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Eosin-B 에 의한 rat 난모세포의 생존성 검사

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Assessing the Viability of Rat Oocyte by Use of Eosin B

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적 요

Eosin B 의 사용에 의한 rat 난모세포의 생존성 및 발달가능성을 검사하였다. 염료는 Brinster's Medium for Ovum Culture plus glucose (BMOC)-2 medium ml 당 eosin B 1.0 mg 을 함유하는 stock solution 으로 준비하였다. 난모세포연구를 위하여 BMOC 로 stock solution 을 희석하여 만든 3가지의 염료 농도들이 선택되었다 : 0.12 mM, 0.60 mM, 1.20 mM.

난소세포들로 부터 난모세포들을 채취하고, 보존기간 및 열 처리의 다양한 조건하에서 eosin B 를 사용한 dye exclusion assay 로 검사하였다. 생존한 난모세포는 eosin B 를 배척하고, 사멸한 세포는 eosin B 를 배척하지 않는 것으로 제시되었다.

I. INTRODUCTION

Efforts to fertilize *in vitro*-matured follicular oocytes have met with variable success (Sreenan, 1970; Shea et al., 1976; Trounson et al., 1977; Leibfried et al., 1987; Fukui et al., 1988). To enhance the success rate of the fertilization, assays for the viability of oocytes are required before fertilization. A various approaches have been to assess the viability of mammalian embryos during the preimplantation period of development. In practice, the morphological appearance of the embryo remains as the primary and most widely used tool used for the evaluation of embryo viability.

The viability of mammalian preimplantation embryos was assessed on the basis of dye metabolism (Umbreit et al., 1972; Alley et al., 1982), vital staining (Schilling et al., 1979; Hoppe and Bavister., 1984), nonvital staining (Linder et al., 1982; Freshney, 1983; Hutz et al., 1985), and dye-exclusion assays (Kaltenbach et al., 1958; Dooley, 1984). Unfortunately, many of these tests are cumbersome and expensive to use, produce unreliable results, or damage the embryos.

Dye-exclusion tests, utilizing dyes such as

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eosin B and Y, erythrosin B and trypan blue have been used as indicators of viability for a number of cell types(Hanks and Wallace, 1958; Holmberg, 1961; Phillips, 1973), including spermatozoa(Dott and Foster, 1972; Dooley, 1979) and embryo(Dooley, 1984), but they have not been developed for the assessment of the oocyte viability. Rapid and inexpensive techniques that permit or enhance the discrimination between viable and nonviable oocytes which can be performed simultaneously or in conjunction with the morphological evaluation of the oocyte, are badly needed.

II. MATERIALS AND METHODS

1. Preparation of Oocytes

Oocytes were collected from the ovarian follicles of rats (100 to 200 days; 200~325 g) which were sacrificed by cervical dislocation. Oocytes were placed in culture dishes containing Brinster's Medium for Ovum Culture plus glucose(BMOC)-2 medium (Brinster, 1971) for storage, heat treatment, and exposure to eosin B. Oocytes were maintained in an incubator at 37°C in a humidified 5% CO_2 atmosphere.

2. Dye Formulation

Dye was prepared as a stock solution to contain 1.0 mg eosinB(Polysciences Inc., Warrington, PA) per ml of BMOG. The powdered eosin B contained 88% eosin B, thus the actual concentration of eosin B in a 1.0 mg/ml solution corresponded to 1,410.0 μM. Three dye concentrations were selected for the oocyte studies by dilution of the stock solution with BMOC: 0.12 mM, 0.60 mM and 1.20 mM.

3. Evaluation of the Staining Response

The staining response of the dye-exposed oocytes was determined at room temperature (20~22°C) by direct microscopic observation (40~160 ×) using a didymium filter in the light path. To determine optimal temperature for assessing oocyte viability, oocytes were exposed for 30 minutes to a control(37°C) and one of two treatment temperatures (40°C or 45°C). After 30 minutes heat treatment, oocytes were exposed to 0.12 mM, 0.60 mM or 1.20 mM eosin B for 15 minutes and the staining response was evaluated. To assess the change in oocyte viability according to the duration of preservation, oocytes were exposed for variable duration(0, 12, 24, 36, 48, and 60 hours). After lapse of each duration of preservation, oocytes were exposed to 0.12 mM eosin B for 15 minutes and the staining response was evaluated. The oocytes were classified as follows: unstained (U), none of the cytoplasm appeared to be stained; partially stained (PS), part of cytoplasm did not stain in an oocyte where staining was evident; completely stained (CS), cytoplasm of oocyte was completely stained

III. RESULTS

1. Response of Eosin B Staining by Heat Treatment

A total 175 oocytes were evaluated for the influence of heat treatment on the viability of oocytes. As shown in Table 1, none of the oocytes was stained at 37°C; variable staining response (U, PS, and CS) resulted at 40°C; all of oocytes except partially stained oocytes were completely stained at 45°C. No remarkable differences between concentrations of eosin B were observed.

2. Response of Eosin B Staining by the Duration of Preservation

A total of 337 oocytes were evaluated for the influence of duration of preservation on the viability of oocytes. As shown in Table 2, none

Table 1. Respones of eosin B staining in heat-treated rat oocytes.

Treatment	No. of oocytes	Concentration of eosin B								
		0.12 mM			0.60 mM			1.20 mM		
		U	PS	CS	U	PS	CS	U	PS	CS
37°C	65	23			22			20		
40°C	58	12	2	5	11	2	7	7	3	9
45°C	52		2	16		1	17			16

U : unstained
PS : partially stained
CS : completely stained

Table 2. Respones of eosin B staining on rat oocytes according to the duration of preservation.

Duration (hours)	No. of oocytes	Concentration of eosin B								
		0.12 mM			0.60 mM			1.20 mM		
		U	PS	CS	U	PS	CS	U	PS	CS
0	48	16			18			14		
12	62	17	3	1	15	3	2	16	2	3
24	57	14	2	8	10		7	9	1	6
36	56	8	5	8	6	8	7	2	4	8
48	65	15	2	6	3	8	11	2	5	13
60	49	3	3	11	2	4	10	1	2	13

of the oocytes was stained at O hour; thereafter the number of stained oocytes tended to increase in proportion to the time lapse; at 60 hours in particular, all of the oocytes except 6 out of total 49 were completely or partially stained. Up to 36 hours, no differences between concentrations of eosin B were revealed but after that time the higher the concentration of eosin B, the better staining of oocytes.

IV. DISCUSSION

The work presented here demonstrated that exposure of rat oocytes to micromolar concentrations of eosin B can be used to estimate the viability of rat oocytes. In these studies two factors on the viability of rat oocytes were considered: effects of thermal shock and influence of duration of preservation. The results of heat treatment clearly demonstrated a negative effects of temperature on the capability of oocytes to exclude eosin B(Table 1). As preservation time increased, capability of oocytes to exclude eosin B decreased proportionally (Table 2).

Fluorescein diacetate(FDA) has been used to estimate embryonic viability in the cow (Church and Raines, 1980; Hoppe and Bavister, 1984;

Schilling et al., 1979), hamster(Hoppe and Bavister, 1984; Hutz et al., 1985), mouse (Jackowski, 1977; Mohr and Trounson, 1980), rabbit(Schilling et at., 1979) and squirrel monkey(Chan et al., 1982). While the fluorescein diacetate response appears to be useful for the evaluation of embryonic viability, its application has been limited by its toxicity to the cell. Furthermore, FDA whose activity depends on hydrolysis by esterase to yield fluorescein, was not considered specific for determination of cellular integrity as 100 enzymes are believed to be necessary for cell life while esterase enzyme is included in the list.

Dye exclusion tests have been routinely applied to many cell types to detect membrane damage and changes in membrane permeability, and have also been used to estimate embryo viability. Unfortunately, they have not been developed for the assessment of the oocyte viability. The basic principle of dye-exclusion is that viable cells impermeable to dye in surrounding medium(Kaltenbach et al., 1958), whereas, dead cells are permeable to dye.

As shown in this work using eosin B for dye -exclusion test, it was assessed as rapid and inexpensive for the viability test of oocytes. This method should prove valuable in animal breeding fertility management, and other relevant researches.

V. SUMMARY

The viability and developmental potential of rat oocyte was assessed by the use of eosin B. Dye was prepared as a stock solution to contain 1.0 mg eosin B per ml of Brinster's Medium for Ovum Culture plus glucose (BMOC)-2 medium. Three dye concentrations were selected for the Oocytes from rat ovarian follicles were collected and assessed under various conditions of heat oocyte studies by dilution of the stock solution with BMOC: 0.12mM, 0.60mM and 1.20mM. treatment and preservation duration by dye exclusion assay using eosin B. It is suggested that live oocyte excludes eosin B whereas dead oocyte does not.

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