Morphological and molecular markers are correlated with maturation-competence of human oocytes

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STUDY QUESTION: Does the position of the germinal vesicle (GV) in human oocytes correlate with molecular and morphological parameters as well as with maturation-competence?

SUMMARY ANSWER: The position of GV in human oocytes correlates with density of microtubule (MT) filaments, concentration of Fyn, nucleolus localization and the ability of the oocytes to complete maturation following GV breakdown (GVBD).

WHAT IS KNOWN ALREADY: Our knowledge is confined to oocytes of young mice where maturation-competence is correlated with a central GV and regulated by MTs and the presence of a chromatin ring. Fyn kinase is localized at the spindle and cortex of mouse oocytes and plays a role in both maturation and MT stabilization.

STUDY DESIGN, SIZE, DURATION: Spatial localization of the GV and nucleolus (central or peripheral), the presence of a chromatin ring, the localization of Fyn, MT density and oocyte maturation were assessed in 153 human oocytes, 335 oocytes from young mice (2-month-old) and 146 oocytes from old mice (12-month-old).

PARTICIPANTS/MATERIALS, SETTING, METHODS: GV human oocytes were donated by consenting female patients (n = 57), 21–45-year-old undergoing IVF/ICSI. As a control, GV mouse oocytes were collected from female mice after injection of pregnant mares’ serum gonadotrophin. Human and mouse GV oocytes allocated for immunocytochemistry were fixed on day of retrieval, stained with specific antibodies and imaged using a confocal laser-scanning microscope. Human and mouse oocytes allocated for maturation were incubated for 48 and 24 h, respectively. GVBD and extrusion of the first polar body (PBI) were assessed using differential interference contrast optics.

MAIN RESULTS AND THE ROLE OF CHANCE: GV location was peripheral and independent of age in 69.9% of the human oocytes, but GV location did vary with age in mice oocytes; it was central in 89.9% of the oocytes retrieved from young mice and peripheral in 52.1% of the oocytes retrieved from old mice (P < 0.05). A central GV, whether in human or mouse oocytes, was highly correlated with a central nucleolus, absence of Fyn at the GV and a dense MT network (P < 0.05), whereas a peripheral GV correlated with peripheral nucleolus, presence of Fyn at the GV and a flimsy MT network. After 48 h in culture, no degeneration was observed in human central-GV oocytes, however, 12/95 (12.6%) of the peripheral-GV oocytes degenerated (P < 0.05). No correlation was observed between GV position and the presence of a chromatin ring. The percentage of human oocytes that extruded the PBI after completing GVBD was significantly higher (73.7%) in central than in peripheral-GV oocytes (45.8%; P < 0.05). In mice oocytes, central location of the GV correlated with maturation competence in young (P < 0.05) but not old mice.

LIMITATIONS, REASONS FOR CAUTION: The fact that the human GV oocytes used in this study were exposed to gonadotrophic stimulation but failed to mature in vivo might be a sign of their low quality and this should be considered when drawing conclusions from the data. Furthermore, our observation that only peripheral-GV human oocytes were degraded may indicate that they are of a lower quality than central-GV human oocytes.

WIDER IMPLICATIONS OF THE FINDINGS: We suggest that the central location of GV within the oocytes, which is associated with an absence of Fyn at the GV and the presence of thick filamentous MTs in the ooplasm, may serve as a predictor of successful maturation and provide new insights for the use of IVM.

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Key words: maturation / germinal vesicle / Fyn kinase / microtubules / oocyte competence
Introduction

Oocytes remain arrested at the prophase of the first meiotic division [termed germinal vesicle (GV) oocytes] from embryonic life (Wassarman and Albertini, 1988). Maturation of oocytes within selected follicles, induced by a surge of LH, is manifested by chromosomes condensation, GV breakdown (GVBD), formation of the spindle at a filamentous actin (F-actin) rich area, segregation of homologous chromosomes, extrusion of the first polar body (PBI), formation of the spindle and an arrest at the metaphase of the second meiotic division (MI; Maro and Verlhac, 2002).

Since fertilization, the oocytes exit from the MI-arrest and extrude the second polar body (Levi et al., 2010a,b) that indicates completion of the asymmetric meiotic divisions. The polarity of the oocytes and the asymmetric meiotic divisions play a role in maintaining organelles, proteins and RNAs within the ooplasm and are important for normal embryonic development.

The mechanisms of asymmetric division in mouse and human oocytes are different. The post-GVBD spindle in mouse oocytes forms at the center and migrates along its long-axis toward the nearest region of the oocyte cortex (Verlhac et al., 2000). The GV within human oocytes was reported to be located peripherally in the majority of oocytes, which allows for a shorter radial migration of the metaphase I spindle (reviewed by Albertini and Barrett, 2004; Brunet and Verlhac, 2011). Brunet and Maro (2007) have shown that competent mouse oocytes exhibit mainly central GVs and suggested that the position of the GV within young mouse oocytes can be used as a morphological marker for predicting their maturational competence. Moreover, they showed that old mice oocytes exhibit fewer central GVs and a reduced ability to progress through meiosis. Bellone et al. (2009) demonstrated that the position of the GV is an adequate marker for predicting oocyte developmental competence in mice but only when associated with the presence of a chromatin ring at the nucleolus periphery.

Other studies showed that GV oocytes lacking the chromat ring remain transcriptionally active and synthesize all classes of RNA, whereas oocytes with a chromat ring are transcriptionally inactive (Debey et al., 1993; Bouniol-Baly et al., 1999) and have higher levels of DNA methylation, as well as histone acetylation and methylation (Kageyama et al., 2007). The chromat ring in mouse oocytes is formed 1 h before GVBD (Schuh and Ellenberg, 2007) at a time when the ooplasmic and GV microtubule (MT) filaments become shorter (Maro et al., 1990; Mattson and Albertini, 1990; Schuh and Ellenberg, 2007). Observations in human and mice oocytes suggest that MT organizing complexes (MTOCs) congregate at the center of the oocyte, where they contribute to a massive increase in the number of MTs after GVBD and form a barrel-shaped acentrosomal metaphase spindle (Kim et al., 1998; Schuh and Ellenberg, 2007). Several lines of evidence indicate that Fyn, a Src family kinase localized at the spindle and oocyte cortex, participates in both GVBD (Zheng et al., 2007; Levi et al., 2010a) and the stabilization of MTs (Talmor-Cohen et al., 2004; Levi and Shalgi, 2010). Stein et al. (1994) and McGinnis et al. (2009) reported decreased fertility in Fyn-null mice.

In vitro maturation (IVM) can serve as an alternative to the conventional IVF treatment for women suffering from polycystic ovary syndrome, ovarian hyperstimulation syndrome or repeated IVF failures, as well as in cases of oocyte banking or donation and fertility preservation in young patients. Yet, despite the significantly improved rate of pregnancies achieved from fertilized IVM human oocytes, IVM treatment is still only partially effective, possibly because meiosis in human oocytes is highly error-prone (Brockmann et al., 2011). Genomes of both the oocyte and the spermatozoon participate equally in creating the embryonic genome. Since the ooplasm becomes the developing embryo’s cytoplasm, while the spermatozoon’s cytoplasmic contribution is minimal, the quality of the oocyte is a key factor that determines the quality of the developing early embryo (see review Martel et al., 2009). Furthermore, no transcription occurs at the early stages of embryo development, so the very first steps of embryogenesis are controlled by maternal information stored in the oocyte. Therefore, there is a clinical necessity for selective markers of oocyte quality and maturation competence. The aim of the current study was to examine the feasibility of several morphological and molecular parameters to indicate the competence of human oocytes to undergo maturation and compare these human data with the extensively studied mouse oocytes.

Materials and Methods

In this study, we examined the following morphological and molecular parameters that could indicate maturation competence: GV spatial position, nucleolus position, chromat organization, MT filament density and Fyn expression and localization.

Human oocytes

GV oocytes included were donated by consenting female patients, 21–45 years of age undergoing IVF/ICSI procedures and induced to ovulate according to the protocol described previously (Shulman et al., 1996). These GV oocytes constitute biological material that would have otherwise been discarded. This study was approved by the national Helsinki committee and informed consent obtained from all participants. GV oocytes were exposed to 80 U/ml hyaluronidase (HSA; Cooper-Surgical, Trumbull, CT, USA) in hydroxyethyl piperazinethanesulfonic buffer in human tubal fluid medium supplemented with 5 mg/ml human serum albumin (Cooper-Surgical), and cultured in human data with the extensively studied mouse oocytes.

Mouse oocytes

C57-black female mice (2-month-old, referred to as young mice, or 12-month-old, referred to as old mice; Harlan Laboratories, Jerusalem, Israel) were housed in air conditioned, light controlled animal facilities of the Sackler Faculty of Medicine, Tel Aviv University. Institutional and national guidelines for the care and use of laboratory animals were followed. Female mice were stimulated to superovulate by an s.c. injection of 5 IU pregnant mares’ serum gonadotropin (Syncro-part, Sanofi, Paris, France), sacrificed immediately after oocyte denudation in order to avoid any effect the medium might have on GV position. Some of the GV oocytes were allocated for immunocytochemistry and were fixed on retrieval day (Supplementary Table S1), while others were allocated for maturation assay and were randomly placed in 25 µl drops of culture media that are routinely used in our laboratory: Preimplantation Stage One Medium (P1; Irvine Scientific, Santa Ana, CA, USA) and/or Quinn’s Advantage Fertilization Medium (Cooper-Surgical) under mineral oil in Falcon cell culture dishes (#3004; BD, Franklin Lakes, NJ, USA). According to Jurema and Nogueira (2006) and Yan et al. (2011), the oocytes were cultured for 48 h at 37°C with 5.5% CO2 in air, after which their developmental status was assessed using differential interference contrast (DIC) optics. After 48 h of culture, oocytes were classified as arrested at the germinal vesicle stage (GVA), arrested after GVBD or completed their maturation with an extruded PBI.
48 h later and the ovaries removed into pre-warmed (37°C) M2 medium (M-7167; Sigma Chemical Co., St. Louis, MO, USA) supplemented with 4 mg/ml bovine serum albumin (Sigma). Complexes of cumulus and GV oocytes arrested at prophase I were released from the follicles into M2 medium. Oocytes were denuded mechanically. As in human oocytes, the position of the GV in mouse oocytes was assessed immediately after oocyte denudation. These oocytes were also randomly allocated for either immunocytochemistry (Supplementary Table SI) or cultured for further development. Oocytes were cultured for 24 h in 25 μl drops of M2 culture medium (Sigma) under mineral oil (Sigma) in Falcon cell culture dishes (#3004; BD) and at 37°C in air. The use of M2 culture medium in air (CO₂ was not added to air and thus is around 0.03%) is routinely used in our laboratory and was shown by the authors (Levi et al., 2010a) and others (Brunet et al., 2003; Brunet and Maro, 2007) to be a highly successful maturation system for mouse oocytes. At the end of the culture period oocyte developmental status was assessed by DIC optics and they were characterized as GVA, GVBD or PBI oocytes.

**GV and nucleolus position**

We examined the location of the GV in live, freshly retrieved and denuded oocytes by DIC optics of Zeiss LSM-510 microscope (Carl Zeiss Micro Imaging, Oberkochen, Germany; 40X C-apochromat water-immersion lens; NA-1.2). The GV was defined as ‘central’ when the distance from the center of the oocyte to the center of its GV was less than half the difference in the lengths of the oocyte and GV radii (Fig. 1Aa). The GV was defined as ‘peripheral’ when this distance was more than half the radii difference (Fig. 1Ab). The nucleolus was defined as ‘central’ when the distance from the center of the GV to the center of the nucleolus was less than half of the difference in lengths of the GV and nucleolus radii. Nucleolus was defined as ‘peripheral’ when this distance was more than half of the difference in lengths of the GV and nucleolus radii.

**Immunocytochemistry and confocal laser-scanning microscopy of human and mice oocytes**

GV mouse oocytes were fixed for 10 min at room temperature in a solution of 3% paraformaldehyde and 0.01% glutaraldehyde (Polysciences, Warrington, PA, USA), washed in 3% fetal calf serum (FCS; Biological Industries, Beit-Haemek, Israel) in Dulbecco’s phosphate-buffered saline (Biological Industries; DPBS/FCS) and zona pellucidae were removed by a brief exposure to a solution of 0.25% pronase (Sigma) in DPBS/FCS prior to an additional wash in DPBS/FCS. GV human oocytes were first exposed to 0.25% pronase, washed in medium and fixed as indicated above. Oocyte plasma membranes were permeabilized for 10 min in a solution of 0.05% Nonidet P (NP)-40 in DPBS/FCS and washed in 0.005% NP-40 in DPBS/FCS. All further treatments were performed in DPBS/FCS. Oocytes were immunostained (90 min at room temperature) with either rabbit anti-Fyn (1:100; b-tubulin (1:5000; T8328; Sigma) primary antibodies, prior to a 45 min incubation at room temperature with AlexaFlour488-conjugated goat anti-rabbit (1:400; A11034; Invitrogen—Molecular Probes, Carlsbad, CA, USA) or Cy-3-conjugated donkey anti-mouse (1:400; 715-165-151; Jackson Immunoresearch Laboratories, West Grove, PA, USA) secondary antibodies, respectively, mixed with Hoechst 33342 (1 μg/ml; Sigma).

Immunostained oocytes were visualized and photographed using a Zeiss-LSM-510 confocal microscope equipped with Diode (405 nm excitation wavelength line; output 30 mW, imaging chromosomes in blue), Argon (488 nm excitation wavelength line; output 30 mW; imaging Fyn in green) and Diode-pumped solid-state (561 excitation wavelength nm line; output: 15 mW; imaging MTs in red) lasers. Three-dimensional images...
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\((x, y, z)\) were created by the movement of the objective along the z-axis \((\Delta z = 7 \, \mu\text{m})\) over a total depth of 70 \, \mu\text{m}, and processed and analyzed using the Velocity 5 software (Perkin Elmer, Courtaboeuf Cedex, France).

To reduce auto-fluorescence and immunofluorescence background, GV oocytes that served as a negative control were incubated with secondary antibodies only \((\text{Cy}-3\)-conjugated donkey anti-mouse (Supplementary data, Fig. S1A) or Alexa Flour \(488\)-conjugated goat anti-rabbit (Supplementary data, bodies only \((\text{Cy}-3\)-conjugated donkey anti-mouse (Supplementary data, Fig. S1B)) and were used to calibrate the offset in the Zeiss-LSM-510 confocal microscope imaging (Supplementary data, Fig. S1C).

The thickness of filamentous MTs was measured as the ratio between the average pixel intensities at the ooplasm and at the GV, as measured through the 561 nm channel. Fyn concentration at the GV was measured as the ratio between the average pixel intensities at the GV and at the ooplasm, as measured through the 488 nm channel of the Zeiss LSM-510 microscope.

Statistical analysis

Data were evaluated by Pearson’s \(x^2\) test for independence, or by independent, two-sample t-test for unequal sample sizes and unequal variances (Microsoft Excel 2007, Microsoft Israel, Raanana, Israel). \(P < 0.05\) was considered significant. A one way analysis of variance statistical analysis showed similar results. For an association test between categorical and continuous variables, we used the Two-tailed Point Biserial Correlation coefficient (VasarStats, Poughkeepsie, NY, USA).

Results

In the current study, we assessed the correlation between GV location, Fyn localization and the MT network and GVBD and PBI extrusion in human oocytes and in mouse oocytes serving as a control model of mammalian oocytes.

Human oocytes

One hundred and fifty-three oocytes were retrieved from 57 women participating in the study. Oocytes can be divided into three groups according to GV location: I. Central-GV oocytes retrieved from eight women with an average age of 28.2 ± 1.52 years and an average of 2.1 retrieved immature oocytes; II. Peripheral-GV oocytes retrieved from 35 women with an average age of 30.2 ± 0.20 years and an average of 1.8 retrieved immature oocytes \((P > 0.05\) for both parameters compared with group I); III. Oocytes with both central-GV and peripheral-GV were retrieved from 14 women with an average age of 35.8 ± 1.00 years \((P < 0.05\) compared with groups I and II) and an average of 3.7 retrieved immature oocytes.

The human cumulus–oocyte complexes were aspirated from large antral follicles \((\text{diameter} \geq 12 \, \text{mm})\) 36 h after hCG administration in a routine IVF procedure. The diameter of central-GV oocytes \((101.6 \pm 1.6 \, \mu\text{m})\) was similar to that of peripheral-GV oocytes \((99.9 \pm 1.2 \, \mu\text{m}; P > 0.05\). Age also had no bearing on oocyte diameter, which was similar in oocytes from young women \((34\)-year-old; 101.1 ± 1.7 \, \mu\text{m}) and old women \((34\)-year-old; 98.3 ± 1.2 \, \mu\text{m}).

Thirty percent of human oocytes presented a central GV at the time of retrieval \((\text{Fig. Ia and B}).\) GV location in human oocytes was not dependent upon age: oocytes with central GV were retrieved from women with a mean age of 32.2 ± 0.9, whereas those with peripheral GV from women with a mean age of 32.4 ± 0.8 \((\text{Fig. Ic}; \text{Two-tailed Point Biserial Correlation coefficient} = 0.02; P = 0.849630).\) Central-GV oocytes exhibited denser filamentous MTs in the ooplasm \((\text{Fig. Ia and B}; \text{Supplementary data S1})\) than peripheral-GV oocytes \((\text{Fig. IAb and B}; \text{Supplementary Data S1}; P < 0.05).\) Central-GV oocytes exhibited a lower concentration of Fyn in the GV \((\text{Fig. 2Ca and D}; \text{Supplementary data S1})\) than peripheral-GV oocytes \((\text{Fig. 2Cb and D}; \text{Supplementary data S2}; P < 0.05).\) A significant difference was observed in the degeneration rate between the two groups. After 48 h in culture, no degeneration was observed in central-GV oocytes, however, 12/95 \((12.6\%; P < 0.05)\) of the peripheral-GV oocytes degenerated. Central nucleoli were observed in 87.5% of central-GV oocytes but only in 21.4% of peripheral-GV oocytes \((\text{Fig. 3A and B}; P < 0.05).\) No correlation between the presence of a chromatin ring within the GV and spatial location of the GV within the oocyte was observed \((\text{Fig. 3C}).\)

The rate of GVBD was similar in central- \((19/37; 51.4\%); \text{Fig. 4}) and peripheral-GV oocytes \((59/95; 62.1\%).\) However, once GVBD occurred and the maturation process started, a significantly higher percentage of central-GV oocytes completed this process and extruded PBI \((14/19; 73.7\%)\) compared with peripheral-GV oocytes \((27/59; 45.8\%; P < 0.05).\)

Mouse oocytes

Our observations show that unlike human oocytes, GV location is age-dependent in mouse oocytes. The proportion of central GV was higher in oocytes of young (2-month-old) mice than old (12-month-old) mice \((\text{Fig. 1B}; P < 0.05).\) Similar to our findings in human oocytes, central-GV mouse oocytes exhibited denser filamentous MTs at the ooplasm \((\text{Fig. 2B})\) and a lower concentration of Fyn in the GV \((\text{Fig. 2D})\) than peripheral-GV mouse oocytes \((\text{Fig. 2B and D}; \text{respectively}).\) A central GV correlated with a central nucleolus in oocytes of young but not old mice \((\text{Fig. 3B}; P < 0.05); no correlation was observed between the presence of a chromatin ring within the GV and its spatial location within the oocyte, regardless of the mouse age \((\text{Fig. 3C}).\) We also observed high frequency of central GV in cumulus-enclosed oocytes and in oocytes of young, non-stimulated female mice \((\text{over 90%}; \text{data not shown}).\) The incidence of GVBD and PBI extrusion was higher in central-GV oocytes originating from young mice than in peripheral-GV oocytes \((\text{Fig. 4}; \text{both} P < 0.05).\) The rate of maturation in central versus peripheral GV oocytes was similar in stimulated or non-stimulated mice \((\text{data not shown}).\) The spatial location of the GV within oocytes retrieved from old mice had no significant bearing on maturation rate \((\text{Fig. 4}).\)

Discussion

A prerequisite for the development of an embryo is the penetration of a spermatozoon into a mature MII oocyte. It is common knowledge that not all oocytes collected during IVF treatments are mature. Furthermore, retrieval of immature oocytes followed by IVM is an alternative to the conventional IVF treatment \((\text{Chian et al., 2004}).\)

The purpose of the current study was to assess the correlation between the GV location and morphological and molecular parameters of human oocytes, in an attempt to use them as predictors of oocyte maturation competence. The location of GV in human oocytes, as demonstrated in this study, is independent of age and a peripheral location is the most common. The present study adds an important clinical observation, indicating that once GVBD occurs in central-GV human oocytes and the maturation process starts, the percentage of oocytes that will complete this process and extrude PBI is significantly higher than in peripheral-GV oocytes.
Data indicate that the developmental competence of the in vitro matured oocytes must be compromised by some inherent but unidentified problem in the maturation process in vitro rather than being directly related to reduced follicle size, at least for follicles ≥ 6 mm in diameter (see review by Trounson et al., 2001). To minimize the effect of follicle size variable on maturation, the human cumulus–oocyte complexes in our study were aspirated from large antral follicles (diameter ≥ 12 mm).

Being an adequate model for studying the fundamental biological processes that control oocyte maturation (Brockmann et al., 2011), we used mouse oocytes as the reference species. Our mouse model results, in accordance with those of Brunet and Maro (2007), show that the central location of the GV can be used as a morphological marker for predicting maturation success in oocytes of young, but not old, mice.

Most classical morphological studies performed on intact ovaries document a relatively central GV in mammalian oocytes confined within quiescent primordial follicles (Hertig and Adams, 1967). MTs are known as the main contributors to the central localization of the GV. We observed that the filamentous MTs in human oocytes are denser in the ooplasm of central-GV oocytes than in the ooplasm of peripheral-GV oocytes, so that the existence of an insufficient or flimsy MT network may cause a change in the central location of the GV. GV location in maturing mouse oocytes depends on opposite forces formed by the two main types of cytoskeleton: a centripetal force created by MTs and a centrifugal force created by F-actin, as demonstrated using drugs (Alexandre et al., 1989). It is likely that the major reason for a decrease in female fertility is the decrease in oocyte quality along with the increase in maternal age (Carnevale, 2008; Jones, 2008). The reason for the age-related peripheral translocation of GVs within mouse oocytes is not known; however, two possible factors that affect the oocyte MTs and polarity are Myosin 10 and RAs-
related nuclear protein, which are down-regulated with increasing maternal age (Hamatani et al., 2004; Pan et al., 2008).

MTOCs regulate MT arrangement in mammalian oocytes (Brunet and Maro, 2005). Several MTOCs are present in GV mouse oocytes (Schuh and Ellenberg, 2007) but none are observed in human oocytes (Battaglia et al., 1996). This difference may account for the different behavior of the MT forces and hence the different location of the GV in oocytes in the mouse and human.

We demonstrated for the first time the localization of Fyn in human oocytes and compared it and the density of MTs in central- and peripheral-GV oocytes. We showed that central-GV human oocytes have lower concentrations of Fyn inside the GV than peripheral-GV oocytes. Several lines of evidence in mice oocytes indicate that Fyn takes part in GVBD (Zheng et al., 2007; Levi et al., 2010a), stabilization of MTs (Campbell et al., 1998; Talmor-Cohen et al., 2004; Nishida et al., 2005; Meng et al., 2006; Sulimenko et al., 2006; Levi and Shalgi, 2010), organization of F-actin (Thomas et al., 1995; Ng et al., 2005; Levi et al., 2011b) and degradation of cyclin-B1 (Tomashov-Matar et al., 2007). Holt et al. (2010) found that cyclin-B1 concentration rises within the GV and induces GVBD in mouse oocyte, while Heil-Chapdelaine and Otto (1996) showed that F-actin translocates to the GV shortly before GVBD in starfish oocyte. Furthermore, several studies demonstrated that Fyn is localized at the pronucleus of the mouse zygote and is necessary for nuclear envelope breakdown (NEBD; Meng et al., 2006; McGinnis et al., 2007; Levi et al., 2011a, 2012). Taken together, these findings suggest that Fyn takes part in nuclear processes in general, and in signal transduction pathways leading to both meiotic GVBD and mitotic NEBD, possibly by affecting other key factors, such as MTs, F-actin or cyclin-B1. However, the mechanism by which Fyn is involved in these processes is yet to be determined. It is possible that the molecular signal for Fyn entry to the GV was achieved in peripheral-GV oocytes, and not in central-GV oocytes; although Fyn entered the GV, Fyn-dependent maturation processes, such as GVBD, were not accomplished due to the activity of other checkpoint factors; implying oocytes of a lower quality, also suggested by their higher degradation rate.

Figure 3 Nucleolus location and chromatin organization within the GV. Oocytes were collected and denuded as indicated in legend of Fig. 1. DNA was stained with Hoechst, the spatial position of the nucleolus within the GV and the presence of a chromatin ring were assessed by the merge image of the DIC optic and 405 nm channels of Zeiss-510 laser-scanning microscope. The nucleolus was defined as central when the distance from the center of the GV to the center of its nucleolus was less than half of the difference in lengths of the GV and nucleolus radii. (A) Three representative types of oocyte: central nucleolus with a chromatin ring (a), peripheral nucleolus with a chromatin ring (b) and nucleolus without a chromatin ring (c). (B) Percentage of central nucleoli in oocytes with central or peripheral GVs, retrieved from mice and from women. Numbers in bars indicate number of oocytes scored. *Significantly different between oocytes with peripheral GV and oocytes with central GV (P < 0.05 in Pearson’s χ² test for independence). (C) Percentage of GV chromatin ring in oocytes with central or peripheral GVs retrieved from mice and from women. Numbers in bars indicate number of oocytes scored. No significant differences in the percentage of chromatin ring in oocytes with peripheral or central GVs were found between any of the groups in Pearson’s χ² test for independence.
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Authors’ roles

M.L. and Y.G. participated in study design, execution, analysis, manuscript drafting and critical discussion. A.S. and R.S. participated in study design, manuscript drafting and critical discussion.

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Conflict of interest

We declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

References


