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# Ovulation *in Vitro* in the Rat Ovary

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*(With 8 Textfigures and 1 Table)*

The mechanism of the discharge of the egg from the ovary —ovulation— is a matter of scientific interest in biology, as well as a subject of practical importance for the understanding to the principle of sexual reproduction in animal breeding. Studies on induced ovulation *in vitro* have been carried out rather extensively in amphibians by several authors mainly from physiological standpoints (Heilbrunn, Daugherty and Wilbur 1939, Samartino and Rugh 1945, Wright 1945, 1946, 1950, Nadamitsu 1953). In those animals *in vitro* ovulation is caused to occur in excised ovaries by a comparatively simple method with the addition of pituitary gland suspension into media. There are some discrepancies whether *in vitro* ovulation is induced by direct action of the anterior pituitary hormone on the gonad, or not (Samartino and Rugh 1945). Wilbur and McPhail (1944) reported that the ovulation-inducing principle of the *Rana pipiens* anterior pituitary hormone *in vitro* was potentiated by adding potassium fluoride.

Interest was shown by Nadamitsu (1957) in inducing ovulation *in vitro* in mammalian ovaries. Mouse ovaries excised in the oestrus stage and exposed to pituitary gland suspension did not respond by ovulation, while *in vitro* ovulation successfully was brought about in the ovaries which were exposed to saline solution containing potassium fluoride with previous injection of gonadotrophic hormones. The present author undertook a study of the same nature in rat ovaries being excited by an interest in the potentiating action of potassium fluoride on inducing ovulation *in vitro*. This paper is to report the results of his investigation.

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## Material and methods

Adult rats (*Rattus norvegicus*) of the *cbbh* strain were adopted as experimental animals for this study. The gonadotrophic hormone used was "Hypohorin"; a saline solution of extract of mammalian pituitaries (Teikoku Hormone MFG., Co.). Potassium fluoride solution was prepared in concentrations ranging from

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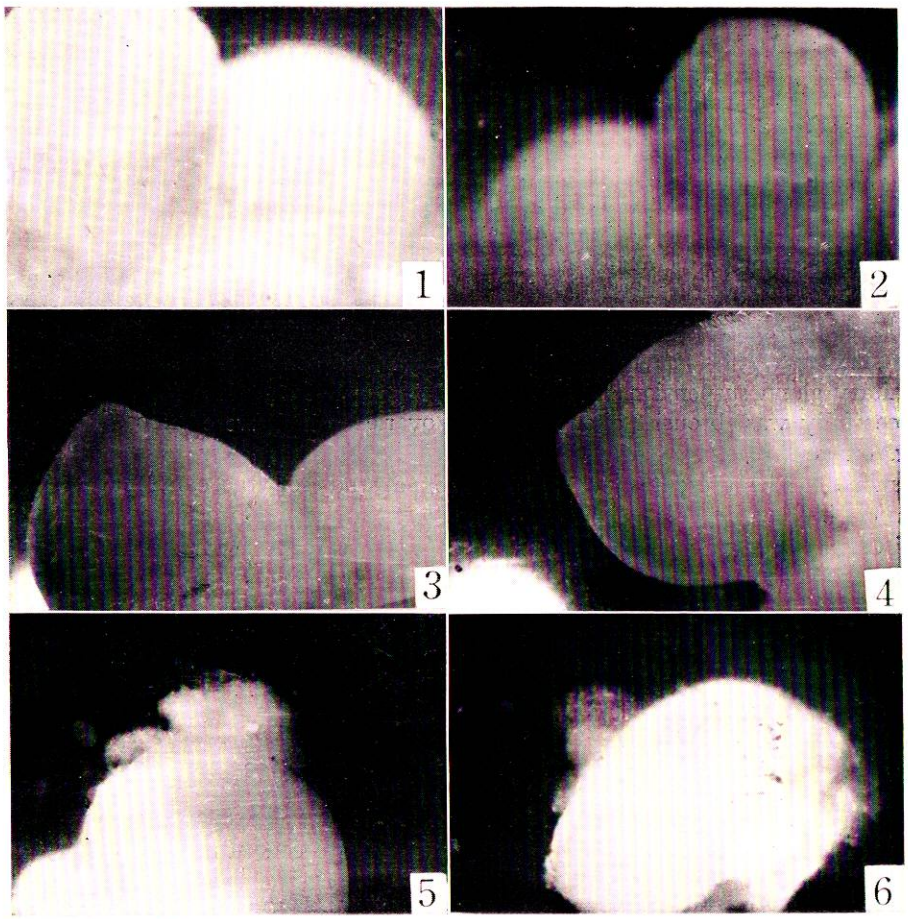
1×M/200 to 3×M/200 in saline. As the control, the following four solutions were used; saline solution alone, saline solution containing 5.0 rabbit units Hypohorin, saline solution containing M/200 potassium fluoride, and Ca-free saline. The rats received the injection of 5 to 12 rabbit units Hypohorin in total dosage during the period from 12 to 24 hours before operation. They were sacrificed in various stage of the oestrus cylce. One side of the ovaries of each animal was immersed in 20 cc saline solution containing M/200 to 3×M/200 potassium fluoride, and the other side ovaries were placed in control solutions as mentioned above.




Figs. 1–5. Successive changes in the cone-like elevation of a follicle and its rupture, observed on the surface of an ovarian follicle immersed in potassium fluoride saline solution. Fresh material. ×30.

Fig. 6. Rupture of a follicle. Fresh material. ×30.

The ova were investigated *in vitro* with the aid of phase optics. When necessary, they were fixed and stained by the hematoxylin-eosin method in order to enable observation with the ordinary microscope.

## Results

Induced ovulation sets on to take place in excised ovaries a few hours after their immersion in potassium fluoride saline solution. Figures 1 to 5 show successive changes observed on the surface of an ovary placed in the medium. An elevation like a small cone makes its appearance on the surface of the smooth ovarian surface at the onset of ovulation (Figs. 1–3). Then the rupture of the cone-like elevation takes place, and an ovum with the follicular fluid is found extruded in the medium (Figs. 5–6). There were a few instances in which the follicular fluid was run out without discharging any mature ovum. In Table 1 are shown the data and results of *in vitro* ovulation in the saline solution containing various concentrations of potassium fluoride. It is evident from this table that ovulation *in vitro* occurs in higher frequency in a medium containing M/200 potassium fluoride than in the media of other concentrations, and that 5 to 10 rabbit units of Hypohorin are favourable doses to induce ovulation in rat ovaries.

Table 1. Results of *in vitro* ovulation in rats

| Dose of Hypohorin (rabbit u.) | Stage of oestrus cycle | | Number of ova extruded | | |
|---|---|---|---|---|---|
| | At the time of injection | At the time of operation | Concentration of KF | | |
| | | | 3×M/200 | 1.5×M/200 | 1M/200 |
| 5 | Dioestrus | Dioestrus | — | — | 0 |
| 5 | Preoestrus | Oestrus | 0 | — | 2 |
| 5 | Oestrus | Oestrus | — | 1 | — |
| 5 | Oestrus | Metooestrus | 0 | — | 0 |
| 10 | Dioestrus | Dioestrus | — | — | 0 |
| 10 | Dioestrus | Preoestrus | — | 2 | 2 |
| 10 | Preoestrus | Oestrus | — | 3 | 6 |
| 10 | Preoestrus | Oestrus | — | 1 | 7 |
| 10 | Oestrus | Metooestrus | 1 | — | 1 |
| 15 | Dioestrus | Prooestrus | — | 1 | 2 |
| 15 | Dioestrus | Dioestrus | — | 0 | 1 |
| 15 | Prooesfrus | Oestrus | 2 | 3 | 6 |
| 15 | Metooestrus | Dioestrus | — | 0 | 0 |
| 15 | Oestrus | Oestrus | 1 | — | 3 |

The control data were obtained from experiments with the following materials: 1) ovaries immersed in saline solution with previous injections of 15 rabbit units hormone, 2) those placed in M/200 potassium fluoride without previous hormone injection, 3) those immersed in saline solution without previous hormone injection, 4) those placed in saline solution containing 5.0 rabbit units Hypohorin, and 5) those placed in Ca-free saline solution alone.

None of these control materials extruded an egg at all, though the follicular

puncture was observed in ovaries treated with potassium fluoride saline solution without Hypohorin injection.

The diameter of ova obtained in *in vitro* ovulation measures from 73 to 80 micra. It is apparent that the egg diameter is approximately similar between natural and artificial ovulations, since the egg diameter ranging from 70 to 80 micra was observed in the eggs from natural ovulation (Okigaki 1958). The number of eggs extruded varies from 1 to 7.

With the purpose to examine the maturation stage of eggs, extruded eggs in induced ovulation were fixed with Allen-Bouin's solution and stained with Heidenhain's or Delafield's hematoxylin. It was found that most of extruded eggs under study showed the second polar spindle persisting at metaphase (Figs. 7–8).

## Discussion

It has been shown by experimental ways that the anterior pituitary hormones induce ovulation in certain mammals and some other animals. But, the results of *in vitro* experiments indicate that no eggs were ovulated in the rat ovaries excised from animal which had received injections of gonadotrophic hormones and placed in saline solution. Wilbur and McPhail (1944) found that potassium fluoride possessed a potentiating effect on the anterior pituitary gland

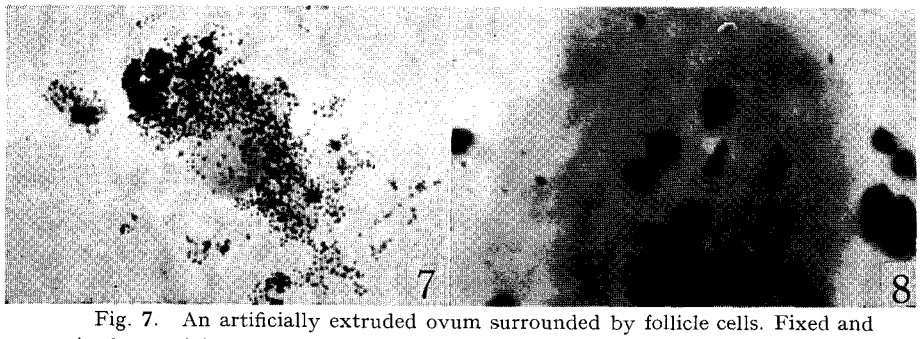



Fig. 7. An artificially extruded ovum surrounded by follicle cells. Fixed and stained material. ×100.

Fig. 8. Second polar spindle at metaphase, observed in an extruded ovum in *in vitro* ovulation. Fixed and stained material. ×800.

to induce *in vitro* ovulation in frogs. Nadamitsu (1957) also reported inducing ovulation *in vitro* in mouse ovaries through the use of a combination method of gonadotrophic hormone and potassium fluoride according to the principle of Wilbur and McPhail (1944). The present author similarly achieved the induction of *in vitro* ovulation in rat ovaries by following the method of Nadamitsu (1957) with a slight modification. Based on the evidence presented it seems probable that the mechanism of ovulation is not essentially dissimilar between amphibians and

mammals.

A consideration was offered by Wilbur and McPhail (1944) and Nakamitsu (1957) that the ova may start their maturation divisions under some effect of gonadotrophic hormones, and that potassium fluoride may break down the follicles by some chemical means. According to Wilbur and McPhail (1944) fluoride may exert its action through a binding of calcium which causes destruction of the stability of the intercellular cement substance of the follicle.

The number of extruded eggs which varies from 1 to 7 is less than that observed in natural ovulation, since in the latter 4 to 11 eggs were obtained (Okigaki 1958). Nadamitsu (1957) reported 1 to 21 eggs in *in vitro* ovulation in mouse ovaries.

The eggs extruded in *in vitro* ovulation show nothing different from those of natural ovulation, not only in their diameter but also in the state of maturation. The evidence seems to suggest that the eggs obtained in *in vitro* ovulation are, capable of fertilization and development, an item of the subject which calls one's attention for future study.

## Summary

In the present paper are presented some results of artificial ovulation investigated *in vitro* in rat ovaries. It was shown that eggs were ovulated in the rat ovaries which were excised from animals previously received injections of gonadotrophic hormones, and were placed in saline solution containing M/200 potassium fluoride. The data are as shown in Table 1.

The egg extruded in *in vitro* ovulation show nothing dissimilar from those obtained in natural ovulation, so far as their diameter and stage of maturation are concerned.

No ovulation was achieved in the ovaries placed in : 1) saline solution with or without hormone injection, 2) saline solution containing M/200 potassium fluoride without hormone injection, 3) saline solution containing gonadotrophic hormones, and 4) Ca-free saline solution.

## Literature cited

Heilbrunn, L. v., K Daugherty and K.M. Wilbur 1939. Initiation of maturation in the frog eggs. Physiol. Zool. 12 : 97-100.

Nadamitsu, S. 1953. Ovulation *in vitro* in several species of amphibians. J. Sci. Hiroshima Univ. Ser. B, Div. 1, 14 : 151-157.

———— 1957. Artificial ovulation of mammalian ovaries *in vitro*. J. Sci. Hiroshima Univ. Ser. B, Div. 1, 17 : 47-50.

Okigaki, T. 1959. Some microscopical obserations on natural and artificial ovulation in mature and immature albino rats (*Rattus norvegicus*). Jap. J. Genet. 34 : 15-22.

Samartino, G.T. and R. Rugh 1945. Frog ovulation *in vitro*. J. Exp. Zool. 98 : 153-159.
Wilbur. K.M. and M. K. McPhail 1944. Production of ovulation by fluoride *in vitro*. Nature 154 : 83-84.
Wright, R.A. 1945. Factors affecting *in vitro* ovulation in the frog. J. Exp. Zool. 100 : 565-575.
———— 1946. Sensitization of the frog ovary following hypophysectomy. Physiol. Zool. 19 : 359-364.
———— 1950. Time relationships in frog ovulation. J. Exp. Zool. 114 : 465-474.