

This Week's Citation Classic®

Brinster R L. A method for *in vitro* cultivation of mouse ova from two-cell to blastocyst. *Exp. Cell Res.* 32:205-8, 1963.
[King Ranch Laboratory of Reproductive Physiology, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA]

In order to manipulate mammalian eggs, it was first necessary to culture them *in vitro*. This paper describes a simple, reliable method for culturing mouse eggs. It was followed by a series of four papers in which this method was used to establish detailed characteristics of a good culture medium and to identify pyruvate as the central energy source for mouse eggs. [The SCI® indicates that this paper has been cited in over 350 publications.]

Mouse Egg Culture, Manipulation, and Transgenesis

R.L. Brinster
Laboratory of Reproductive
Physiology
School of Veterinary Medicine
University of Pennsylvania
Philadelphia, PA 19104-6009

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While training at the University of Pennsylvania School of Veterinary Medicine, I became interested in the serious problem of embryonic loss during pregnancy. Because much of this loss occurred early in gestation, my attention was directed to the early stages of mammalian development. As a result, in 1960 I entered PhD training at Penn to learn more about fertilization and early egg cleavage. I thought that an ideal approach would be to study embryo development *in vitro*. Although there were several reports describing the culture of eggs, none was reliably reproducible. After trying more than a dozen techniques for egg culture, I finally devised a system employing microdrops of medium under liquid paraffin oil that proved to be surprisingly simple and efficient. This was reported in the above paper, and over the years it has come to be the most widely used system for mammalian egg culture, including human eggs during *in vitro* fertilization.

I first employed the method to establish precise characteristics of culture medium for mouse eggs, which were published in a series of four articles. The last described the composition of an effective culture medium,¹ and these parameters have served as the basis for almost all subsequently described media used to grow or manipulate mouse eggs, as well as eggs of other mammalian species. A surprising finding from this work was the critical role that pyruvate, rather than glucose, played in supplying energy to

the egg.¹ The simple culture system and the identification of the importance of pyruvate made it possible for us and others to grow, manipulate, and eventually modify the genotype of mammalian eggs.

During the late 1960s and early 1970s, we used the culture system to study energy, protein, and nucleic acid metabolism in mouse eggs to define the biochemical events that characterize the first days of mammalian development. However, I was always intrigued by the possibility of modifying the genotype of the egg as a way to study development, and I simultaneously used the culture system in a variety of approaches to egg manipulation, including nuclear transfer, cell-egg fusion, and injection of totipotent stem cells into blastocysts. The last of these approaches proved successful, and in 1974 we reported² that embryonal carcinoma (EC) cells, microinjected into a mouse blastocyst, would participate in the formation of the resulting animal.

This was a new and potentially valuable approach to the study of development that stimulated considerable excitement. However, the frequency with which the EC cell colonized the germ line (sperm and eggs) was very low, and a new cell was sought to increase efficiency. After much effort, the embryonic stem (ES) cell was identified in 1981 independently by the laboratories of Martin Evans in England and Gail Martin in California. Targeted integration of foreign DNA in ES cells has recently been achieved, and this approach to making transgenic mice has become increasingly important in the last year.

While this work was under way during the late 1970s, we were also using the culture system to study gene expression in one-cell eggs by microinjecting nucleic acids. We first evaluated translation of microinjected RNA in fertilized mouse eggs,³ then used the same system to demonstrate that injected DNA was faithfully transcribed.⁴ With this work as a background, we initiated our experiments in which foreign genes were microinjected into the nuclei of fertilized mouse eggs to create transgenic mice.⁵

For each of the three phases in our studies of eggs and development, the culture system, EC cell colonization, and transgenic mice, many years separated the initial pilot experiments from the definitive publication. In the beginning these studies seemed like long shots but also very intriguing and valuable if successful. In fact, their value has been much greater than I ever expected, both for my own research interests and those of others. The simple culture system and medium provided the wherewithal for all of us to manipulate and modify the egg, an approach I had always felt had enormous power to enhance our understanding of mammalian development. It is very gratifying to see the results of this early research so widely used.

1. Brinster R L. Studies on the development of mouse embryos *in vitro*. IV. The interaction of energy source. *J. Reprod. Fertil.* 10:227-40, 1965. (Cited 195 times.)
2. ———. The effect of cells transferred into the mouse blastocyst on subsequent development. *J. Exp. Med.* 140:1049-56, 1974. (Cited 185 times.)
3. Brinster R L, Chen H Y, Trumbauer M E & Avarbock M R. Translation of globin messenger RNA by the mouse ovum. *Nature* 283:499-501, 1980. (Cited 25 times.)
4. Brinster R L, Chen H Y & Trumbauer M E. Mouse oocytes transcribe injected *Xenopus* 5S RNA gene. *Science* 211:396-8, 1981. (Cited 25 times.)
5. Brinster R L, Chen H Y, Trumbauer M E, Senear A W, Warren R & Palmiter R D. Somatic expression of herpes thymidine kinase in mice following injection of a fusion gene into eggs. *Cell* 27:223-31, 1981. (Cited 205 times.)