Morphologic indicators predict the stage of chromatin condensation of human germinal vesicle oocytes recovered from stimulated cycles

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Objective: To assess germinal vesicles (GV) recovered from stimulated cycles by means of morphometric and morphologic examination (using contrast-phase and image analysis) and chromatin configuration (using fluorescent DNA imaging), and to evaluate the relevance of morphometric and morphologic parameters as forecasters of chromatin status.
Design: Experimental study.
Setting: University-affiliated infertility clinic.
Patient(s): One hundred and thirty-one GV oocytes donated to patients for intracytoplasmic sperm injection.
Intervention(s): We evaluated 131 GVs by means of morphology and morphometry with the use of contrast phase microscopy. They were subsequently fixed, DNA stained, and assessed by fluorescent microscopy. Compiled data were retrospectively grouped according to three models.
Main Outcome Measure(s): Model A: ova were grouped according to chromatin condensation (noncondensed vs. condensed). Model B: ova were grouped according to chromatin distribution in relation to the nucleolus-like body (NLB) (not surrounding vs. surrounding and/or absent) but regardless of the condensation stage. Model C: GV oocytes were grouped according to the combination of both of the previously mentioned parameters (chromatin condensation and distribution in relation to the NLB).
Result(s): According to the GV classification of model A, nucleoplasm, nucleus position, nuclear envelope continuity, and oocyte size were shown to be relevant and were included in a mathematical model for predicting chromatin condensation stage.
Conclusion(s): Noninvasive analysis of GV oocytes using contrast-phase microscopy maintains oocytes in a viable state and allows the chromatin condensation status to be predicted. (Fertil Steril® 2010;93:2557–64. ©2010 by American Society for Reproductive Medicine.)

Key Words: Chromatin configuration, germinal vesicle, morphologic predictors, nuclear envelope continuity, nucleolus-like body, oocyte size

At birth, mammalian oocytes are arrested at the diplotene stage of the first meiotic prophase. After delivery, one or more follicles abandon the ovarian reserves, and the first phase of concerted follicle and oocyte growth commences. During the second phase, the follicle enlarges, principally through formation of an internal cavity (the antrum), while the oocyte, though under meiotic arrest, possesses a large nucleus named the germinal vesicle (GV), which is characterized by a completely compact nucleolus known as the nucleolus-like-body (NLB). At this point, having achieved sexual maturity and receiving gonadotropic support, follicle growth continues, while oocyte size increases slightly and acquires competence (namely, the ability to resume meiosis and sustain the first cleavage divisions after fertilization). Thus, after the ovulatory LH surge of each cycle, the oocyte reinitiates its first reductive meiosis division, which culminates in the extrusion of the first polar body (PB). The mature oocyte is once again meiotically arrested at the metaphase II (MII) stage and remains so until fertilization.

In humans, after the application of controlled ovarian hyperstimulation protocols, oocytes isolated from follicles of different sizes display certain heterogeneity within the same follicle size category (12 to 20mm). Of the dozen recovered oocytes, most are mature (85% at the MII stage), while other immature examples are at either the metaphase I (MI: 4%; oocytes without both GV and first PB) or the GV stage (11%) (1).

Early attempts at in vitro maturation (IVM) in human oocytes date back to 1965 (2), but the first successful birth was reported in 1991 (3). However, clinical outcomes are variable and continue to be poor after transfer of embryos derived from in vitro matured oocytes with respect to those derived from in vivo matured oocytes (4). These variations have been attributed to intrinsic differences among recovered oocytes.

Detailed analysis by fixation, specific Hoechst staining, and ultraviolet exposure of fully grown mammalian oocytes recovered from large antral follicles have revealed that immature GV oocytes constitute a heterogeneous population in terms of chromatin configuration (5, 6), which is related to the subsequent meiotic progression

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(5, 7, 8), cytoplasmic maturation, and developmental competence of the oocyte (4, 5, 9, 10).

The most detailed works on human GV oocytes are those by Combelles et al. (4) and Miyara et al. (11). These investigators established four GV oocyte categories according to both chromatin configuration and NLB dynamics after fixation and Hoechst staining. Both groups agreed that modifications in chromatin organization consist of a shift from a decondensed, dispersed configuration around several NLB that are small or heterogeneous in size to a condensed chromatin, organized around a single well-defined nucleolus. In mammals, these chromatin modifications correspond to a transition from a transcriptionally active state to an inactive one as the end of the growth phase of oogenesis approaches (4, 6, 10, 12, 13). Thus, the definition of morphologic markers of chromatin stage in fresh (nonfixed and stained) GV oocytes before IVM represents an initial step toward future studies on the developmental competence of GV oocytes.

We first aimed to characterize a population of immature GV oocytes recovered from stimulated cycles through morphometric and morphologic examination of different parameters using phase-contrast and image analysis, and to determine the configuration of the GV chromatin by means of fluorescent DNA imaging. Second, we defined three models with which to assess the value of selected morphometric and morphologic parameters as predictors of chromatin status.

MATERIALS AND METHODS

This work was approved by both the ethics committee of the Instituto Universitario IVI (Valencia, Spain) and the Valencian regional government (Consellería de Sanitat, Generalitat Valenciana). Eggs were acquired after obtaining the written informed consent of donors.

Oocyte Collection

The oocytes employed in this study were obtained from healthy donors aged between 18 and 34 years (average: 27.7 years, standard deviation [SD] ±3.9 years) and with no family history of chromosomal diseases. They underwent a complete gynecologic examination, karyotype, and screening for infectious diseases such as human immunodeficiency virus, hepatitis B and C, gonococci, and syphilis. Oocytes were recovered after controlled ovarian hyperstimulation treatment and follicle punction, and were incubated in vitro for 4hours in 50μL of human tubal fluid medium (hTF; IVI Barcelona, Barcelona, Spain), under standard culture conditions (at 37°C and 5% CO_2 in a humidified atmosphere). Cumulus cells were then gently removed by hand pipetting after a brief period of incubation in 40IU/mL of hyaluronidase (Sage In Vitro Fertilization Inc, Tumbull, CT).

Cumulus-free oocytes were classified as mature (MII) or immature. The latter included oocytes with no PB or nuclear structure (MI) (Fig. 1D) and those with a GV structure with no PB in the perivitelline space (see Fig. 1A–C). Mature oocytes were used for reproductive purposes, whereas GV oocytes were donated to be used in this research.

The GV oocytes were collected from 66 donors and cultured in vitro in hTF medium for 1 to 3hours. The nuclear maturation state of eggs was confirmed so that only oocytes at the germinal vesicle stage were included in the study.

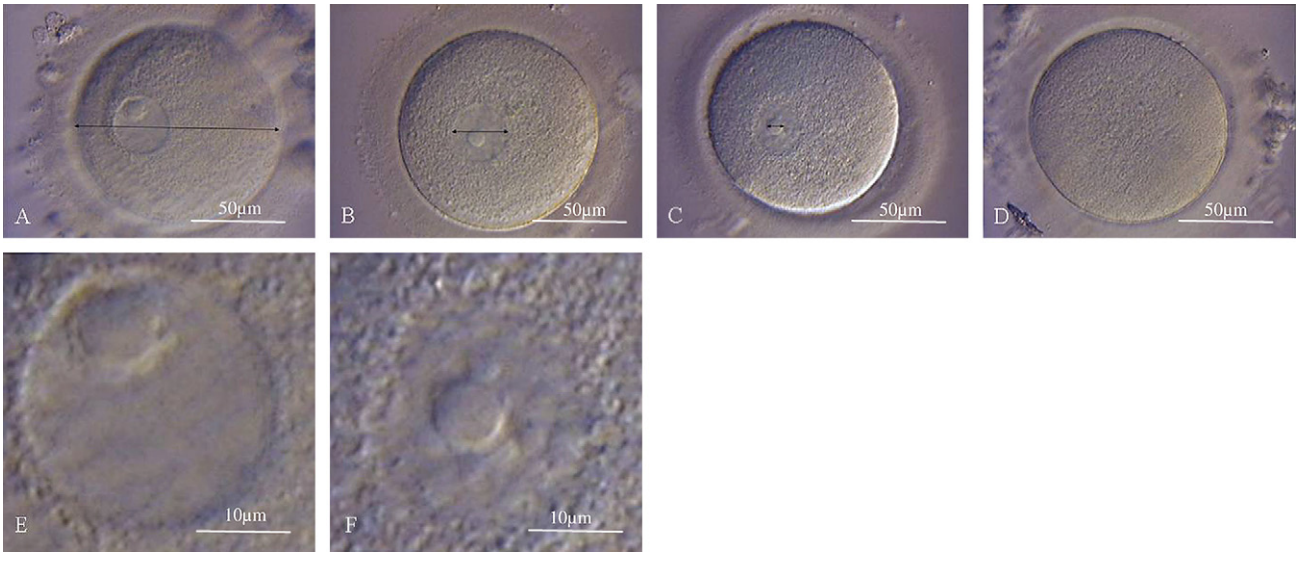
Morphologic GV Observations

Individualized immature GV oocytes were morphologically examined by contrast-phase microscopy at ×400 magnification (Olympus, Barcelona, Spain).

For every oocyte (see Fig. 1), at least three images were recorded for subsequent image analysis. The image capture process lasted no more than

FIGURE 1

(A–D) Contrast-phase microscopy images at ×40 magnification and (E, F) detailed nuclear observation of immature oocytes at the germinal vesicle stage (GV: A–C) or metaphase I stage (MI: D). In images A, B, and C, arrows represent diameters of the (A) oocyte, (B) nucleus, and (C) nucleolus. (**A**) GV oocyte with a well-defined nuclear envelope (grade 1) and one large eccentric NLB. (**B**) GV oocyte with an irregular nuclear envelope (grade 2). Nucleus is centrally located in the ooplasm. (**C**) GV oocyte with a discontinuous nuclear envelope in some areas (grade 3) that defines a nuclear area containing an clearly visible NLB. (**D**) Immature oocyte at the MI stage. Note the absence of both the nucleus and the first polar body in the perivitelline space. (**E**) Detailed image of oocyte from A. Note smooth appearance of nucleoplasm. (**F**) Note rough appearance of nucleoplasm of GV oocyte from C.



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