

This Week's Citation Classic®

CC/NUMBER 2
JANUARY 13, 1986

Chang M C. Fertilizability of rabbit ova and the effects of temperature *in vitro* on their subsequent fertilization and activation *in vivo*.
J. Exp. Zool. 121:351-81, 1952.

[Worcester Foundation for Experimental Biology, Shrewsbury, and Department of Biology, Boston University, MA]

Rabbit eggs were capable of fertilization up to eight hours after ovulation, but their fertilizability dropped sharply after the fourth hour. Some eggs were still fertilizable when kept at various temperatures for various times and then transferred into the tubes of recipient rabbits. The proportion of parthenogenetically cleaved eggs increased as the temperature of treatment was decreased. [The SC¹® indicates that this paper has been cited in over 200 publications since 1955.]

M.C. Chang
Worcester Foundation for
Experimental Biology
Shrewsbury, MA 01545

October 23, 1985

The late G. Pincus was well known for his report of fatherless rabbits and test-tube babies in the early 1940s.¹ When he offered me a fellowship to work in his newly established Worcester Foundation for Experimental Biology, I was very pleased to have the opportunity to study parthenogenesis and *in vitro* fertilization. From 1945 to 1950 I was frustrated by my failure to get rabbit eggs fertilized in a test tube, but my work on the storage of eggs, transplantation of eggs, and fertilization was more successful.

I completed my PhD under the direction of the late Sir John Hammond and the late A. Walton, School of Agriculture, Cambridge University. One of Hammond's major contributions was the determination of the

fertile life of rabbit eggs after ovulation.² During a time of frustration when I could not get anywhere on *in vitro* fertilization, I tried to extend Hammond's work on the fertile life of rabbit eggs by a different and more accurate method and at the same time tried to extend Pincus's work on the effect of low temperature to induce parthenogenesis. That was how I started this work. As far as I can remember, I did not encounter any specific obstacles, difficulty, or frustration in carrying out this study, and Pincus did not complain about the 200 rabbits I used.

Personally, I do not think this paper represents one of my best works, yet it has been frequently cited. This is probably because very few people were working on mammalian eggs and fertilization in the early 1950s, and the importance of this study was to investigate fertilization and activation of mammalian eggs by a combination of *in vitro* and *in vivo* methods. Moreover, a method for examining a large number of mammalian eggs mounted and stained *in toto* on one slide was first described in this paper. This was a very important advance in the study of mammalian eggs and fertilization.

Based upon personal impressions, I often think that my most significant works are on the importance of synchronization between embryos and endometria for implantation,³ the development of the fertilizing capacity of sperm in the female tract (the capacitation of sperm),⁴ and the fertilization of rabbit eggs *in vitro*.⁵ At this juncture, I cannot help but mention that one very important paper that was published by Pincus and me,⁶ which led to the development of the oral contraceptives commonly used nowadays, is not a frequently cited paper. Thus, the popularity of any article partially depends on the time of publication and the general trend of research activity at a particular time. As for my own research activity, I have published a more recent review paper.⁷

1. Pincus G & Shapiro H. The comparative behavior of mammalian eggs *in vivo* and *in vitro*. VII. Further studies on the parthenogenetic activation of rabbit eggs. *Proc. Amer. Phil. Soc.* 83:631-47, 1940.
2. Hammond J. The fertilization of rabbit ova in relation to time: a method of controlling the litter size, the duration of pregnancy and the weight of young at birth. *J. Exp. Biol.* 11:140-61, 1934. (Cited 65 times since 1955.)
3. Chang M C. Development and fate of transferred rabbit ova or blastocysts in relation to the ovulation time of recipients. *J. Exp. Zool.* 114:197-226, 1950. (Cited 175 times since 1955.)
4. Fertilizing capacity of spermatozoa deposited into the Fallopian tubes. *Nature* 168:697-8, 1951. (Cited 310 times since 1955.)
5. Fertilization of rabbit ova *in vitro*. *Nature* 184:466, 1959. (Cited 90 times.)
6. Pincus G & Chang M C. The effects of progesterone and related compounds on ovulation and early development in the rabbit. *Acta Physiol. Lat. Amer.* 3:177-83, 1953. (Cited 55 times since 1955.)
7. Chang M C. Experimental studies of mammalian fertilization. *Zool. Sci.* 1:349-64, 1984.