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Enhanced polarizing microscopy as a new tool in aneuploidy research in oocytes

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Abstract

Chromosomal non-disjunction in female meiosis gives rise to reduced fertility and trisomy in humans. Human oocytes, especially from aged women, appear especially susceptible to non-disjunction. The oocyte spindle is crucial for high fidelity of chromosome segregation at meiotic divisions, and alterations in spindle morphology are therefore indicators of adverse conditions during oocyte development that may result in meiotic aneuploidy. In the past, oocytes had to be fixed for spindle analysis, precluding direct non-invasive identification of aneugens and adverse maturation conditions that affect spindle integrity and chromosome behaviour. Aneuploidy research for detection of spindle aberrations was therefore mainly focused on *in vivo* or *in vitro* exposed, fixed animal oocytes or cytogenetic analysis of spread oocytes. Orientation independent enhanced polarizing microscopy with nearly circularly polarized light and electronically controlled liquid crystal compensator optics is a new tool to study spindle morphology non-invasively *in vivo* for qualitative as well as quantitative analysis. Image generation by polarization microscopy depends on the intrinsic optical properties of the spindle with its paracrystalline microtubule lattice. When polarized light passes through such a lattice it induces a splitting of the beam and shift in the plane of vibration and retardation of light (termed birefringence and retardance). Studies of animal oocytes and follicle-cell denuded human oocytes fertilized by intracytoplasmic sperm injection for assisted conception have demonstrated the safety and efficacy of enhanced polarization microscopy. The method can be employed in aneuploidy research for non-invasive dose–response studies to detect spindle aberrations, for instance, in combination with cytogenetic analysis. Due to the non-invasive nature of the technique it may be employed in routine analysis of human oocytes to assess risks by lifestyle factors, and occupational and adverse environmental exposures.

Keywords: Spindle; Oocyte; Maturation; Aneuploidy; Polarizing microscopy

1. Introduction

Aneuploidy resulting in inheritable human genetic disease, e.g. trisomy, originates predominantly from non-disjunction during oogenesis [1–3]. Errors in chromosome segregation at first meiosis are the major cause for implantation failure, congenital abnormality, spontaneous abortion and trisomy [4–9].

Studies in rodents, especially in mouse oocytes, demonstrated that the risk for non-disjunction and aneuploidy is increased by exposures interfering with the dynamic polymerization kinetics of microtubules in spindle formation (e.g. [10–18]). In humans there is ample evidence that maternal age is the major aetiological factor associated with chromosome non-disjunction at oocyte meiosis, possibly as a result of spindle aberrations [1,4,9,19–21]. Still, it is unknown whether and to what extent adverse exposures, lifestyle, hormonal imbalance and genetic background may contribute to the genesis of aneuploidy in human oocytes [3,7,17,21]. Most errors in chromosome segregation at oogenesis occur at meiosis I. Studies in mice and humans showed that postovulatory ageing may also increase the risks for meiotic errors at anaphase II. Ageing may induce precocious separation of chromatids (predivision) [22]. The latter and the reduction in expression of genes in control of the spindle checkpoint [23], as well as spindle degeneration [24,25], may predispose to errors at second meiosis. Chromatids may not correctly orient and assemble at the spindle equator (termed congression failure). It is unknown whether and to what extent pre- and

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postovulatory ageing sensitizes oocytes to exposures to aneugens.

Disturbances in chromosome attachment and tension on kinetochores/centromeres of chromosomes are sensed by the spindle assembly/attachment checkpoint (SAC). This may lead to meiotic arrest, thus protecting from induction of heritable chromosomal aberrations (for discussion, see: [26]). A reduction in mRNA coding for genes in the spindle checkpoint, relaxed cell cycle control and reduced cohesion between sister chromatids may contribute to the high susceptibility of aged human oocytes to meiotic error [27–29]. As a consequence, this may increase the sensitivity to mutagens (e.g. [30–34]). It is difficult to assess the possibly synergistic effects of age and exposures in animal studies since the maternal age-effect is much less dramatic in other mammals compared to humans. Due to ethical and legal considerations only very limited information has therefore been obtained about changes in spindle morphology in human oocytes in response to age or adverse exposures. In assisted reproduction it is paramount that oocyte maturation conditions or handling do not induce disturbances (for references see: [7,35]). Furthermore, it has been difficult to assess the consequences of changes in spindle dynamics and morphology inducing non-disjunction in human oocytes in real-time in a non-invasive manner.

2. Induction of spindle aberrations by aneugens in animal oocytes

The mechanisms leading to aneuploidy in mammalian oogenesis can involve non-disjunction, anaphase lagging and/or precocious separation of chromatids, presumably with different thresholds as in mitosis [36–42]. Experimental data are mainly obtained from *in vivo* or *in vitro* maturing mouse oocytes. They suggest that formation of asymmetric and aberrant, or mono- and multipolar spindles [37,43–50], disturbances in tubulin turnover or activity of motor proteins [51], and ablation of surveillance mechanisms sensing chromosome attachment and spindle integrity [29,52,53] occur frequently in association with aneuploidy in oocytes and preimplantation embryos. From genetic models it is obvious that disturbances in presence, localisation and numbers of chiasmata [3,54,55] may influence spindle function and susceptibility to meiotic errors during oogenesis. In addition, disturbances in the regulation of chromatin and epigenetic modifications [56,57] or expression and function of microtubule motor- and regulatory proteins [58–60] are critical. Frequently, chromosomes fail to align properly at the spindle equator (congression failure) when oocytes are maturing in the presence of aneugens [43,44]. This often involves alterations in spindle organization. Alterations in the alignment of microtubules and in oocyte spindle organization are thus hallmarks of an exposure to aneugens and increase the risks for chromosomal non-disjunction and aneuploidy [16,17,43,61–64]. In the past, studies on correlations between aberrations in spindle morphology and chromosome behaviour causing aneuploidy were mainly obtained from fixed animal oocytes, for instance, those of the mouse [37,43,61–64].

Human oocytes studied for spindle abnormalities [25,65–67] and chromosomal constitution (e.g. [20,68]) were predominantly unfertilized ones. They mainly came from stimulated cycles of assisted reproduction. Most represented the presumably low quality oocytes, left after selection of the morphologically best ones for *in vitro* fertilization (IVF) or those failing to become activated. Accordingly, many of the fixed oocytes had aged in culture media for 1 day or longer before they were fixed. Due to the invasive methodology in conventional electron microscopy or tubulin immunofluorescence, it has been impossible or difficult to detect direct correlations between spindle aberrations, aneugen exposures, and abnormal chromosomal constitution and reduced developmental potential of human oocytes [68].

3. Enhanced polarizing microscopy to view spindles in oocytes

Polarization microscopy is useful to analyse the organization of high order structures in non-biological and biological specimens. For instance, macromolecules in membranes, microtubule or microfilament bundles and other molecularly ordered components of cells might be visualized. Paracrystalline or crystalline structures, including cell organelles with such high order composition of components, cause splitting of the beam, alter the plane of vibration and retard the polarized light, a property termed birefringence [69,70]. Only those parts of the unpolarized light vibrating in one plane will pass through linearly polarizing filters. Accordingly, light passing through perpendicularly oriented polarizer and analyser lenses will not pass through the optical axis and the field of view is dark. A birefringent structure becomes visible when it is placed in the light path, and the compensator optics are rotated such that the polarized light changed in its plane of vibration by the specimen can pass through. Using this principle, polarized light microscopy has been employed to effectively study the submicroscopic molecular order in living cells for several decades [71,72]. Inoué was the first to observe the filamentous structure of the spindle apparatus in animal oocytes with polarizing optics [73]. His group demonstrated that microtubules within the spindle were at the basis of the birefringent properties of this cell organelle [74].

Conventional polarized light microscopy is orientation-dependent. The specimen can only be viewed in one specific orientation relative to the polarization axes of the microscope using the compensator optics. When a light beam enters a birefringent body, it is split into two beams whose vibration planes are perpendicular to each other. This is due to a difference in the refractive index of the birefringent body for the two orthogonally polarized light beams [69]. Polarization microscopy can be used to measure the relative change in phase between the two polarized beams, termed retardance, to quantify the birefringent property of the sample. Conventional polarization microscopy using linearly polarized light has only a low sensitivity to detect changes in retardance in the order of 5–10 nm [70]. Biological materials have comparatively little birefringence, in the range of only a few nanometres [75–79], unlike mineralogical and metallographic specimens. Orientation-independent polarizing microscopy (PolScope) has been a breakthrough to view