PRO EXPERIMENTIS

Simple methods for observing cortical granules in living mammalian eggs

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Summary. Simple light microscopic methods for routine observation and checking cortical granules of vital mammalian eggs will be described. By using interference contrast and dark field techniques, even small cortical granules can easily be examined. These methods are time-saving compared to the usual ones using an electron microscope. They make distribution studies possible without difficulties.

Cortical granules in mammalian eggs were first described by Austin[1] in hamster eggs, using phase contrast microscopy. This is the only light microscopic technique used so far. Since then cortical granules in eggs of many mammalian species were demonstrated by electron microscopic methods. The formation of cortical granules during the development of eggs and their structure were described[2–6]. Cortical granules are thought to play an important role in the fertilization process. The enzymes stored in these granules are released, while sperms penetrate the vestments of the eggs. These enzymes seem to induce alteration in the surface of the vitellus and the zona pellucida. The alterations prevent penetration of more than one sperm and establish the block to polyspermy[7]. The exact mechanism of the block to polyspermy and the action of the cortical granule enzymes is a complex phenomenon and not yet completely understood. Studying cortical granules during the egg-spermatozoon interaction is of the utmost importance[8].

Compared to light microscopic methods, the electron microscopic visualization of cortical granules is technically difficult and time-consuming. The necessary procedures for producing specimens for electron microscopic examinations allow only a small insight into distribution and number of cortical granules per egg.

For these reasons we looked for other techniques. Phase contrast microscopy, as described by Austin[1], has only a limited value because of the small size of cortical granules of many mammalian species. We therefore developed a technique for light microscopic examinations by an interference contrast and dark field method. To test these techniques for their practicability, we used hamster eggs as an example for eggs with large cortical granules and mouse eggs for small ones.

Material and methods. Eggs were recovered from superovulated MRI-mice and field-hamsters 9 h after ovulation by flushing the isolated oviducts. The cells of the corona radiata were removed with 0.1% hyaluronidase-solution

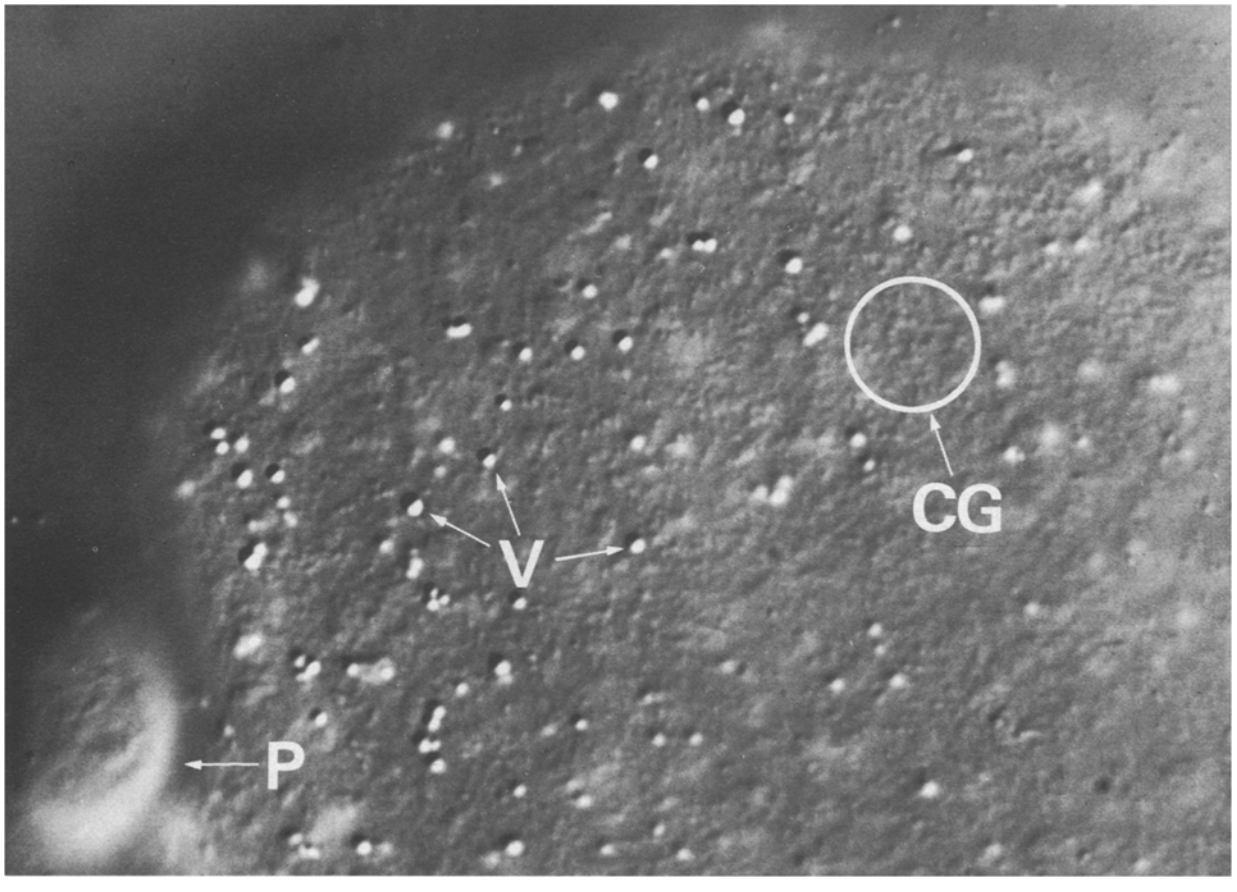

Fig. 1. Cortical granules in an unfertilized mouse egg using interference contrast microscopy (CG: cortical granules; V: vesicles emerging from the perivitelline membrane; P: polar body).

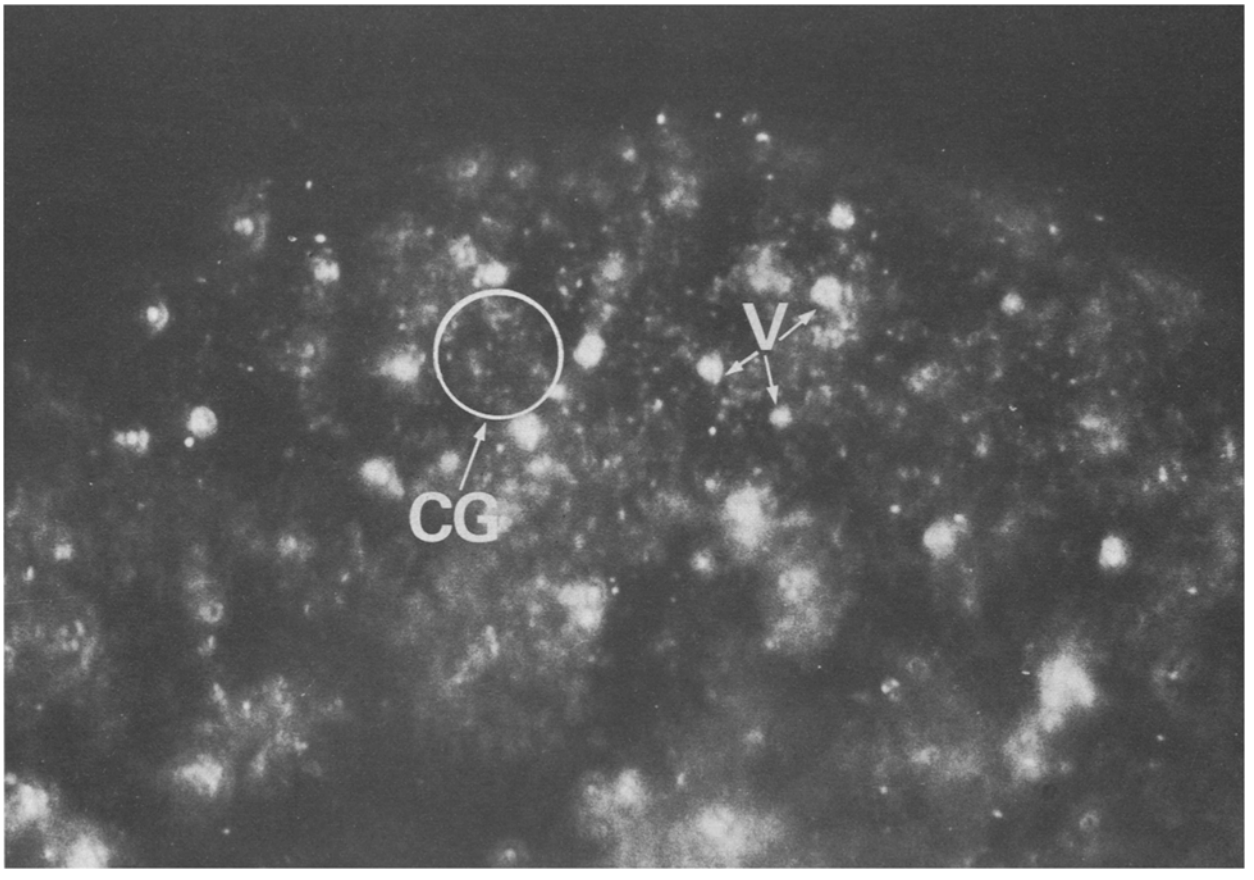

Fig. 2. Cortical granules in an unfertilized mouse egg using dark field technique (CG: cortical granules; V: vesicles).

(Merck) in a modified Dulbecco-phosphate-buffered saline. The same procedure was employed for getting fertilized eggs. Females and males were put together after HCG injection and the eggs were isolated 12 h after ovulation. Cortical granules were observed by mounting the eggs on a slide, covering them with a vaseline-edged cover glass. The interference contrast technique [9] (E. Leitz, Wetzlar) and dark field illumination [9] were used with oil-immersion objectives (100×). The dark field techniques make slight compression of the eggs between slide and cover glass necessary. The upper surface of the egg is focused and the cortical granules underneath the perivitelline membrane examined.

Results and discussion. With the aid of interference contrast microscopy cortical granules can be recognized as small spots, which are evenly distributed over the whole surface of the egg (figure 1). Using dark field illumination, the same distribution of cortical granules can be seen (figure 2). Each of the granules is imaged as a bright point and the picture of the granules is brilliant.

Phase contrast microscopy of cortical granules was used again lately by Gwatkin et al.[10]. Interference contrast microscopy and dark ground techniques have to our knowledge not been described so far for observation of cortical granules.

All 3 light microscopic methods have the advantage that unfixed and whole living eggs can be observed. Directly during or after external stimulation of the eggs (incubation with sperms, alteration of ionic concentration, artificial activation) changes in the number of cortical granules can be examined. In contrast to electron microscopical methods, it is possible to give quantitative results for the number of granules per μm^2 resp. per egg.

The phase contrast technique has the disadvantage of limiting the optical thickness of the specimen to about 5 μm. The axial and radial resolving power is limitted by the halo of the phase contrast technique. For these reasons this method is only practicable for eggs with relatively large cortical granules, for instance those of hamsters (0.4 to 0.5 μm diameter).

The dark field technique allows the visualization even of the very small cortical granules of the mouse (diameter 0.1 to 0.2 μm). However, only granule counting is possible; the method is not suitable for linear measuring.

The interference contrast technique has optimal axial and radial resolving power based on full illumination-aperture. Additionally this technique allows the optical sectioning which permits the observation of each level in an object in focus. Therefore it does not depend on the thickness of the object. Consequently it is very suitable for examination of living eggs. The technique allows a direct observation of the reaction of cortical granules to external stimuli.

The methods described are simple and may play an important role in future for solving the many problems concerned with the function of cortical granules in the fertilization process.

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