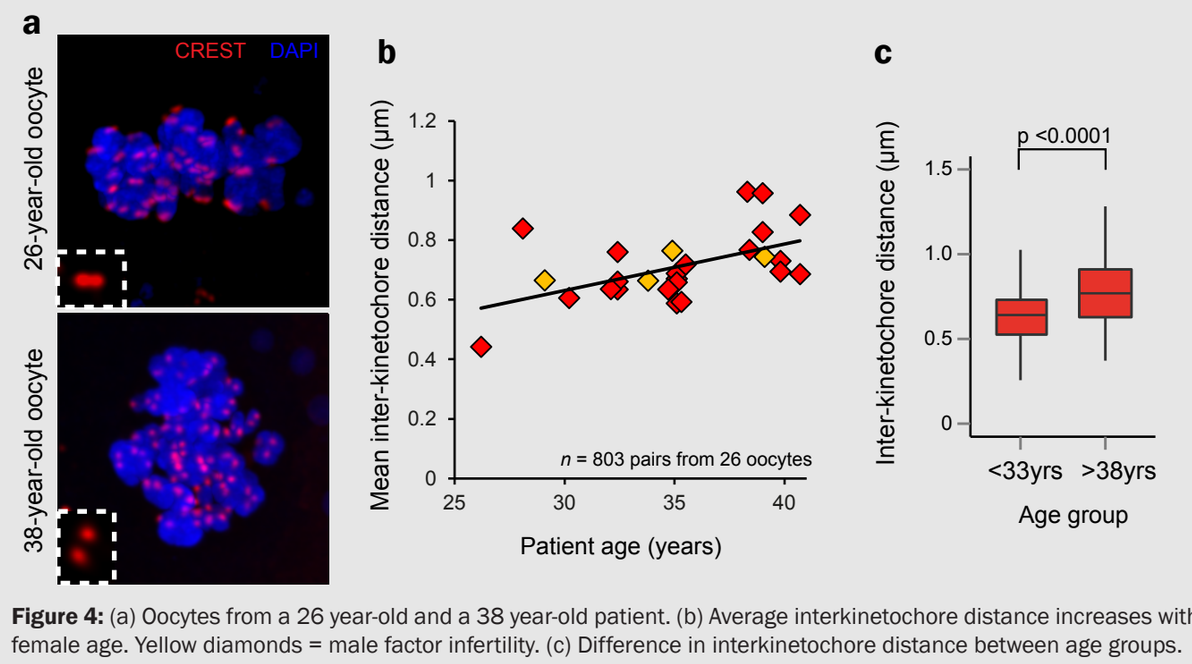


Figure 4: (a) Oocytes from a 26 year-old and a 38 year-old patient. (b) Average interkinetochore distance increases with female age. Yellow diamonds = male factor infertility. (c) Difference in interkinetochore distance between age groups.

Summary

Separation of sister kinetochores in human oocyte MI, coupled with their ability to act as separate attachment sites, may make formation of stable attachments difficult and hence increase the chances of chromosome missegregation. This effect may be exacerbated as sister kinetochores come further apart with female age.

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