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SHORT TAKE

# Chromosome cohesion decreases in human eggs with advanced maternal age

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## Summary

**Aneuploidy in human eggs increases with maternal age and can result in infertility, miscarriages, and birth defects. The molecular mechanisms leading to aneuploidy, however, are largely unknown especially in the human where eggs are exceedingly rare and precious. We obtained human eggs from subjects ranging from 16.4 to 49.7 years old following *in vitro* maturation of oocyte-cumulus complexes isolated directly from surgically removed ovarian tissue. A subset of these eggs was used to investigate how age-associated aneuploidy occurs in the human. The inter-kinetochore distance between sister chromatids increased significantly with maternal age, indicating weakened cohesion. Moreover, we observed unpaired sister chromatids from females of advanced age. We conclude that loss of cohesion with increasing maternal age likely contributes to the well-documented increased incidence of aneuploidy.**

**Key words: aneuploidy; cohesion; egg; human; maternal age; meiosis.**

Fertility decreases with advanced maternal age (Van Voorhis, 2007). This decrease is primarily due to poor egg quality, as successful pregnancies increase significantly in females of advanced maternal age when eggs from young, fertile donors are used (Van Voorhis, 2007). An aneuploid egg is known to be of poor quality, and in humans, egg aneuploidy is associated with advanced maternal age and occurs most often because of chromosome segregation errors at meiosis I (Hassold & Hunt, 2001).

Meiosis in females is particularly error prone, likely a consequence of being protracted and involving two cell cycle arrests. In humans, oocytes enter meiosis during fetal development and arrest in prophase of meiosis I (prophase I). This prophase arrest is maintained until ovulation when, in response to hormonal cues, meiotic resumption occurs. Homologous chromosomes are segregated with completion of meiosis I, and the oocyte arrests again at metaphase of meiosis II (MII) at which point it is called an egg. If the egg is fertilized, MII is completed with separation of sister chromatids. Because the physiologically relevant follicle pool is thought to be nonrenewable, a primordial follicle activated to grow in a woman of advanced maternal age contains an oocyte that has been arrested in prophase I for decades.

Several molecular mechanisms have been proposed to explain the meiotic origins of aneuploidy, including errors in recombination, improper spindle formation and microtubule–kinetochore interactions, and defects in the spindle assembly checkpoint (reviewed in Hunt & Hassold, 2008; Hassold & Hunt, 2009; Eichenlaub-Ritter *et al.*, 2010; Jones & Lane, 2012). Recent findings in mouse suggest that deteriorating chromosome cohesion that occurs during the extended prophase I arrest is also likely a significant cause of age-associated aneuploidy (reviewed in Chiang *et al.*, 2012; Jessberger, 2012; Jones & Lane, 2012). Chromosome cohesion, mediated by a multiprotein cohesin complex, is established along the chromosome arms and at the centromere and serves to keep homologous chromosomes and sister chromatids together until completion of meiosis I and II, respectively (Watanabe, 2005; Holt & Jones, 2009). Current evidence suggests that in the oocyte, cohesins load during S phase prior to recombination during fetal development and that little, if any, turnover occurs after this time (Revenkova *et al.*, 2010; Tachibana-Konwalski *et al.*, 2010). Thus, chromosome cohesion must remain functional for months in the mouse and years in the human to ensure faithful chromosome segregation. In several mouse strains, cohesion function is compromised in eggs from animals of advanced maternal age (Chiang *et al.*, 2010; Lister *et al.*, 2010; Chiang *et al.*, 2011; Merriman *et al.*, 2012). These eggs have reduced levels of chromosome-associated REC8, a meiotic-specific cohesin, resulting in increased inter-kinetochore distances (Chiang *et al.*, 2010; Lister *et al.*, 2010; Merriman *et al.*, 2012). These cohesion changes precede and predict the most commonly observed chromosome segregation errors (Chiang *et al.*, 2010). These findings are also consistent with functional gene deletion studies in which loss of the cohesin component, SMC1β, results in aneuploidy that is exacerbated with age (Revenkova *et al.*, 2004; Hodges *et al.*, 2005).

Although cohesin proteins are conserved between humans and mouse, it is not known whether a similar functional deterioration of these components occurs in the human with advanced reproductive age (Garcia-Cruz *et al.*, 2010). Such studies have been hampered by the difficulty in obtaining mature gametes from reproductively young and older women. Here, we collected a total of 166 oocyte-cumulus complexes (OCCs) from 18 subjects who had their ovaries removed for medical indications (Fig. 1A,B and Data S1, Supporting information). The number of OCCs collected per subject decreased with age, and only one OCC was collected from subjects 40 years or older (Fig. 1B). In addition, fewer OCCs were collected from subjects who had prior cancer therapy compared to untreated subjects of similar age (Fig. 1B). We performed *in vitro* maturation (IVM) using 112 OCCs from ten subjects and observed that 28.2 ± 5.8% of the oocytes reached MII (Fig. 1C and Data S1). Maternal age did not impact meiotic competence as 29.2 ± 7.5% of oocytes within OCCs from subjects under 30 years resumed meiosis and reached

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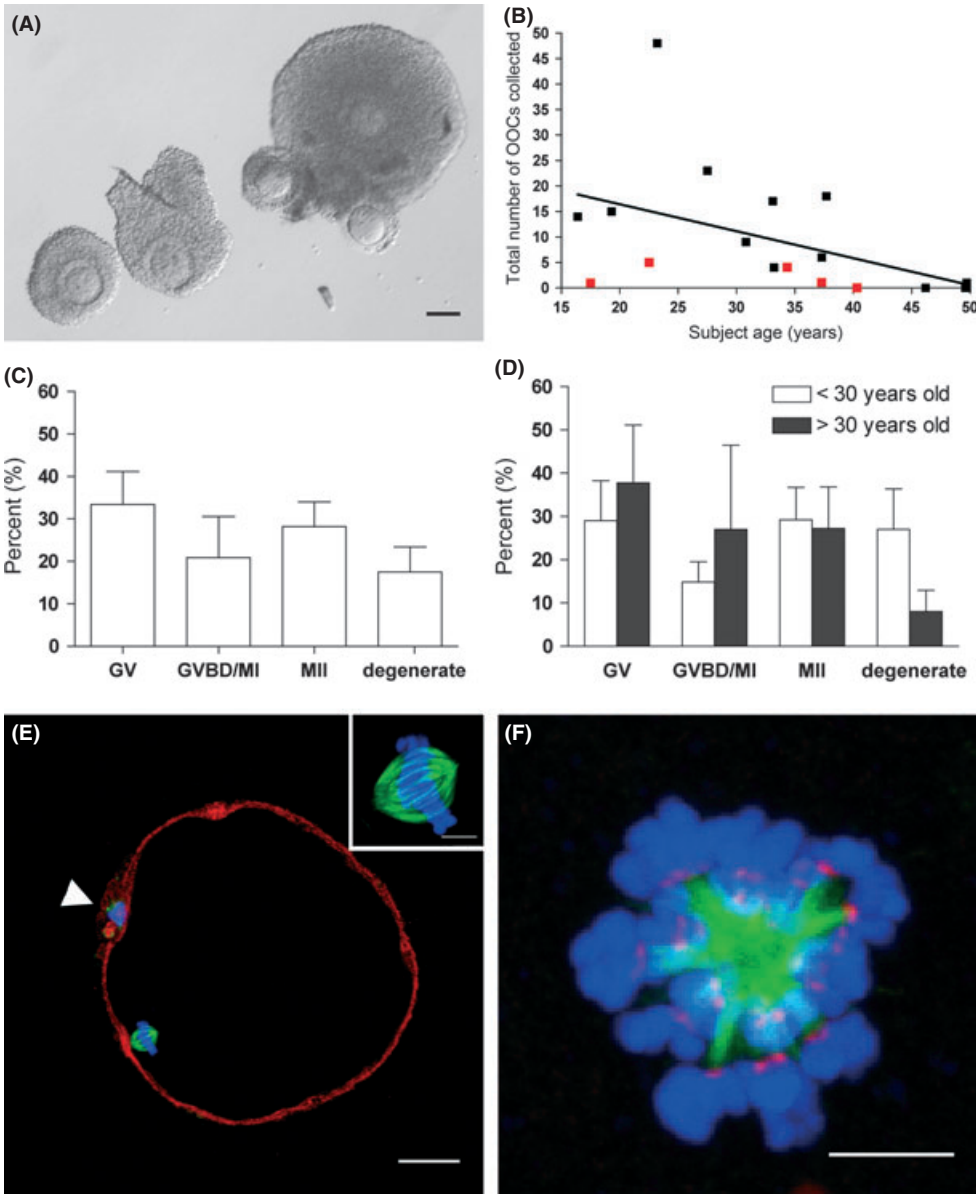


**Fig. 1** Human MII eggs can be obtained following *in vitro* maturation (IVM) of oocyte-cumulus complexes (OCCs) isolated directly from surgically removed ovarian tissue. (A) Morphology of OCCs from small antral follicles. Scale bar = 100 μm. (B) The effects of age and previous cancer therapy (red squares) on the number of OCCs collected per subject. (C, D) The percent of cells in each stage of meiosis following IVM for 36–42 h (GV, germinal vesicle-intact; GVBD/MI, germinal vesicle breakdown/metaphase of meiosis I; MII, metaphase of meiosis II) reported as an (C) overall total or (D) separated according to age (> or < 30 years old). There were no statistical differences between the groups. (E) The cytoskeleton in a human MII egg following IVM. (F-actin = red; α-tubulin = green, DNA = blue). The polar body is marked by an arrowhead, and the inset highlights the bipolar metaphase II spindle. Scale bar = 25 μm. (F) The human meiotic spindle following monastrol treatment (α-tubulin = green, DNA = blue, kinetochores = red). Scale bar = 5 μm.

MII compared to 27.2 ± 9.6% from subjects over 30 years (Fig. 1D; $P > 0.05$). The eggs derived from IVM had characteristic morphology with a small first polar body, a bipolar spindle asymmetrically positioned in the cortex, cortical actin microfilaments that were slightly enriched in the region adjacent to the spindle, and condensed chromosomes tightly aligned on the metaphase plate (Fig. 1E).

We used 18 of the eggs obtained through IVM from a subset of six subjects (ages 16.4, 19.3, 22.5, 27.5, 33.1, and 37.3 years) to assess how chromosome cohesion changes with maternal age. As a readout of chromosome cohesion, we measured the distance between kinetochores of sister chromatids, or the inter-kinetochore distance, in eggs using an *in situ* chromosome spreading technique (Figs 1F and 2A–F, and Data S1) (Duncan *et al.*, 2009). We found that the average inter-kinetochore distances increased gradually and significantly with subject age (Fig. 2A–F). The average inter-kinetochore distance increased from 0.82 ± 0.03 μm in the youngest subject (16.4 years) to 1.1 ± 0.03 μm in the oldest subject (37.3 years) (Fig. 2E; $P < 0.001$). In the human, this absolute increase of 0.28 μm in inter-kinetochore distance between age extremes is consistent with data in two mouse strains in which increases of 0.13 and 0.44 μm were reported (Chiang *et al.*, 2010; Merriman *et al.*, 2012).

In addition to measuring inter-kinetochore distances within individual eggs, we were also able to accurately count total kinetochores in

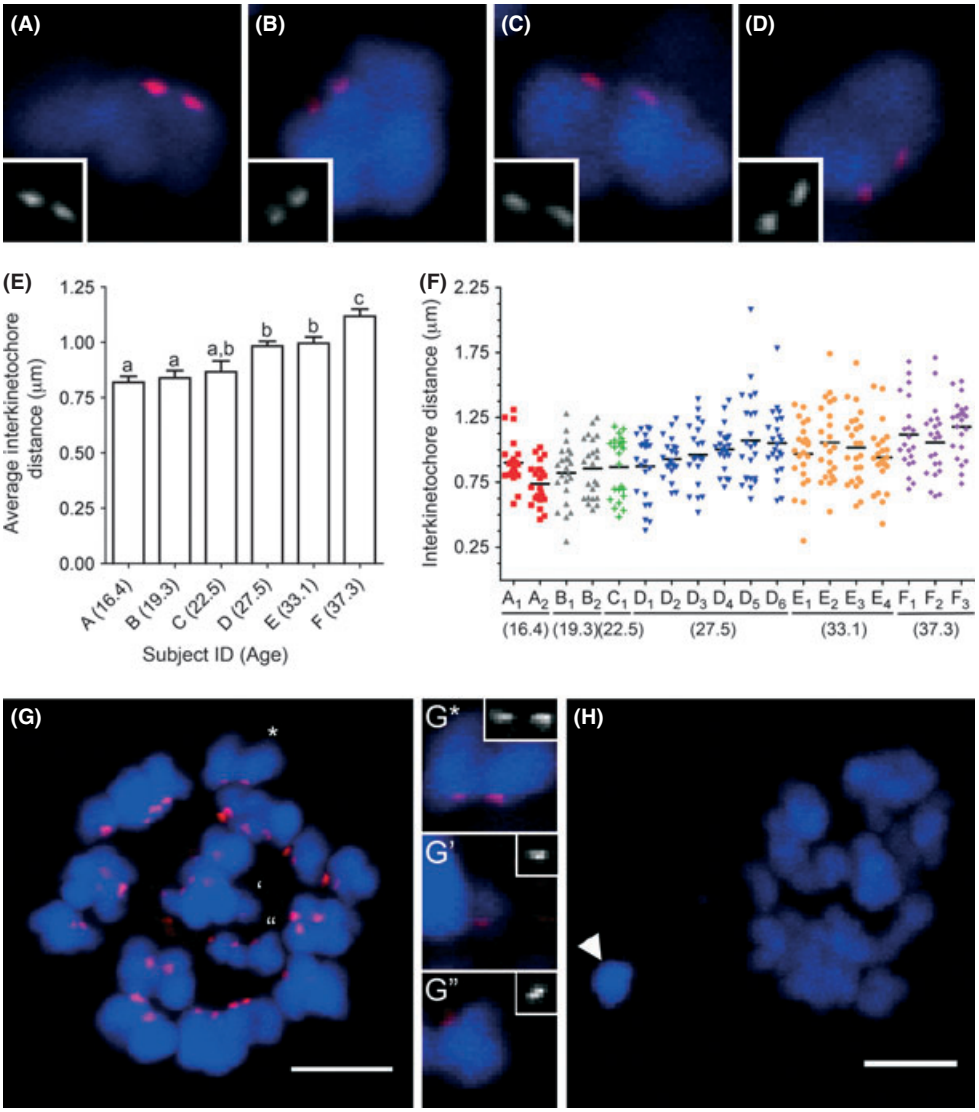


**Fig. 2** Inter-kinetochore distances increase significantly and chromosome segregation errors occur more frequently in human eggs with advanced age. Representative sister chromatids from (A, B) Subject D (27.5 years old) has on average smaller inter-kinetochore distances compared to those from (C, D) Subject F (37.3 years old). (E) The average inter-kinetochore distances of eggs from individual subjects are plotted with increasing maternal age. The uppercase letters correspond to the subject ID, and the subject age in years is indicated in parentheses. Different lowercase letters denote significant differences. (F) All the measured inter-kinetochore distances per individual egg are plotted with increasing maternal age. The uppercase letters, consistent with the labeling in (E), represent the subject ID, and the subscript number indicates an individual egg. Each color represents inter-kinetochore measurements from the same subject. The mean inter-kinetochore distance for each egg is indicated by a black line. Eggs $B_1$, $E_3$, $E_4$, $F_1$, and $F_2$ were determined to have chromosome segregation errors. (G) An egg from Subject F ($F_2$) with a pair of separated sister chromatids. The marked chromosomes are further magnified to show a representative image of an intact sister chromatid pair (G*) and the pair of separated sister chromatids (G′, G″) separated by four 0.2 μm-thick optical sections. (H) Another egg from Subject F ($F_1$) had a chromosome pair (arrowhead) that was separated from the egg DNA in both the *XY*-plane and *Z*-plane (0.8 μm). Kinetochores = red and DNA = blue. The scale bar = 5 μm.

> 85% of the eggs examined (16⁄18) to assess chromosome status. Chromosome segregation errors were observed in 31% (5⁄16) of the eggs following IVM, and four of these eggs were from the two subjects over the age of 30 (Subject E and F; Fig. 2E–H). Subject F, who was 37.3 years old, had two eggs each with a set of unpaired sister chromatids indicative of a total loss of cohesion ($F_1$ and $F_2$; Fig. 2F,G). In addition to a set of unpaired sister chromatids, egg $F_1$ also had an improperly segregated chromosome pair that was separated from the egg chromosomes and was not associated with the polar body DNA (Fig. 2H). This pair resided within the egg because, without including this pair, egg $F_1$ would be hypoploid. Subject E, who was 33.1 years old, instead had two hyperploid eggs ($E_3$ and $E_4$; Fig. 2F). One egg had an extra chromosome pair ($E_3$), whereas the other had an extra unpaired sister chromatid ($E_4$) (data not shown). Subject B, who was 19.3 years old, had one egg ($B_1$) that had an extra unpaired sister chromatid (Fig. 2F and data not shown). The five eggs with chromosome segregation errors were not restricted to those with the largest average inter-kinetochore distance ($B_1$, $E_3$, $E_4$, $F_1$, and $F_2$; Fig. 2F).

Taken together, these results demonstrate for the first time that in human eggs, there is a maternal-age-associated deterioration of chromosome cohesion as indicated by increased inter-kinetochore distances between sister chromatids. In the oldest two subjects, this increased inter-kinetochore distance was also accompanied by chromosome segregation errors. Interestingly, the majority of the observed segregation

errors involved the premature separation of sister chromatids, which is a clear manifestation of a complete cohesion defect. Although the majority of the observed aneuploidies occurred in subjects over the age of 30, there was one instance of aneuploidy in a reproductively young subject, suggesting that factors in addition to age contribute to aneuploidy. The eggs used for these studies were obtained from ovarian tissue, primarily from women with a cancer diagnosis, so future studies to investigate whether similar aging mechanisms are conserved in a noncancer population are warranted. Moreover, understanding whether and how cancer itself and cancer treatments affect chromosome dynamics during meiosis will be important for the field of fertility preservation where such eggs could be fertilized or cryopreserved for a patient's future use (Fasano *et al.*, 2011).

Deteriorating chromosome cohesion is likely a contributing factor in the age-associated meiotic origins of aneuploidy in the human. If chromosome cohesion in humans is only established during S phase (which happens one time in the development of an oocyte) and little turnover occurs after oogenesis as has been reported in model organisms, it is unlikely that supplementation of exogenous cohesin components will rescue the deterioration (Watanabe *et al.*, 2001; Revenkova *et al.*, 2010; Tachibana-Konwalski *et al.*, 2010). Instead, methods that prevent the deterioration of chromosome cohesion are more likely have a greater impact on reducing the incidence of aneuploidy. The findings reported here thus provide important new opportunities for intervention in age-related infertility and are consistent with the progressive aging of an established oocyte pool.

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## Author contributions

All authors contributed significantly to this work.

## Conflict of interest

The authors declare that they have no competing financial interests.

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## Supporting Information

Additional supporting information may be found in the online version of this article at the publisher's web-site:

**Data S1** Experimental procedures.