Studies on the Mechanism of Ovulation in the Fowl

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Introduction

It is established that the ovulation in the fowl is induced at the stigmal region[1)2)3)] by the cyclic release of the luteinizing hormone (LH) from the pituitary[4)]. It is not clear, however, whether LH acts alone on ovulation or it's biological function is brought into activity when it works with other hormones or blood constituents, for example, blood protein. To clear up these problematical points, excised follicles removed from the hen autopsied at the various periods before the expected ovulation were ovulated in vitro by pouring the solution containing HCG alone or both HCG and blood plasma.

By these means were carried out the removal of the somewhat confused factors coming from endogenous hormones or blood constituents in case of LH injection to the fowl and the clarification of the problematical point whether LH acts alone or in concert with other hormones.

Materials and methods

The birds used in this study were S. C. White Leghorn females in their first and second year of egg production. All hens were maintained in laying cages, and were fed commercial laying mash. Their egg productions were recorded hourly from 8:00 A. M. to 4:00 P. M. every day by routine procedure, experimental hens being selected of them. The hens were killed by decapitation, and immediately after, follicles were excised and used for experiment. The follicles used were, in all cases, the second or subsequent ones of a clutch; no initial and terminal ones being used.

The experiments are broadly divisible into three classes in connection with the time before the expected ovulation; 1) experiments on the follicle excised immediately after oviposition, 2) experiments on the follicle removed about 2～3 hours before the time of the expected ovulation and 3) experiments on the follicles removed about more than 24 hours before the time of the expected ovulation. These were shown in Figure 1.

In connection with the treatment, the poured fluid in these experiments may be divided into four categories; Ringer's solution (Control), Ringer's solution added HCG., blood plasma added HCG and blood plasma prepared from the HCG-injected-hen.

The states of ovulations are divided into three types; 1) complete ovulation (ovulation occured as that of the normal hen), 2) incomplete ovulation (ovum was burst in the

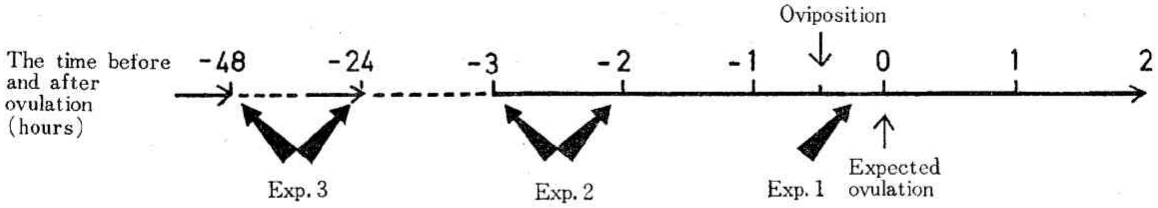


Fig. 1. The procedure in this study

Exp. 1. Several experiments were carried out by using the excised follicles immediately following oviposition

Exp. 2. Several experiments were performed by using the follicles removed 2~3 hours before the time of expected ovulation

Exp. 3. Several experiments were performed by using the follicles removed more than 24 hours before the time of expected ovulation

middle of ovulation) and 3) non ovulation (no ovulation could occur).

The methods of each experiment are as follows;

(1) The study of ovulation in vitro on the follicles removed from the ovary immedia tely following oviposition.

The results reported by Nehr, Olsen and Fraps (1950)[5] that the mature ovarian follicle removed from the ovary some 15 minutes following the oviposition of the preceding egg of the same clutch, ovulated in vitro, were confirmed by us, the percentage of ovulation being used for control to the series of this study.

a) The experiment in the deep Petri dish kept moist with Ringer's solution.

Five hens were sacrificed immediately following oviposition. The largest follicle from each hen was excised at its stalk and was placed in a deep Petri dish (about 9 *cm* in diameter), in which the absorbent cotton saturated with Ringer's solution was spread on the bottom of the dish (Figure 2).

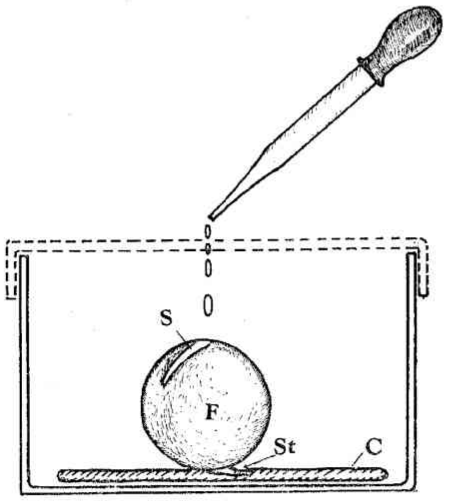


Fig. 2. The Figure of the excised follicle which was poured with fluid
F : Follicle St : Stalk
S : Stigma
C : The absorbent cotton saturated with Ringer's solution

Then it was placed in an electrically heated incubator maintained at the temperature of approximately 42° C. The positions of the stigma and stalk were shown in Figure 2. To protect the surface of the follicle from drying, the exposed portion of it was poured with Ringer's solution at 42°C every 10 minutes, and the possibility of ovulation-occurrence was observed.

b) The experiment in the Ringer's solution maintained at 42°C. As the pouring of solution was somewhat perplexing in the previous experiments, the excised follicle was placed in 300 *ml* Ringer's solution at

42°C and the possibility of ovulation in the fluid was observed (Figure 3).

(2) Possibility of ovulation in vitro on the excised follicle removed about 2~3 hours before the time of the expected ovulation.

a) The experiment in the deep Petri dish kept moist with Ringer's solution.

Seven hens were slaughtered 2~3 hours before the time of the expected ovulation, and the largest follicle in each hen was removed and treated by the same method as in the experiment (1)-a).

b) The experiment in the Ringer's solution maintained at 42°C.

Using 5 hens, the possibility of ovulation in the Ringer's solution maintained at 42°C was observed with the same method as in the experiment (1)-b).

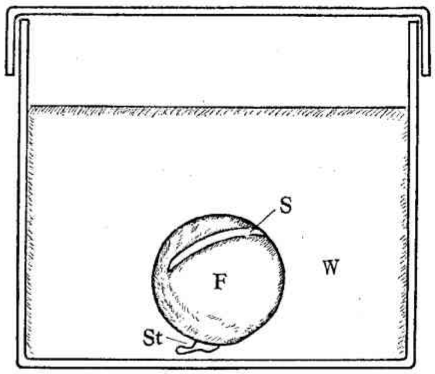


Fig. 3. Figure of the excised follicle in the Ringer's solution
F : Follicle
S : Stigma
St : Stalk
W : Ringer's solution

(3) The effect of HCG on the follicle removed about 2~3 hours before the time of the expected ovulation.

a) The effect of HCG on the excised follicle in the deep Petri dish kept moist with Ringer's solution.

The excised follicles from three experimental hens were poured with 500 *u.* HCG in 10 *ml* of Ringer's solution maintained at 42°C, and whether ovulation occurs or not was observed by the same method.

b) The effect of HCG on the follicle placed in the Ringer's solution.

The excised follicles from 7 hens were placed in 300 *ml* Ringer's solution containing 3.3 *u.* (4 hens) or 250 *u.* HCG (3 hens) at 42°C, and the possibility of ovulation was observed.

(4) The effect of blood plasma on the follicle removed about 3 hours before the time of the expected ovulation.

In this experiment, 4 hens were used. The excised follicle was poured with 10 *ml* blood plasma collected from another hen. Sodium citrate solution (3.8 % $Na_3\ C_6\ H_5\ O_7\ 2\ H_2\ O$) was used to prevent the blood coagulation and blood plasma was prepared by 3000 *rpm* centrifugation.

(5) State of ovulation affected with HCG injected 3 hours before the time of the expected ovulation and circulated for an hour in the blood stream.

Four hens were treated in this experiment. Namely, 500 *u.* or 250 *u.* HCG was injected into their blood vessels 3 hours before the time of the expected ovulation. An hour after injection or 2 hours before the time of ovulation, they were sacrificed to excise the largest follicle.

These excised follicles were placed in a deep Petri dish each with the same manner as in the previous experiment, and they were poured with Ringer's solution. The process of experiment was shown in Figure 4.

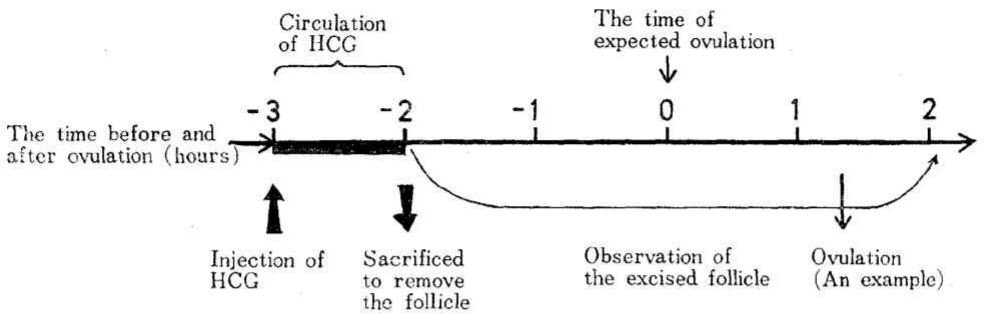


Fig. 4. State of ovulation affected with HCG which was injected 3 hours before the time of expected ovulation and circulated for an hour in the blood stream.

(6) The effect of blood plasma, separated from the blood of another hen in which HCG had been circulating for an hour, on the follicle excised 2~3 hours before the time of the expected ovulation.

At first, to the hens fed with the sole purpose of collecting the blood plasma HCG (500 *u.* or 250 *u.*) was injected in the one of the wing-vein, and after an hour the blood was collected from the other vein to separate the blood plasma. Secondly the experimental birds were sacrificed 2~3 hours before the time of the expected ovulation to excise the largest follicle. The follicle was poured with the above blood plasma. In this experiment, 5 hens were used and the process of experiment was shown in Figure 5.

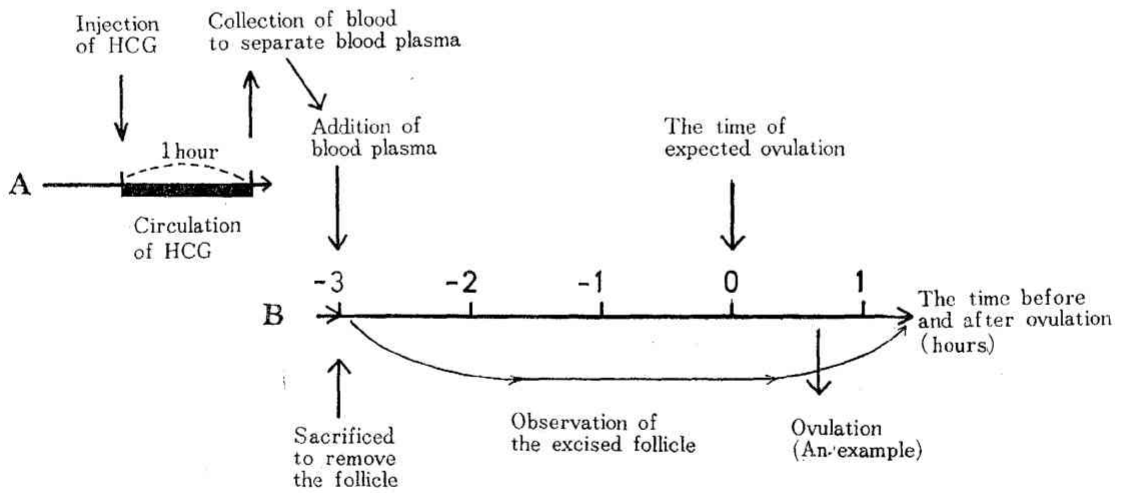


Fig. 5. The effect of blood plasma separated from the blood of another hen in which HCG had been circulating for an hour on the follicle
A: A hen from which blood plasma was collected
B: An experimental hen

(7) The effect of the blood plasma mixed with HCG on the follicle removed 2~3 hours before the time of the expected ovulation.

In this experiment, three hens were used. The largest excised follicle was placed in an

incubator by the usual method. HCG 500 *u.*, 250 *u.* and 125 *u.* were mixed in each 10 *ml* blood plasma collected from another hens and the excised follicles were poured with the mixed blood plasma every 10 minutes.

(8) The effect of the blood plasma mixed with HCG on the follicles removed more than 24 hours before the time of the expected ovulation.

Six experimental hens were sacrificed to excise the first, second and third follicles. These surgical operations were performed quickly.

The respective follicles were placed in the deep Petri dish in an incubator and poured with the blood plasma mixed with 500 *u.* or 250 *u.* of HCG every 10 minutes. The blood plasma was collected from another hens, thoroughly mixed with HCG, warmed at 42°C and used.

(9) The effect of the blood plasma mixed with HCG on the excised follicles removed from the hen treated with PMS previously.

In this experiment, 6 hens were used. They were previously injected in muscles with 500 *u.* PMS to increase the number of the large sized follicles. Then several large follicles were removed about 70～120 hours after single injection of PMS and they were placed in each Petri dish which was put in the incubator maintained at 42°C. And they were poured with the mixed blood plasma (500 *u.* HCG in 10 *ml* of blood plasma) like the experiment 7, the possibility of ovulation being put under observation.

(10) The effect of high temperature on ovulation.

Three experiments were performed to determine the effect of high temperature on ovulation. In each experiment, 3 hens were treated.

The hens previously injected with PMS were sacrificed about 90～100 hours following PMS treatment, and the largest, the second and the third follicles were removed from the ovary. They were placed quickly in each Petri dish situated in the incubator about 43°～44°C.

Following the above process, 3 experiments were performed as follows;

a) the excised follicles were poured only with Ringer's solution,

b) they were poured with the Ringer's solution (10 *ml*) mixed with HCG (500 *u.*),

c) they were poured with the blood plasma (10 *ml*) mixed with HCG (500 *u.*), and the ovulations of them were observed by the same method.

Results and discussion

(1) The study of ovulation in vitro on the follicles removed from the ovary immediately following oviposition.

Results are shown in Table 1. Average time from oviposition to ovulation is 60.4 minutes, ranging from 27 to 91 minutes. Nehr, Olsen and Fraps[5] performed the study of ovulation in vitro, using 75 hens. The follicles of 64 hens were ovulated of them. Moreover they reported that the average time from oviposition to ovulation was 57.8 minutes, ranging 20～130 minutes. The result of our experiment is similar to those of theirs. Adding to this, in the experiment using a follicle immersed in the Ringer's solution, an occurrence of the complete ovulation was observable, though the number of the

Table 1. Ovulation in vitro on the follicles removed from the ovary immediately following oviposition

Bird No.	Interval between oviposition and sacrifice (min.)	Interval between oviposition and ovulation (min.)	State of Ovulation
32	15	32	○
43	15	88	○
3	19	27	○
2	2	64	○
29	4	91	○
Average	11	60.4	

○……Complete ovulation

experiment performed was only one (Experiment 1-b).

From these results we considered that both in deep Petri dish and in Ringer's solution (300 *ml*), the inducement of ovulation was possible, but in the fluid the interval between the beginning and the termination of ovulation was very long as compared with normal ovulation; one to three minutes in the Petri dish and in the normal ovulation and 9.3 minutes in Ringer's solution.

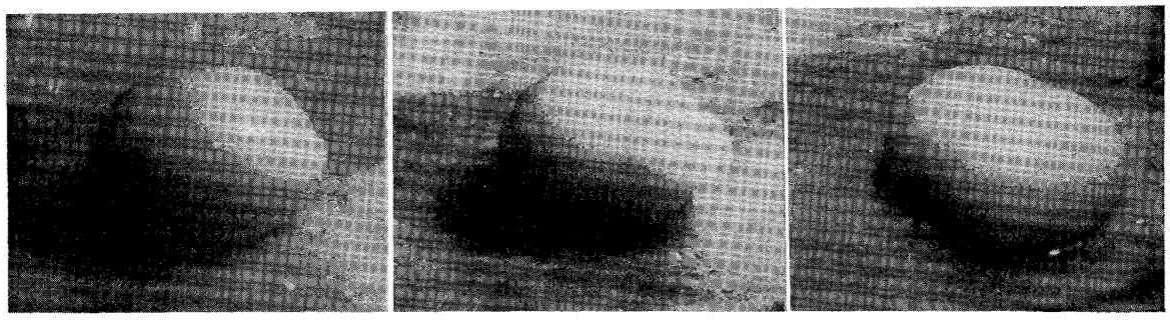
The process of normal ovulation was as follows; At first, one side of the stigma clumsily buldged, and along the stigma the follicle burst completely to another side of the stigma, and an ovum slipped out of the follicle, which collapsed. This condition was shown in plate 1 and the ovulation in the Ringer's solution was represented in plate 2.

As we were able to recognize ovulation in vitro, we used this technique to perform a series of this study.

(2) Possibility of ovulation in vitro on the excised follicle removed about 2~3 hours before the time of the expected ovulation.

In this experiment, the possibility of ovulation on the largest excised follicle in which endogenous LH had not acted sufficiently was determined in vitro.

a) The experiment in the deep Petri dish kept moist with Ringer's solution. The results are summarized in Table 2. According to these data, three follicles could ovulate in vitro, but the other (4 follicles) could not.



1 2 3

Plate 1. Ovulation in vitro (1→2→3)
(in the Petri dish)

b) The experiment in the Ringer's solution maintained at 42℃. As shown in Table 3, ovulation could not occur in all the excised follicles.

These findings suggest that the effect of the endogenous LH to induce ovulation is not satisfactory on the follicle removed 2 ~3 hours before the time of the expected ovulation. In case of the experiment b), as the follicle was immersed in the Ringer's solution, the external pressure of fluid might be one of the causes to make the ovulation difficult. And among the follicles there might be a considerable difference in the sensitivity to the endogenous LH or in the releasing time of LH.

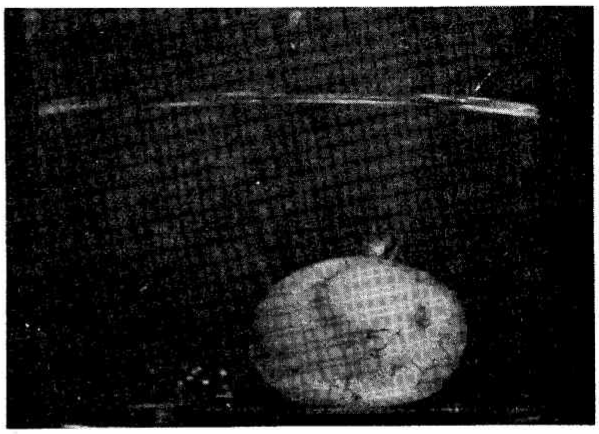


Plate 2. Ovulation in vitro (in the Ringer's solution)

Table 2. Possibility of ovulation in vitro on the follicle removed about 2~3 hours before the time of ovulation

Bird No.	From the time of sacrifice to the time of the expected ovulation (min.)	From the time of sacrifice to the time of ovulation (min.)	State of ovulation
A 48	175		—
A 63	165		—
95	116		—
59	177	127	○
70	195	198	○
54	170	165	○
120	173		—

○……Complete ovulation
—……No ovulation

Table 3. Possibility of ovulation in the Ringer's solution maintained at 42℃ on the follicle removed about 2 hours before the time of the expected ovulation

Bird No.	From the time of sacrifice to the time of the expected ovulation (min.)	State of ovulation
113	120	—
6353	119	—
6495	122	—
28	120	—
115	120	—
Average	120.2	

— ……No ovulation

(3) The effect of HCG on the follicle removed about 2～3 hours before the time of the expected ovulation.

According to the above experiment, it was recognized that the effect of the endogenous LH to the follicle removed about 2～3 hours before the time of the expected ovulation was not sufficient enough to induce ovulation.

If these excised follicle in vitro is poured with the exogenous LH continuously to complement the deficiency of endogenous LH, it is presumed that ovulation of the excised follicle may be induced. Then, instead of LH, HCG having the similar effect to that was added to the excised follicle in the deep Petri dish or in 300 *ml* Ringer's solution and whether ovulation of the excised follicle in vitro might occur or not, was determined.

a) The effect of HCG on the excised follicle in the deep Petri dish kept moist with Ringer's solution.

The results are shown in Table 4. No follicles could ovulate.

Table 4. The effect of HCG on the follicle removed about 2-3 hours before the time of the expected ovulation

Bird No.	From the time of sacrifice to the time of the expected ovulation (min)	State of Ovulation
314	175	—
377	150	—
381	190	—

— ···No ovulation

b) The effect of HCG on the follicle placed in the Ringer's solution. At first, the effect of small amount HCG (3.3 *u.*) was determined, and secondly the effect of large amount HCG (250 *u.*) was examined by the same method. The results are shown in Table 5. In the former case, 3 of 4 follicles could not ovulate, and in the latter case no

Table 5. The effect of HCG in 300cc Ringer's solution on the follicles removed about 2-3 hours before the time of the expected ovulation

Bird No.	Additional HCG (u)	From the time of sacrifice to the time of the expected ovulation (min)	State of ovulation
6374	3.3	120	—
144	〃	120	○
79	〃	105	—
59	〃	120	—
51	250	120	—
163	〃	123	—
106	〃	120	—

○·····Complete ovulation
—·····No ovulation

follicles were able to ovulate. From these results, it was considered that the effect of HCG to induce ovulation, by itself, was scarcely recognizable.

(4) The effect of blood plasma on the follicle removed about 3 hours before the time of the expected ovulation.

In this experiment, the excised follicles were poured with the blood plasma collected from laying hens. This blood plasma was expected to contain small amount of endogenous LH. The occurrence of ovulation was possible in 2 of 4 follicles as shown in Table 6. The size of the ovulated follicles had a tendency to be a little larger than that of the other, so it was considered that their sensitivity might be higher than that of other follicles. From these results, it might be presumed that the blood plasma containing a small quantity of LH had a little effect to ovulate the follicle removed 2~3 hours before the time of the expected ovulation.

Table 6. The effect of blood plasma on the follicle removed about 3 hours before the time of the expected ovulation

Bird No.	From the time of sacrifice to the time of the expected ovulation (min)	From the time of sacrifice to the time of ovulation (min)	State of ovulation
3	196		—
A 57	185		—
39	193	153	○
A 69	195	104	○

○……Complete ovulation
—……No ovulation

(5) State of ovulation affected with HCG injected 3 hours before the time of the expected ovulation and circulated for an hour in the blood stream.

The results are shown in Table 7. In this data, complete ovulation occured in 3 of 4 follicles. So, HCG injected 3 hours before the time of the expected ovulation and circulated for an hour seemed to be very effective to induce the ovulation of excised follicle.

Table 7. The effect of HCG injected 3 hours before the time of ovulation and circulated for an hour in the blood stream.

Bird No	Injected HCG (u)	From the time of injection to the time of ovulation (min)	The time in which HCG circulated (min)	From the time of sacrifice to the time of ovulation	State of ovulation
66	500	175	55	120	○
18	〃	163	50	113	○
13	250	170	58	112	○
120	〃	173	51	122	—

○……Complete ovulation
—……No ovulation

(6) The effect of blood plasma separated from the blood of another hen in which HCG had been circulating for an hour, on the follicle excised 2～3 hours before the time of the expected ovulation.

From the results mentioned previously, it was recognized that the LH, by itself, had little effect to induce the ovulation of excised follicle, but the blood plasma containing LH or HCG had a possibility to induce ovulation. This reason might be the effect of blood plasma combined with or co-operated with LH or HCG. So, in this experiment the follicle excised 2～3 hours before the time of the expected ovulation was poured with blood plasma of another hen in which HCG had been injected an hour before the experiment.

As showned in Table 8, ovulation was induced in all follicles. Three follicles of them could ovulate before the time of the expected ovulation.

Table 8. The effect of the blood plasma treated with HCG on the follicle excised 2～3 hours before the time of ovulation

Bird No	The time in which HCG circulated	From the time of sacrifice to		State of ovulation
		the time of the expected ovulation (min)	the time of ovulation	
27	55	168	163	○
103	57	173	175	○
115	70	148	41	○
89	30	175	72	○
102	35	172	175	○

○……Complete ovulation

In this experiment, though the injected HCG was much larger than the blood level of the circulated LH in intact hen, the quantity of HCG in the used blood plasma was very small, for HCG had circulated for an hour in the blood stream and the collected blood was about 20 *ml.* It was very interesting that this blood plasma was more effective than the addition of large quantity of single HCG.

(7) The effect of the blood plasma mixed with HCG on the follicle removed 2～3 hours before the time of the expected ovulation.

As the blood plasma containing HCG circulated in the blood stream for an hour had a greater effect to induce the ovulation in vitro, in this experiment the blood plasma (10 *ml*) was mixed directly with HCG (500 *u.*, 250 *u.* and 125 *u.*) and it was poured on the excised follicle to induce ovulation in vitro. As shown in Table 9, all follicles were ovulat-

Table 9. The effect of the blood plasma (10ml) mixed with HCG on the follicle removed about 2-3 hours before the time of ovulation

Bird No.	HCG mixed with blood plasma (u)	From the time of sacrifice to		State of ovulation
		the time of the expected ovulation	the time of ovulation	
30	500	170	74	○
1	250	164	88	○
124	125	185	190	○

○……Complete ovulation

ed perfectly. From these experiments, we concluded that the effect of HCG to induce ovulation must increase greatly by being mixed with blood plasma.

(8) The effect of the blood plasma mixed with HCG on the follicles removed more than 24 hours before the time of the expected ovulation.

In experiment 7, we confirmed that the blood plasma containing HCG (or mixed HCG) was most effective to induce ovulation on the excised follicle, then in this experiment, whether the same blood plasma might be in possession of an effect to ovulate on the follicle removed more than 24 hours before ovulation or not was determined. As shown in Table 10, in the case of blood plasma mixed with 500 *u.* HCG, the second follicles of 2 hens were able to ovulate, and moreover, the third follicle of No. 99 was ovulated completely.

Table 10. The effect of the blood plasma mixed with HCG on the follicle removed more than 24 hours before the time of ovulation

Bird No.	Treatment	The first follicle		The second follicle		The third follicle	
		State of ovulation	Sacrificed I.O / E.O	State of ovulation	Sacrificed I.O. / E.O	State of ovulation	Sacrificed I.O. / E.O
A 19	Blood plasma 10*ml* + HCG 500 *u.*	△	120*min* / 163	△	247 *min* / more than 24*hrs*		*min*
A 82	〃	△	112 / 173	△	149 / more than 24*hrs*		
99	〃	○	81 / 158	○	91 / more than 24*hrs*	○	107 / more than 48*hrs*
75	〃	○	75 / 140	○	260 / more than 24*hrs*	△	438 / more than 48*hrs*
70	Blood plasma 10*ml* + HCG 250 *u.*	○	55 / 150	○	60 / more than 24*hrs*	—	
93	〃	○	75 / 175	○	85 / more than 24*hrs*	—	

I. O.……The time of the induced ovulation
E. O.……The time of the expected ovulation
○……Complete ovulation
△……Unnatural ovulation (Ovum was burst in the middle of ovulation)
—……No ovulation

In the case of the blood plasma mixed with 250 *u.* HCG, the second follicles in both hens were also ovulated. It means that ovulation is induced on the follicles removed more than 24 hours before the time of the expected ovulation.

(9) The effect of the blood plasma mixed with HCG on the excised follicle removed from the hen treated with PMS previously.

The second and the third follicles that had ovulated in the experiment 8 had a tendency to be large sized follicles, so it was supposed that the sensitivity of the follicle might be concerned with the size of follicle.

Then, in this experiment, a number of comparatively larger follicles were made by single injection of PMS in 70～120 hours before autopsy. The sizes of follicles were increased with PMS, but most of them were about 2 *cm* in diameter. So, the follicles used for this experiment, 3-4cm in diameter, were only 3 or 4 in number. The fol-

licles were poured with the blood plasma mixed with HCG 500 *u*. The results are shown in Table 11.

Table 11. The effect of blood plasma mixed with HCG on the excised follicle injected previously with PMS

Bird No.	Interval between PMS injection and treatment	Treatment	The first follicle	The second follicle	The third follicle	The fourth follicle
A 13	70	Blood plasma 10*ml* + HCG 500 *u*.	○	○	○	△
A 84	70	〃	—	—	—	
140	71	〃	○	○	○	△
A 72	76	〃	○	○	△	
A 52	86	〃	○	—	—	
75	118	〃	○	○	—	

○ ····· Complete ovulation
△ ····· Unnatural ovulation
— ····· No ovulation

Though there were a few exceptional follicles, the effect to induce ovulation was recognized in a large number of them; 5 samples in the first follicle, 4 samples in the second follicle and 2 samples in the third follicle were completely ovulated. Even in the fourth follicle of No. 140, nearly complete ovulation was achieved, though finally the ovum was broken.

From this result, it is considered that the ovulation in the follicle treated previously with PMS was easily induced.

(10) The effect of high temperature on ovulation.

In the repeatedly performed experiments, we had the impression that ovulation time was promoted when the ovum was put under higher temperature, and this experiment was performed to determine it. The results were shown in Table 12. Ovulation occured only in the experiment of the blood plasma mixed with HCG, and in another experiment ovulation did not occur. From these results, it was considered that the high temperature had no effect on ovulation.

Summary

To clarify the effect of LH on the mechanism of ovulation in the fowl, a series of the following experiments were performed by the method of ovulation in vitro.

1) In the excised follicles removed 2～3 hours before the time of the expected ovulation, whether they could ovulate in vitro without any addition of HCG or not was determined. From the result of this experiment, it was recognized that the follicle scarcely ovulated. So, it was presumed that in 2～3 hours before the time of the expected ovulation the effect of the endogenous LH to induce ovulation was not sufficient.

2) Various experiments of HCG addition on the follicle removed 2～3 hours before the time of the expected ovulation in which the effect of endogenous LH was unsatis-

Table 12. The effect of high temperature on ovulation

Bird No.	Interval between PMS injection and treatment	Treatment	The first follicle	The second follicle	The third follicle
5	72 *hrs*	Ringer's solution 10 *ml*	—	—	—
A 33	94	〃	—	—	—
24	94	〃	—	—	—
7	89	Ringer's solution 10 *ml* +HCG 500*u*	—	—	—
44	90	〃	—	—	—
13	93	〃	—	—	—
83	94	Blood plasma 10 *ml* + HCG 500 *u.*	○	○	—
43	95	〃	○	○	○
34	98	〃	○	—	—

○···Complete ovulation
—···No ovulation

factory were performed. In all experiments, most of follicles did not ovulate, and it indicated that HCG, by itself, had no effect to ovulate the follicle.

3) Then the follicles removed 2～3 hours before the time of the expected ovulation were poured with the blood plasma containing HCG in several experiments. According to these experiments, most of follicles were ovulated. And it was found that the poured fluid was effective and especially the blood plasma 10 *ml* + HCG 500 *u.* was quite effective.

From these results, we concluded that HCG became active when it was mixed or co-operated with blood plasma.

4) Not only the first follicle (the largest one) but the second and the third follicle were poured with the blood plasma mixed with HCG to determine whether ovulation in vitro might occur or not. From the result of this experiment, it was concluded that ovulation could occur in the follicle removed more than 24 hours before the time of the expected ovulation.

5) The large sized follicles (which were smaller than the normal largest follicle) excised 70～120 hours following PMS injection were treated with the blood plasma mixed with HCG in the same manner as the ones mentioned above. According to this result, ovulation could be induced not only in the first follicle but in the second ones.

6) The high temperature had no effect on ovulation.

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