

Fertilization of Undenuded Rat Ova.* (18411)

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It was recently shown that rat ova can be fertilized preceding denudation of their follicle cells(1,2). Since the precise location of fertilization was not reported, it was desired to know if ova, surrounded by follicle cells and located in the oviduct, were still capable of being fertilized. Reports of previous investigators, based on an estimated time of ovulation, suggest that fertilization may occur in the oviduct(3-5), by none has shown that ova were actually in this part of the tract and surrounded by follicle cells at the time fertilization occurred. The present experiment will show that such ova can be fertilized.

Methods. It has been indicated that both ovaries of the mouse probably ovulate within a short time of one another(6,7); the same holds true for the rat(8). With these observations as a basis, the experimental procedure was to (a) breed rats late in heat, (b) immediately remove and examine one oviduct for the presence of ova, and (c) after a suitable period of time recover ova from the other oviduct and determine if fertilization had occurred. Rats which were in heat in the evening, as observed by lordosis when tested with males, usually remained in heat until at least 8 o'clock the following morning. Since the rat ovulates during late estrus(9) the animals in this experiment were bred between

5 and 7 o'clock of the morning following observed evening heat. Within a few minutes after ejaculation the oviduct and ovary were removed from one side. Those animals in whose oviducts no ova could be found were discarded. Four to 9 hours after copulation the animals were killed, and the remaining oviduct removed. The loop containing the ova was then transferred to a microscope slide and the ova pressed out into a saline solution. They were examined for the disposition of follicle cells, which, if present, were removed by hyaluronidase(10). The occurrence of fertilization was then determined by the presence of spermatozoa within the ova.

Results. Nineteen animals had ovulated at the time of copulation, and 98 ova were obtained from the remaining oviducts of these animals. Table I shows that 34 of the ova were fertilized, and that all but 2 of these were enveloped by follicle cells when recovered.

TABLE I. Fertilization of Rat Ova.

Animal No.	Bred before sunrise*	Hr between copulation and autopsy	Follicle cells surrounding ova	Ova recovered	
				Fertile	Non-fertile
1	+	7	+	2	3
2	+	7	+	4	1
3	+	7	—	0	4
4	+	7	+	9	1
5	+	7	—	2	3
6	+	7	+	2	0
7	+	7	+	4	0
8	+	7	+	0	6
9	+	9	+	1	5
10	—	6	+	5	1
11	—	6	—	0	5
12	—	9	—	0	6
13	—	5	+	0	3
14	—	5	—	0	3
15	—	5	+	0	7
16	—	5	+	0	4
17	—	5	+	0	6
18	—	4	+	5	0
19	—	5	+	0	6

* Period of experiment: Feb. 24 to May 6, 1950.

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Lewis and Wright(7) expressed the view that the mouse ovum is always fertilized before loss of its follicle cells, and Leonard, Perlman, and Kurzrok(1), as well as Austin(2) demonstrated that the recently fertilized rat ovum is usually found still enclosed by these cells.

In these previous experiments, however, no attempt was made to determine where fertilization occurred. There exists the possibility that as the ovum is forced from the ovary the associated follicle cells might be pulled aside sufficiently to facilitate entrance of the awaiting sperm. A subsequent closing-in of these cells would then give the impression that the sperm had worked its way through the entire surrounding follicle cell mass. In the present experiment, however, it was known that the ova surrounded by their follicle cells were in the ampulla of the oviduct before arrival of the spermatozoa, and yet the sperm were able to effect fertilization.

It will be noted in Table I that the first 9 animals yielded 24 fertilized ova out of a possible 47 recovered, whereas of the remaining animals, only 2 possessed ova with sperm. These first 9 animals were all bred before sunrise, but due to progression of the spring season the remaining rats were bred after sunrise. It is possible that with the approach of longer

days, the ovulation time was shifted to an earlier period with the result that the inseminations may have taken place too long after ovulation for fertilization to succeed. It is true that the lapsed time between insemination and examination of the ova for sperm was somewhat shorter in the animals bred after sunrise, but one rat had ova fertilized by 4 hours post-coitus. It could not be for the lack of sperm that fertilization failed in these animals since spermatozoa were observed in all of the oviducts but one. Blandau and Money(11) have reported spermatozoa in the ovarian portion of 100% of rat oviducts examined one hour after copulation.

Summary. Rats were bred late in estrus and the presence of ova in the oviducts determined by immediate removal and examination of one of the ducts. By later removal and examination of ova from the other oviduct, it was possible to demonstrate that rat sperm could fertilize non-denuded ova within the oviduct. The incidence of fertilization was markedly lowered by copulation late in estrus.

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Determination of Urea in Saliva. (18412)

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Although the concentration of urea plus ammonia ($U + A$) in saliva is known on the average to approximate that in blood(1-5), the determination of $U + A$ in saliva has not come into clinical use, probably on account of

the wide variations encountered in individual samples.

Various methods for blood urea have been applied to saliva. Whatever the chemical technic used, the manner of collection of saliva samples is more important. In most previous studies, the volume of saliva required for analysis was relatively large, and, to obtain sufficient samples, artificial stimulation of the salivary glands by chewing paraffin was resorted to. The increased rate of flow due to

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