Surface Characteristics of Oocytes from Juvenile Mice as Observed in the Scanning Electron Microscope

Nathalie von Weymarn*, R. Guggenheim**, Hj. Müller*

** Labor für Raster-Elektronenmikroskopie, Universität Basel, Schweiz

Summary. The in vivo and in vitro development of the zona pellucida as well as the vitelline membrane surface of oocytes isolated from juvenile mice aged between 8 and 30 days p.p. were investigated by scanning electron microscopy.

In vivo a consistent development of the zona pellucida surface can be observed, namely the formation of a fibrous network like structure interspersed with numerous pores.

After enzymatic removal of the zona pellucida the surface of the vitelline membrane was studied. In “normal” oocytes with intact germinal vesicles, microvilli were distributed over the entire oocyte surface in all age groups investigated. After resumption of meiosis in vitro a characteristic differentiation on the vitelline membrane occurs. A glabrous polar region appears in primary oocytes and a glabrous polar body in secondary oocytes.

The same differentiation in surface organization could be observed in the in vivo precociously matured oocytes.

Key words: Surface characteristics – Oocytes – Mouse – Scanning Electron Microscopy.

Introduction

We have previously shown (von Weymarn et al. 1980) that along with oocytes containing an intact germinal vesicle, a considerable number of degenerating oocytes could be isolated from ovaries of mice aged between 8 and 30 days p.p. by mechanical disruption of the tissue. The “normal” oocytes were shown to be partly able to resume meiosis in vitro if obtained from mice aged more than two weeks. Several different degeneration types were characterized by inverted light-microscopical as well as cytogenetic observations.

Offprint requests to: Dr. Hj. Müller, Abt. Genetik, Basler Kinderspital, Römergasse 8, Postfach, CH-4005 Basel, Schweiz

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In this study, development and degeneration of the zona pellucida as well as of vitelline membrane surface in vivo and in vitro was investigated, using scanning electron microscopy.

Material and Methods

Oocyte isolation, light-microscopical characterization of the different oocyte types isolated and the culture techniques were described in a previous paper (von Weymarn et al. 1980).

The scanning electron microscopical investigations are based on at least 20 oocytes per age group and oocyte type, both for zona pellucida and vitelline membrane.

Removal of the Zona Pellucida

To study the vitelline membrane surface, the zona pellucida was removed by incubating the oocytes for 10 min at 37°C in a solution of PBS (phosphate buffered salt solution) containing 5 mg/ml each of hyaluronidase (Sigma, H 2374), collagenase (Sigma C 0130) and lysozyme (Sigma L 7001). After this enzymatic treatment the oocytes were washed once in PBS and put in culture or prepared for scanning electron microscopical investigations. The enzymatic treatment had no influence on the capacity to resume meiosis in culture.

Preparation for Scanning Electron Microscopy

Oocytes were transferred to a BEEM capsule (LKB, 4865-02) containing 2% glutaraldehyde in 80% PBS (pH 7.1, 315 mOsmol) and concentrated at the bottom of the capsule by centrifuging for 10 min at 300 g.

After a fixation time of at least two hours at a temperature of 4°C, they were washed three times in PBS and distilled water. The samples were dehydrated within the capsule either in a graduated series of acetones (30%, 40%, 50%, 60%, 70%, 80%, 90% and three times 100%) or in 2,2-di-methoxy-propane (DMP) (6) (Merck, No. 80 2936). The oocytes were dried in the capsule by the critical point method using CO2. The upper part of the capsule was then cut off and the oocytes were sputtered with 200–300 Å of gold-palladium and viewed in a Cambridge “Stereoscan” Mark 2 A operated at 10 keV.

Results

Zona Pellucida Surface

1. Development in vivo. The zona pellucida surface of normal oocytes (i.e. oocytes that show a germinal vesicle by direct observation in the inverted light-microscope), isolated from four age groups (8, 15, 21 and 30 days p.p.), was studied. Depending on age, the following changes in surface structure could be observed.

As Fig. 1a shows, oocytes recovered from 8 day old mice have an unstructured surface of the zona pellucida. In oocytes from mice aged 15 days (Fig. 1b) a trace of a smooth fibrous network-like structure with some small pores can be detected. This fine fibrous network as well as the pores are more pronounced in mice aged 21 days (Fig. 1c) and even more prominent in oocytes from 30 day old mice (Fig. 1d).