THE *IN VITRO* CULTURE OF RAT OVA

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Lewis & Gregory (1929) were the first to culture the fertilized ovum of laboratory animals *in vitro*, using rabbit ova. Hammond (1949) cultivated 8-cell mouse ova to the blastocyst stage. This method was repeated and further developed by Whitten (1956), McLaren & Biggers (1958), Tarkowski (1961) and Brinster (1963), among others. However, there have been no reported attempts to culture rat ova *in vitro*.

Ovum culture in the Sprague-Dawley rat was developed in our laboratory to test the viability of ova recovered from rats previously treated with anti-fertility compounds. The method was also required for the *in vitro* testing of such compounds upon rat ova with or without subsequent transfer to recipient rats.

Attempts were made to culture 2-, 4- and 8-cell rat ova using various media, energy and nitrogen sources, and several tissue culture systems. The successful culture of rat ova, from the 8-cell to blastocyst stage, was achieved using the method of Brinster (1963), with sodium pyruvate and lactate as the energy source and with bovine serum albumin as the source of nitrogen (Thomson, 1966).

The 8-cell ova were recovered from rats on the 4th day of pregnancy (first day = day of observing spermatozoa in the vaginal lavage), by flushing them from the reproductive tract with the culture medium delivered from a 30-gauge blunt-ended needle attached to a 1-ml syringe. Sterile technique was used throughout the ovum-recovery procedure.

The ova were carefully scrutinized beneath the microscope and any morphologically abnormal ova were rejected. The remaining ova were transferred to the culture medium using a disposable micro-pipette (Drummond, Broomall, Penn.) attached to a Hamilton microsyringe. The culture system consisted of small drops (0.1 ml) of the Brinster medium under sterile equilibrated mineral oil contained in plastic petri dishes of 6 cm diameter. The dishes containing the ova were incubated at 37° C in a humidified atmosphere of 5% carbon dioxide. Twenty-four hours later the cultured ova were examined, using an inverted Plankton microscope (Carl Zeiss) to determine the number of cells or to observe the presence of blastocysts.

In seven consecutive experiments, 197 morulae or blastocysts were produced from 219 ova (Plate 1). None of the cultured blastocysts lost the zona pellucida, as do mouse ova in culture. Continued culture of the rat blastocysts for up to 3 days after the initial 24 hr still showed the zonae intact.

Attempts are being made to test the viability of the cultured ova by transferring them to the uteri of rats on the 4th day of pseudopregnancy. Preliminary results of six experiments indicate that the cultured ova will progress to mature

TABLE 1
THE IN VITRO CULTURE OF 8-CELL RAT OVA TO MORULAE OR BLASTOCYSTS

No. of ova			% ova successfully cultured (B/A × 100)	No. of ova developed into:	
Recovered from donor rats	Cultured in vitro (A)	Progressed in culture (B)		Morulae	Blastocysts
42	38	33	87	9	24
36	23	18	78	—	18
62	54	48	89	—	48
61	35	30	86	—	30
24	15	15	100	—	15
50	38	37	97	9	28
34	16	16	100	3	13
Total 309	219	197	90	21	176

foetuses after transfer. Further work is being done with the *in vitro* culture of 2- and 4-cell ova in the rat. Studies have also begun on the effects of various steroids and antifertility compounds upon rat ova using *in vitro* culture and ovum transfer techniques.

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PLATE 1

The growth of an 8-cell rat ovum (a) through morula (b) and early blastocyst (c) to the blastocyst stage (d) during *in vitro* culture. $\times 700$.

(Facing p. 146)