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Perspectives in Biology and Medicine, Volume 18, Number 1, Autumn 1974, pp. 86-93 (Article)

Published by Johns Hopkins University Press
DOI: 10.1353/pbm.1974.0003

PINCOGENESIS—PARTHENOGENESIS IN RABBITS BY GREGORY PINCUS

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Dwight J. Ingle, previously editor, now advisory editor of this journal, wrote the biographical memoir of the late Gregory Pincus for the National Academy of Sciences [1]. A copy of that memoir reached my desk in the spring of 1973 and, since the man of whom he wrote had been my teacher, colleague, adviser, and dearest older friend from 1931 to 1967, I read it with great interest. I found that, of all the commentaries on Pincus I had heard or read, this one gave the most accurate picture of the man that I knew. But I was shocked when I read the section that dealt with Pincus's induction of "artificial" parthenogenesis in rabbit eggs ("Pincogenesis") in the 1930s. Ingle claimed that there was skepticism among some other workers in this field, since no one had repeated the work, and hence considered Pincus's claims to be questionable.

In a mixed mood of appreciation for an excellent description of my mentor's career and anger at the fact that colleagues doubted an unequivocal fact of accomplishment on his part, I wrote to Ingle saying in effect that I was there, I *helped* make it happen. Perhaps the fact that in the preceding period I had learned that two other accomplishments of Pincus's laboratory prior to 1950 had not yet been repeated lent extra vehemence to my language.[1]

*Department of the Navy, Office of Naval Research, 495 Summer Street, Boston, Massachusetts 02210. Professor R. Christian Johnson, on leave from the University of Wisconsin—Green Bay, is writing a book, *Science and Social Reform: The Search for a Scientific Contraceptive,* in which the story of Pincus and the parthenogenic rabbits will be told in the context of Pincus's other work. We thank Professor Thomas Wegmann and Mr. James Reed of Harvard University for their advice.

[1]Pincus described in 1937 the culture of a fertilized rabbit egg from its earliest possible removal from the tubes through postimplantation stages. Thirty years later no one else had done this. The objective was to prove that development through the blastocyst stage and further required only a sufficiency of unknown growth requisites present in serum. The culture system devised permitted use of large quantities of flowing rabbit serum while retaining the growing embryo in the circumscribed area [2]. In a national conference [3, p. 393] several speakers had commented on the inability of maintaining organs in vitro for 24 hours let alone observing growth and repair. An appropriate system had been described in 1949 [4]. Pincus had initiated this work in 1935. A portion of human uterus bleeding from a curetted endometrium was observed to regrow endometrium under the influence of estrogen. From that point forward the system was used as a routine research tool.

Despite the fact that Ingle and I had had many conversations at Laurentian Hormone Conference meetings since its earliest days, he did not know of my intimate involvement in these studies. He made a point of this in his reply to my letter and his invitation to prepare a rebuttal to the critics. It is important to explain why, although a major portion of my time was spent in this work over a 7-year period, my name appeared only in the "thank you" paragraph at the end of the papers describing parthenogenesis in rabbits.

Pincus's research had three facets. One was genetics, the second was gamete behavior, and the third was regulation of the reproductive system. From the third developed his renown as an endocrinologist. This was my area. However, in those days at Crozier's department a student ordinarily did two separate lines of research. One was his professor's research, in which the student acted as a super-technician and received credit for research assistance. This was the price the student paid for support of his own thesis research. If his professor helped in this thesis work (guidance or collaboration at the bench), the professor's name appeared on the papers, usually as senior author. If not, the papers were published under the name of the student, provided the professor judged the research worthy of publication.

Today's reader of this polemic must be reminded of the state of reproductive physiology when these studies were done. They began in the late 1920s and terminated in the 1940s. During that time the elucidation of the structure of the sex hormones began and ended. At first "FSH" and "LH" were not well known, and chemists were fussing about the structure of "estrogen." (The structure of androgens and corticoids became known in the 1930s.) Human chorionic gonadotropin became available for injection during this period.

The regulation of the reproductive systems of laboratory animals had become reasonably clear at the start of this period. Pincus was familiar with the reproductive physiology of the rabbit, since he had obtained his degree under Dr. William E. Castle at Harvard before doing postgraduate work in John Hammond's laboratories in Cambridge, England. Pincus had been trained in genetics and had experience working with rabbits. Dominant and recessive genes controlling fur quality, nature, and color he knew so well that he could have written a breeders' handbook from memory.

When he returned to Harvard, he continued the study of genetics by working with William Crozier on the inheritance of tropisms in the rat. He also began intense study of the reproductive system in several species.

I first met Pincus when I was an undergraduate at Harvard in the spring of 1931. His course in physiology was the best I had taken, and it was quite challenging to me as the rare undergraduate in the course. I

received my B.S. in 1933 and began graduate work with Pincus. For 6 years I worked for him in the laboratory, manipulating eggs, setting up equipment, sectioning tissue, doing surgery on rabbits, and cleaning cages, until I received my Ph.D.

Pincus had been much impressed with Jacques Loeb's work with amphibian eggs and determined to try to extend Loeb's success by bringing about parthenogenesis in rabbit eggs. Pincus's primary aim was to "determine what happens when the sperm penetrates the egg." His question could not be answered by an anatomical description at the cellular level; he wanted to be able to delineate the dynamics of the operating systems. Loeb's work on the amphibian egg had shown that what the sperm could do to an egg could be replicated with a needle [5]. Eggs stimulated by a needle prick, or by other means, began to divide and produced apparently normal females. Thus, if a similar finding could be made on a mammalian egg, it would seem clear that the systems involved were similar.[2]

Soon after Pincus joined the Department of General Physiology at Harvard, he began a series of studies with E. V. Enzmann on the behavior of the rabbit egg in situ and in vitro [9–14]. These studies led him to feel that parthenogenesis would be feasible with a rabbit egg. Pincus's choice of the rabbit instead of the rat or mouse was based on several observations. Rats and mice show an estrus cycle and ovulate during each cycle. But coitus is required for the corpus luteum to complete its development. Furthermore, not all copulations are adequate. Last but not least, one must continuously observe the female to know when ovulation is about to occur. These difficulties are not present in the rabbit.

In Pincus's laboratory, time was always at a premium. Thus an animal that could be put alone into a cage when it was purchased, checked for vaginal signs of heat, and kept with confidence that it would be in heat when needed lowered the work load. Equally important was the fact that with a rabbit one could predict with precision just what the eggs would be doing and where they would be, in tube or uterus, at any time after copulation.

Thus our practice (and mostly my duty) during these studies was to mate the animals at a specified time in the evening. We moved vasectomized, experienced males to the females' cages. The female who was designated to be a host mother was usually content before I left the room. On those rare occasions when we were trying to save time and attempted to mate a female too soon after her arrival in the colony, the bucks "informed" us of the inadequacy of our choice.

Of equal import here, too, on the point of a complete vasectomy in the

[2]Pincus got more publicity than he wanted from his attempts to extend Loeb's work. See especially [6–8].

experienced bucks is the fact that not infrequently the following day we failed in our efforts at the bench. For example, more potential host mothers were sometimes mated than were needed because superovulation of immature females produced too few eggs for planned experiments. Therefore a transplantation of treated eggs to the host mother (mated the night before) was not attempted. The female would be returned to the colony and used again after she had gone through pseudopregnancy. Additionally, as can be gleaned from the publications, few attempts at inducing parthenogenesis *and* pregnancy were successful; and, of course, no embryos or young were ever noted in those cases, even though the female had mated with the vasectomized buck.

Thus when in his papers on parthenogenesis Pincus refers to the use of vasectomized males before 1937, he is referring to males that I vasectomized and used in many, many matings. I know that they were sterile. If not, many more litters containing males would certainly have been produced. Moreover, the litters should have shown the expected fur type and color of the vasectomized buck and host mother's genetic patterns. But these rabbits came from commercial sources. Well "buried" recessives just might have merged in a rare sperm and host mother's egg to confound the result.

Pincus was a firm believer in Pasteur's dictum on the matter of trying to disprove your own positive findings. Therefore, he decided to spend his sabbatical and final year as a Harvard assistant professor on leave in England. He took me with him to the Animal Research Laboratory at Cambridge, then directed by the late John Hammond. The objective in going to England was in part to produce a live birth that was unquestionably due to an artificially activated egg. Hammond had developed an inbred strain of rabbits that could be used to identify parenthood with certainty.

I emphasize that while Pincus and I had no doubt that the vasectomized males we used at Harvard were sterile, there is no way to prove this fact in retrospect. One can mate such a male 100 times and obtain no offspring, but a skeptic can say that on the one-hundred-first occasion there could have been a few sperm in the ejaculate. By adding ultrastrict genetic controls to one's experiment with vasectomized males, even if there were doubts that they were sterile, the *females* born to the host mothers could be checked for the purity of their female ancestry [15; 16, chap. 8; 17].

Animal breeders know that there are both poor and proficient males and that after surgery or other stressors males may lose their vigor. It was for this reason that early in 1937 I asked Pincus what considerations he had given to having competent vasectomized males on hand soon after our arrival in Cambridge. He grinned in reply and said that Ham-

mond would have suitable males on hand when we arrived to begin work in the fall. In fact, some had already been prepared.

During 1937 a dependable preparation of human chorionic gonadotropin (CG) finally became available commercially. We had learned a great deal in our laboratory of the relationship between progesterone concentration and embryonic survival in the rabbit [18, 19]. We learned that a rabbit's corpus luteum induced by exogenous gonadotropin would be as effective in maintaining an embryo as was one produced by endogenous hormone released from the pituitary after mating.

Equally important, we knew that the time course of events induced by chorionic gonadotropin was the same as that induced by sterile mating. So we switched to its use, after many experiments had been performed in Cambridge, England, with sterile (so we assumed) bucks. The experiments using CG seemed absolutely foolproof, for we again obtained live births. This is the crucial point. No other investigator openly questioned it at the time. Until I read Ingle's comments, I was unaware that some friends of Pincus professed "uncertainty about the validity of the claim."

The day after Chamberlain and Hitler declared "Peace in our Time," I supervised the loading of a large crate of rabbits onto a freighter in London. Some were the offspring of host mothers who had received activated eggs. In addition, there was the nucleus of a colony derived from the strain of rabbits Hammond had developed. Pincus, before he left Cambridge, had selected the animals he wanted sent home. Spending the summer on the Continent with my family, I had gone from Luxembourg to Cambridge, where I found the crate made up and ready for me to ship home. The animals were identified by tattoos, labels, etc., to prevent error. Meanwhile, events had taken place which importantly affected both Pincus's and my careers as well as our experiments with parthenogenesis in the rabbit.

We had gone to England secure in the belief that upon our return in the fall of 1938 we would be working in a well-supported institution that approximated heaven. This dream was shattered in April 1938 by the "Roosevelt Recession." The expected grants and appointments were not forthcoming. Neither of us had an income in sight. We cut expenses to the bone and utilized every available device to increase the purchasing power of funds on hand. With his wife and daughter, Pincus returned to the States and sought a laboratory and funds to support his work. Pincus's son, my wife, and I stayed with a branch of my family in Luxembourg.

Eventually, after wasting money on cables back and forth, we sent John Pincus back to New York and settled down to await orders. When we reached Boston, my wife and I found that our new position was to be in Worcester, Massachusetts. Pincus became a visiting professor at Clark University, and I, his assistant. The laboratory was established in the

abandoned men's locker room of the defunct gymnasium. My salary was $25 per week. Five dollars per week went to repay Pincus the loan on which I had returned to the States. (Putting things in perspective, the anticipated appointment, which evaporated, would have paid me $50 per week, passage home [$100], and moving expenses to boot! The dollar has obviously depreciated since that time.)

The animals were kept in their crate until Dr. Mark Graubard and I built cages and moved them into their quarters, where Graubard and I cared for them. This was the beginning of the Worcester Foundation for Experimental Biology.

Hammond had been checking the ejaculates of his vasectomized males. Males had turned up in the litter of a host mother that had been served by one of them. There should have been none, since parthenogenesis produces only females. He found sperm in the ejaculate of one of the males. The females in this litter might have come from artificial parthenogenesis. Pincus ran through the genetic patterns involved and surmised that one of the females born in the suspect litter fitted the pattern appropriate for one descended solely from its mother and was thus parthenogenic in origin. Two other rabbits born at full term and reported in the same article [20] owe their foster mother's ovulation and preparation for pseudopregnancy to chorionic gonadotropin. The mothers had *not* been mated. Thus these females *were* undoubtedly parthenogenic.

About this time, Herbert Shapiro joined the group that was expanding at Clark under the efforts of Hudson Hoagland and Gregory Pincus. Now, in 1939 and 1940, there was no question of using anything but chorionic gonadotropin to induce ovulation. Shapiro and Pincus performed a critical experiment. Nothing could be simpler, cleaner, or more unequivocal. They induced ovulation in a female, they cooled the eggs in the fallopian tubes, and then sewed up the rabbit. They examined the uterus a few days later and found embryos. Eventually they allowed the mother to go to term and produced a live female rabbit [21]. Shapiro left the group at Worcester, and work on parthenogenesis ceased.[3]

[3]See Pincus's claim [20] and R. A. Beatty's later comments [22]. D. R. Austin in 1961 [23] said that there is "no certain evidence" of live births of parthenogenic rabbits, but Beatty argued convincingly that while the female rabbits produced in a litter derived from an improperly vasectomized male are suspect, five female rabbits brought to term must be accepted in the absence of massive proof to the contrary. These five rabbits include one produced in work with Herbert Shapiro [21] on cold-activated eggs. Parthenogenic, full-term rabbits were born, then, in separate experiments in Cambridge, Massachusetts, Cambridge, England, and Worcester, Massachusetts, as reported by investigators Pincus and Shapiro. Pincus's possible error of scientific reporting lay in claiming certainty for one of the three female rabbits reported as part of the mixed male-female litter from England. Note that the claim of parthenogenesis is *not* in dispute, only the production of full-term young. The latter Pincus accomplished.

Funds for research were scarce in the early 1940s unless one held a post in a well-supported university or research institute. This was infinitely more true then than today. Research on parthenogenesis was given minimal funding. Maintenance and experimentation on large numbers of rabbits has never been cheap.

Pincus was interested in endocrinology, especially as the endocrine secretions affected reproductive functions. Research funds were available for research on the steroids. A number of clinicians had proven the value of steroid hormones in human therapy. Drug houses were then as important a source of funds for endocrine research as are the National Institutes of Health today, but parthenogenesis was outside their field of interest. Since there was no money to support the work, it stopped. There was no reason then for Pincus to feel that additional work was needed merely to confirm what he had already done in Cambridge, Massachusetts, Cambridge, England, and Worcester, Massachusetts. If anyone questioned the work then, I didn't hear of it, nor, to my knowledge, did Pincus.

Later, when funds were at hand and the Worcester Foundation expanded, I was responsible for the conversion of existing buildings on the property of the Foundation and for the construction of new laboratories. Twice I built a unit at Pincus's request so that we could again take up parthenogenesis; both times he was too busy and the space was reworked for someone else. Funds were increasingly available, but time was not. That is why, when others had trouble repeating what Pincus had done so well, he did not do it again. Nor did I.

A number of rabbits who had no father were born of foster mothers in Pincus's laboratory. One of the requirements for proof of a proposition is that the supporting evidence be confirmed by others upon demand. This is the only gap in the proof for parthenogenesis. I hope that this account of the methods used will encourage interested scientists to repeat them.

There is only one sure way to go about certifying that a published report cannot be repeated. That is to go to the first author's laboratory and repeat the experiments under his direction. Much of science, especially in the biological area, requires special skills or undescribed local conditions that influence results. It took years, for example, for our research journals to exclude the term "room temperature." It varies too much around the world and from season to season.

It was fascinating for me to read, during the final preparation of this paper, an article by Jean L. Marx in *Science* [24]. It was under the heading of "Research News." Much of what is discussed in the article can be traced back to research initiated by Pincus. Toward the end of the article Brackett's work is discussed as follows (my italics):

Brackett says that it is frequently difficult to select suitable criteria to prove that a sperm has actually fertilized an egg. Some eggs can divide, *when appropriately stimulated,* even though they have not been fertilized (a process called parthenogenesis) or they may undergo degenerative changes that resemble those of a fertilized egg. The *ultimate criterion* is transplantation of the resulting embryo to a foster-mother and its subsequent development to a fetus. This criterion has been satisfied for the mouse and rabbit, but not for the human —although it has been tried.

It should be noted that Brackett is commenting in that passage on the difficulty of proving in vitro *fertilization.* In this research parthenogenesis is regarded as a potential source of error. Thus *female* offspring are suspect and require genetic proof of a father.

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