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PARTHENOGENETIC DEVELOPMENT OF OVULATED MOUSE OVA UNDER THE INFLUENCE OF ETHYL ALCOHOL

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KEY WORDS: ethyl alcohol; parthenogenesis; ovum; C57Bl/CBA mice.

Several investigations, the results of which have been published in important surveys [1, 5, 7, 9], have been devoted to experimental parthenogenesis in mammals. Various methods of activating mouse ova for parthenogenetic development have been described [2], but a common defect of these methods is either the poor reproducibility of the results or the need to use a laborious technique for culturing oocytes in vitro.

During the study of the embryotoxic and teratogenic action of alcohol in the writers' laboratory, it was found that intraperitoneal injection of ethyl alcohol into nonpregnant mice activates the ovulating ova and induces their parthenogenetic development. These observations are described below.

EXPERIMENTAL METHOD

Experiments were carried out on 221 female hybrid (C57BL × CBA)F_1 mice, obtained at the age of 4 weeks from the "Rappolovo" nursery. Before the beginning of the experiment the animals were kept for not less than 2 weeks under conditions of 17 h daylight and 7 h darkness. The mice were mated with vasectomized males a few hours before the end of the dark period and ovulating females with vaginal plugs were selected. Under these conditions of illumination ovulation was synchronized and took place 1 h after the end of the dark period. The mice were given a single intraperitoneal injection of 0.35 ml of 25% ethyl alcohol at different times after ovulation. Alcohol (0.6 ml) was given to one group of experimental mice via gastric tube. The control group of females with vaginal plugs did not receive ethyl alcohol and was used for testing the sterility of the vasectomized males. Ova or cleaving embryos were flushed out of the genital tract of the control and experimental animals with warm medium No. 199 and investigated in the living state. Air-dried preparations

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TABLE 1. Activating Action of Ethyl Alcohol on Ovulated Ova

Conditions of administration / Indices	Time after ovulation, h	Number of females	Total number of ova and embryos investigated	Including					
				nonactivated (metaphase of meiosis II)		fragmentary		activated	
				absolute	%	absolute	%	absolute	%
Intraperitoneal injection (dose 0.35 ml)	2½	10	88	72	81,8	5	5,7	11	12,5
	5	38	235	103	43,8	27	11,5	105	44,7
	6	35	238	78	32,8	23	9,6	137	57,6
	7	41	258	59	22,9	14	5,4	185	71,7
	8	10	75	28	37,3	7	9,3	40	53,1
	9	10	77	49	63,6	6	7,8	22	28,6
Control	—	22	118	113	95,8	5	4,2	—	—
Intragastric injection (dose 0.6 ml)	7	27	140	122	80,0	28	20,0	—	—

were obtained from some of them [8] for metaphase analysis and for counting the nuclei. Some ova were fixed with 10% neutral formalin, taken through alcohols, and stained with 0.25% lacmoid [4], after which they were examined and photographed in phase contrast with an "Opton" photomicroscope.

EXPERIMENTAL RESULTS

Altogether 1229 ova from ovulating mice were used (Table 1). All the ovulating ova from females not receiving ethyl alcohol, but mated with vasectomized males, were at the metaphase stage of the second maturation division. Consequently, if spontaneous parthenogenesis did occur in our colony of mice [10], it was so rare that it could not be observed in the number of animals used in the control group. These observations, and also experiments with intragastric administration of alcohol, are also evidence of the complete sterility of the vasectomized males.

After intragastric injection of alcohol, 80% of the ova remained in metaphase of the second maturation division and 20% degenerated, i.e., intragastric administration of alcohol activated a small percentage of ova, but they all died after one or two divisions [3].

After intraperitoneal injection of ethyl alcohol, ova which had completed meiosis and commenced parthenogenetic development were regularly found in all the animals. Freshly ovulated ova were activated by alcohol in small numbers, but with an increase in the time elapsing after ovulation, the sensitivity of the ova to the action of alcohol increased and reached a maximum on the 7th day after ovulation, when alcohol induced parthenogenetic development of 71% of ova. The sensitivity of the ova was sharply reduced when alcohol was injected 9 h after ovulation, when only 28% of ova were activated (Table 1). Under the influence of alcohol all types of parthenogenetic embryos described in the literature developed [5, 7, 9], but the predominant types (70%) were ova with one pronucleus and a separate second polar body. Cytogenetic analysis showed that the pronucleus had 20 chromosomes and that the haploid state was preserved in the nuclei of the cleaving blastomeres. In 15.5% of cases the activated ova underwent so-called "uniform rapid division," i.e., they divided into two cells of equal sizes, one of which was the analog of the second polar body. These embryos also were haploid, i.e., in more than 85% of cases alcohol induced haploid parthenogenesis.

In their external appearance and number of blastomeres the cleaving parthenogenetic embryos were indistinguishable from normal. However, in the course of development many of the embryos died and only 11.6% of the ova activated by alcohol survived to the blastocyst stage. All these parthenogenetic embryos died during or immediately after implantation, i.e., at the same stages as parthenogenetic mouse embryos obtained by other methods [5, 7, 9].

Tribromoethanol (Avertin) and certain other general anesthetics are known to induce parthenogenetic development.

In the present experiments activation of ova took place only after intraperitoneal injection and not after intragastric administration of alcohol, although in both cases the anesthetic action of alcohol was observed. It can thus be tentatively suggested that the parthenogenetic development of ova is due not to the general anesthetic effect, but to the direct action of alcohol on the ovulating ova.

These experiments thus showed that ethyl alcohol activates the overwhelming majority of ovulating ova in mice and that the parthenogenetic embryos can undergo normal preimplantation development. This simple technique can thus be successfully used to obtain and study induced parthenogenesis in mice.

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PARTICIPATION OF BONE MARROW STROMAL PRECURSOR CELLS IN BONE REGENERATION IN TRANSOSSEOUS OSTEOSYNTHESIS

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The authors' observations over many years have shown that prolonged bone formation during distraction osteosynthesis takes place with the active participation of bone marrow. The rapid endosteal reaction is maintained throughout the period of distraction, after which it gradually subsides over a long period, during which time foci of proliferation of osteogenic cells can be observed not only in the "zone of growth," but also throughout the bone marrow of the limb on which the operation has been performed. The intensity of the endosteal reaction depends on various conditions, for example, on the greater or lesser degree of preservation of integrity of the intraosseous vessels and stability of fixation of the bone fragments.

On the basis of the foregoing facts, investigations [1, 4, 7-9] in which high osteogenic activity of stromal precursor cells (SPC) of bone marrow was demonstrated are of considerable interest. The model of distraction osteosynthesis which we have developed, in our opinion affords a basically fresh approach to the study of these cells, the principles governing the function of their reserves, and the elucidation of their role in the regeneration of bone tissue. For this purpose, in the investigation described below, using a technique of monolayer culture, the number and proliferative activity of bone marrow SPC from the long bones were determined during leg lengthening by Ilizarov's method.

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