Electron Microscope Study of the Atretic Oocytes of the Rat

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Received February 20, 1967

Summary. The ultrastructural aspect of ovular and follicular atresia were studied in 6—60 days old white rats. In pre-antrum folliculi two types of follicular cells were encountered, one, the more numerous, is a round clear cell, the other is dark and elongated. In antrum folliculi, vacuolization of the cytoplasm and nuclear pyknosis are seen.

The disappearance of the prolongations of the follicular cells and ovular microvilli occur in all ages.

The oocytes of atretic follicles may present the following changes: 1. lamellar alteration of the cytoplasmic matrix. It consists of clear zones devoid of cytoplasmic organelles (initial aspect) in which the disordered fibrous matrix organizes gradually in parallel lamella. Each lamella shows a regularly spaced longitudinal striation. 2. Changes in the distribution of the oocyte organelles: mitochondria, Golgi bodies, multivesicular bodies and clumps of vesicles which in the normal growing oocytes form a cortical and perinuclear layers are distributed at random in the cytoplasm like it occur in fertilized eggs. 3. Anomalous segmentation. Many oocytes undergo a process of segmentation. The segments are of unequal size, some of them are nucleated, others are devoid of a nucleus, Nuclei in an apparent process of amitotic division are frequently found, mitotic divisions are rare.

Final stages of atresia are characterized by loss of contact of the oocytes with the corona cells, increase of the density of the cytoplasm in some cases or decrease in others. In the oocyte cytoplasm vacuolization and increase in density of the mitochondria are very intense. The oocyte outline changes as a consequence of a general shrinking of the oocyte. The last remnant of the follicle and oocyte within the ovary is the zona pellucida.

Introduction

The ultrastructure of mammalian oocytes has been extensively investigated. At present the list of the animals studied includes: Guinea pig by Andersen and Beams (1960); rabbit by Trujillo-Cenoz and Sotelo (1959), Blanchette (1961), Hadek (1963) and by Zamboni and Mastroianni (1966a, b); human by Wartemberg and Stegner (1960); mouse by Yamada et al. (1957). Several ultrastructural aspects of the maturation cycle of the rat oocyte as well as the fertilization stage have been studied. Sotelo and Porter (1959) reported the fine structure of the oocyte and ova during maturation and fertilization (up to the 2-cell stage) and Sotelo (1959) described the cytoplasmic and nuclear components of primary oocytes. Odor (1960) reported some aspects of oocytes in prepuberal and adult animals. Szollosi and Ris (1961) studied the fine structure of the oocyte membrane at the time of sperm penetration. Franchi (1960) studied the close relationship between the corona cell and the oolemma. Izquierdo and Vial (1962) investigated the cytology of the mature and fertilized eggs. Franchi and Mandl (1962) made a systematic study of the nuclear components at the period of meiotic prophase. However, a survey of all the above cited papers reveals the scarcity of knowledge on ultrastructural aspects of atresia.
In mammals atresia is the normal process by which a large part of the original oocyte population perish. In the rat many oocytes undergo atresia and disappear before ovulation. A systematic examination of the prepuberal rat ovaries was planned taking this fact into account. The main purpose was to describe differences between normal oocytes and those undergoing the first stages of degeneration. However, alterations in the rest of the follicle were necessarily taken into account since atresia is a progress in which all follicular components are involved. The electron microscopic research reported here confirms many facts already known from light microscopic research. Furthermore, it adds some new facts concerning the organization of the cytoplasmic matrix during atresia.

**Material and Methods**

White rats were lightly anaesthetized, the abdominal wall opened and the ovaries exposed. Fixative was first dropped over the ovary surface. After 5 min, they were removed, cut into small pieces and immersed in 1% OsO$_4$ dissolved in Palade buffer at pH 7.2. The age of the animals ranged from 6 to 60 days. Embedding was carried out in Araldite and in n-butylmethacrylate as described by Izquierdo and Vial (1962) and by Wetstein and Sotelo (1963). Sections were cut with a Porter-Blum MT-1 ultramicrotome. In some cases short series of 30—90 sections were cut from Araldite embedded material. Examination was performed in an RCA 2C and a Siemens Elmiskop I electron microscopes.

**Observations**

The most significant involutional alterations are grouped as follows: 1. those modifying the follicular cells and their relationship to the oocyte; 2. those affecting oocytes: a) those altering primarily cytoplasmic components, b) those of anomalous segmentation.

1. **Follicular Cells.** The changes affecting follicular cells depend on whether it is a pre-antrum follicle or a vesicular one. In pre-antrum follicles undergoing atresia two types of cells can be observed. One, (type I), the more numerous, is a round clear cell. The other (type II) is dark, elongated and irregular in outline. Their difference in density is mainly due to the amount of ribosomes that each cell possesses. Fig. 1 is a light microscope photograph of a methacrylate embedded young follicle in which the distribution of these cells in the thickness of the follicular wall is seen. In the light microscope and the electron microscope as well, both cells are readily distinguished because of their different density. It is considered that those having less ribosomes are in a more advanced degenerative stage. The cytoplasmic organelles, such as mitochondria or Golgi bodies, do not appear affected even in the cells in an advanced degenerative stage (Fig. 2). In contrast with normal follicles the prolongation of these presumably degenerating cells are short, and do not penetrate deep in the zona pellucida. Therefore the oocyte has lost contact with the follicular cells. The cytoplasm of type II cells is charged with ribosomes and is as electron dense as the nucleus. Very clear vacuoles of different size are found scattered in both types of cells (Fig. 2).

In some follicles, the oocytes may not show alterative changes although the follicle cells may be altered to an advanced degree. The inverse situation is also noticed; i.e. the follicle layer may look normal whereas the oocyte is definitely abnormal. Other changes such as a vesiculated appearance of lipids droplets in