The Capacitation of the Fowl Spermatozoa

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Introduction

In rats and rabbits some descriptions were made by Chang (1951)[1] (1955)[2] and Austin (1952)[3] on the necessity for the freshly ejaculated spermatozoa to reside in the female genitalia for a certain length of time before their acquisition of the capacity to fertilize the ovum, this necessity hitherto having been known as "capacitation".

Namely, when introduced immediately or shortly after ovulation it was seldom that the spermatozoa could fertilize the ovum {Chang (1951)[1] (1955)[2]}, though when introduced into the Fallopian tubes of rabbits 5 to 6 hours before ovulation they could fertilize it {Austin (1948)[4] (1949)[5]}.

Since the confirmation of capacitation not a small number of studies about those of other domestic animals have been carried out by several workers, and the requirement for capacitation of spermatozoa was described in the golden hamster[6] and ewe. Austin and Bishop (1958)[7] reported that capacitation was represented by the induction of changes leading to the detachment of the acrosome. Moreover it was shown by Chang (1957)[8] that the fertilizing capacity developed in the uterus was lost when spermatozoa were treated with seminal plasma, but the capacity was to be recovered when they were permitted to remain in the female tract longer. Noyes, Walton and Adams (1958)[9] demonstrated that capacitation can occur, to some extent at least in non-genital organs, namely, bladder, colon and the anterior chamber of the eye. Contrarily Charles et al. (1967)[10] indicated that the capacitation in the rabbit was limited to the female reproductive tract.

But in the fowl, the existence of capacitation has been clarified scarcely. The aim of this investigation is to examine whether capacitation is necessary for fowl spermatozoa, or not.

Materials and Method

In this trial, S. C. White Leghorn chicken were used and two trials were conducted. In the first trial, three cockerels and ten virgin pullets, and in the second trial, 5 males and 10 females were used.

They were kept in the individual cages and were received laying mash.

50 pullets were previously investigated for laying frequency and type of laying cycle. Of these pullets, only the ones which were expected to lay at least two eggs successively, on the day and the next day of the insemination of semen, were used for trial.

Shortly after laying, the pullets in the first trial were inseminated with 0.1*ml.* of diluted semen into the lower part of the infundibulum by the method mentioned below.

In the second trial, 0.1*ml* of undiluted semen (3.7 million/mm^3) was inseminated.

The fertlity of the eggs laid on the following day after insemination was determined to investigate capacitation, and the duration of fertility was also observed (during the two-week-period) for reference.

The details of techniques are as follows ;

1. Semen collection and assessment of semen quality: Dense clear semen (5–8 million spermatozoa/mm^3) was collected from several cockerels, by the selective collection technique[11], the modified method of the abdominal massage technique by Burrows and Quinn (1937)[12]. After the collection, spermatozoal concentration was determined by the optical density method and the semen was diluted with Ringer solution up to the density of 1 million spermatozoa per cubic millimeter. Semen collection and dilution were carried out just before the insemination.

After the insemination was completed, the motility of spermatozoa was scored on the basis of 0–5.

Spermatozoal concentration of the diluted semen was again determined by the routine microscopical method to ascertain the actual number of the spermatozoa inseminated.

2. Insemination of semen :

The pullet was fastened on a board and an incision was made from behind the left last costa, then the wound was opened. From the wound, the site of insemination or lower part of the infundibulum was confirmed, the part having been sustained previously by the injection of acid violet solution in the preliminary experiment (Fig. 1).

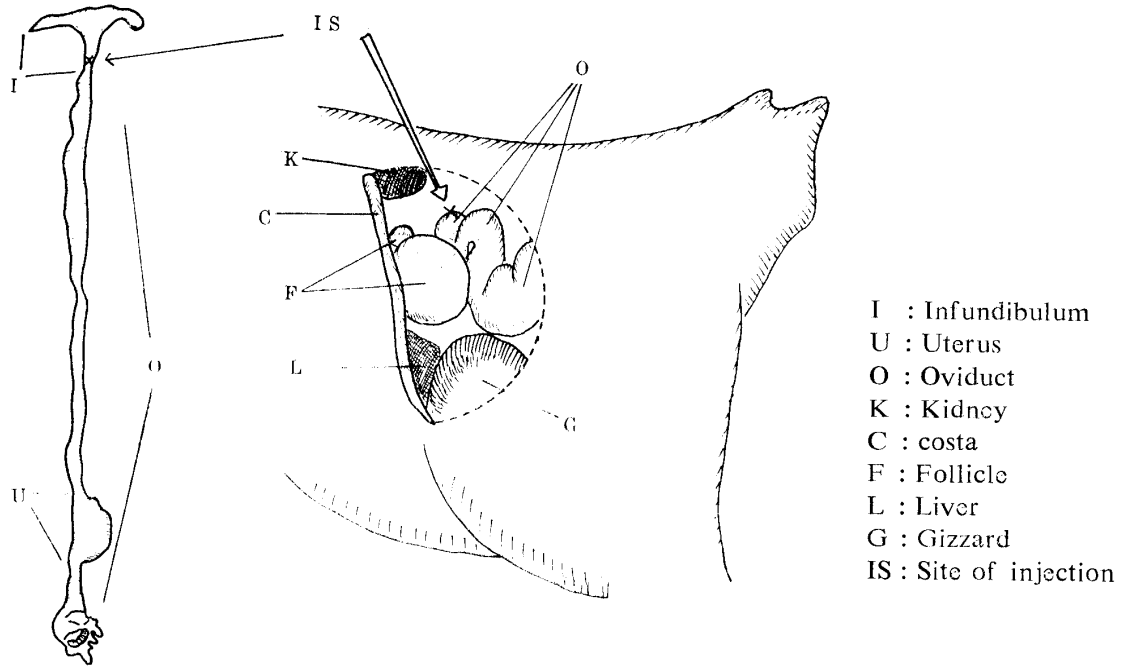

Fig. I Site of infundibulal insemination

0.1 *ml.* of the diluted semen was inseminated with minute care into the lower part of the infundibulum with a tuberculin syrings. Then, the wound was stitched.

3. Fertility determination :

Fertility was determined by the method described in the previous paper[11].

Results and discussion

The laying time, the insemination time to the infundibulum and the semen characteristics in both of the trials were presented in Table 1.

Table 1. Data on the observed fowl concerning the following items i. e. laying time, insemination time, interval from laying to insemination and semen quality

Trial	Bird's No.	Time of laying (a.m.)	Time of insemination (a.m.)	Minutes between laying and insemination	Number of sperm injected	Semen quality	
						Initial motility	Spermatozoal number
					million/mm^3		million/mm^3
I	166	8 : 25	8 : 56	31	0.98	3	6.97
	43	8 : 33	9 : 03	30	0.92	5	6.85
	6336	10 : 07	10 : 29	22	1.06	5	7.90
	40	8 : 52	9 : 24	32	1.03	5	7.10
	53	8 : 27	8 : 53	26	1.02	5	6.93
	11	10 : 35	10 : 50	15	1.04	4	7.82
	125	9 : 36	10 : 08	32	0.82	3	5.24
	43	9 : 35	9 : 47	12	1.06	4	8.80
	37	9 : 35	10 : 00	25	1.13	5	6.40
	177	10 : 07	10 : 17	10	1.13	5	6.40
	Mean			23.5±8.4	1.02±0.09	4.4±0.8	7.05±0.93
II	103	9 : 35	10 : 05	30	3.64	5	3.64
	17	11 : 15	11 : 43	28	3.03	4	3.03
	99	10 : 10	10 : 30	20	3.90	4	3.90
	19	9 : 25	10 : 05	40	3.50	4	3.50
	141	8 : 45	9 : 00	15	3.82	5	3.82
	138	9 : 00	9 : 25	25	3.38	5	3.38
	97	8 : 20	8 : 55	35	3.12	5	3.12
	1	8 : 57	9 : 10	13	4.80	3	4.80
	155	8 : 50	9 : 15	25	4.20	3	4.20
	5	9 : 20	9 : 40	20	3.80	3	3.80
	Mean			25.1±8.5	3.72±0.52	4.1±0.9	3.72±0.52

In the first trial, the spermatozoal number of the diluted semen was approximately 1 million spermatozoa/mm^3, therefore, total number of 100 million spermatozoa (in 0.1*ml.* semen) scoring 4 to 5 in motility was inseminated.

In the second trial, the concentration of spermatozoa introduced was approximately 3.7 million/mm^3, and the motility was 4.1 in average.

The average interval from laying to insemination was 23.5±8.4 minutes in the first trial and was 25.1±8.5 minutes in the second trial, and ovulation did not occur during the operation.

Laying frequency of the pullets following the infundibulal insemination and their fertility were shown in Table 2. The fertility of the eggs laid on the next day after insemination was also shown in Table 2.

Table 2. Data on the observed fowl concerning the following items i.e. laying cycle, the number of fertile eggs and the fertility rate of the eggs laid on the lst day after the infundibulal insemination

Trial	Bird's No.	Days after insemination 1	2	3	4	5	6	7	8	9	10	11	12	13	14	Duration of fertility	No. of eggs laid during 2 weeks	No. of fertile eggs during 2 weeks	Fertility rate	Laying rate	Fertility rate on the 1 st day after insemination
																day			%	%	%
I	166	◎	×		—	—	×	—					×	×	×	1	9	1	11.1	64.3	
	43	◎					×	◎		×	×	×	×	×		7	8	2	25.0	57.1	
	6336	×	◎	◎						◎		◎		○	○	23	7	6	85.7	50.0	
	40	×		◎								◎		×	×	11	5	2	40.0	35.7	
	53	◎	◎		—	×	×		×	×	×	×	×	×		2	11	2	18.2	78.6	
	11			◎			×							×		3	3	1	33.3	21.4	77.7
	125	◎	◎		◎											4	3	3	100.0	21.4	
	45	◎	◎				◎			—	—			—		6	6	3	50.0	42.9	
	27	◎			×			×	◎	×		×	×		×	8	8	2	25.0	57.1	
	177	◎		◎	◎	◎		◎	◎	◎	○	◎	◎	×		12	11	10	90.9	78.6	
	Mean															7.7±6.5	7.1±2.9	3.2±2.8	47.9±32.6	50.7±20.6	
II	103						◎	◎		◎	○	◎		×	×	11	7	5	71.1	50.0	
	17									◎	◎	×		◎		13	4	3	75.0	28.5	
	99	◎		◎	◎		◎	◎		◎	◎		×	×	×	10	10	7	70.0	71.4	
	19	◎		◎		/															
	141	◎		◎	◎		/														
	138	◎		◎		◎		×		◎	×					9	6	4	66.6	42.8	87.5
	97	◎	◎		◎			×	◎							8	5	4	80.0	35.7	
	1	◎	◎	○			×	◎	×	×	×		×	×		7	10	4	40.0	71.4	
	155	×	◎		◎	◎		◎	◎	◎						9	7	6	85.7	50.0	
	5	◎	◎	◎		◎	◎	◎		◎	○	◎		◎	×	16	11	10	90.9	78.5	
	Mean															10.4±2.9	7.5±2.6	5.4±2.3	72.4±15.5	53.5±18.3	

Note : The symbol ◎ indicates a normal fertile egg, ○ a fertile egg with dead germs and × an infertile egg. No eggs were laid on the days corresponding to blank spaces in the Table. No. 19 and No. 141 were sacrificed in 5 to 6 days after insemination to observe the spermatozoa in the genitalia.

According to this results, all hens except one bird (No. 11) in the first trial and eight out of ten birds in the second trial, laid normal eggs at the expected time on the next day after insemination.

The rates of fertility of all eggs laid on the next day after insemination were 77.7 % in the first trial, and 87.5 % in the second trial. Then, the time schedule was presumed from the result of this trial and from those of other workers (Fig. 2).

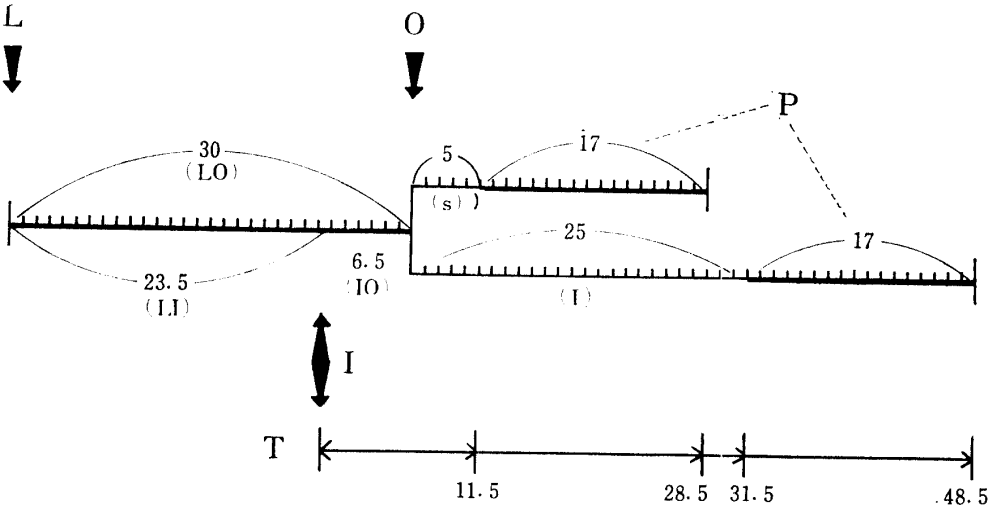

Fig. 2 Minutes between infundibulal insemination and fertilization

L : Laying
O : Ovulation
I : Insemination
(LO) : Interval between laying and ovulation (30 min)
(LI) : Interval between laying and insemination (23.5 min)
(IO) : Interval between insemination and ovulation (6.5 min)
(s) : The shortest interval between ovulation and the time of enclosing the ovum (5 min)
(I) : The longest interval between ovulation and the time of enclosing the ovum (25 min)
P : Passing time of infundibulum
T : Interval between insemination and the expected time to meet ovum (minutes)

As it was reported by Warren and Scott (1935)[14] and Phillips and Warren (1937)[15] that the ovulation occurred about 30 minutes after laying,, though it varied with individuals, we used 30 minutes, in this time schedule, as the interval from laying to ovulation (LO in Fig. 2). Warren and Scott (1935)[14] showed also that ovulated ovum was caught by the infundibulum in 5-25 minutes. So, as the interval between ovulation and the time in which the infundibulum enclosed the ovum, we adopted 5 minutes as the shortest time (s in Fig. 2) and 25 minutes as the longest time(I in Fig. 2).

Moreover, we adopted 17 minutes as the time spent by the ovum in the infundibulum (P in Fig. 2), since this time was reported by Warren and Scott (1935)[14].

From these data, it was ascertained that the interval from ovulation to the finished passing of the ovum through the infundibulum was 22 minutes in case of the shortest (5 min. +17 min.) and 42 minutes in the longest (25 min.+ 17 min.).

In the first trial, the interval between infundibulal insemination and ovulation was

estimated to be 6.5 minutes (30 min.–23.5 min.), then the interval between insemination and the time at which the infundibulum caught the ovum was estimated to be ranging from 11.5 minutes (6.5 min. + 5 min.)to 31.5 minutes (6.5 min.+25 min.).

So the intervals between insemination time and the termination of ovum's passage through the infundibulum were in the range of 28.5 minutes (11.5 min.+17 min.) and 48.5 minutes (31.5 min.+17 min.).

From the result of this trial we assumed that the spermatozoa had met the ovum during 11.5 to 28.5 minutes after insemination in case of the shortest time, and 31.5 to 48.5 minutes in case of the longest time.

In the second trial, the similar result was obtained, too.

On the other hand, the rates of fertility of the eggs laid on the next day after insemination were 77.7 % and 87.5 %.

This means that the spermatozoa which resided in the infundibulum for a very short time had already acquired the fertilizing capacity.

Van Drimmelen, G. C. (1951)[16] reported that the sperm introduced directly into the ovarian pocket of the fowl just prior to ovulation produced immediate fertility, so that traversing the oviduct was unnecessary for their fertilizing capacity.

Hence it was concluded that capacitation was scarcely necessary for fowl spermatozoa and even if it might be existing in this species, it may be a factor of quite a small significance.

The duration of fertility, number of fertile eggs during two weeks and percentage of fertile eggs in the first trial were inferior to those in the second trial. The difference may be attributed to the number of spermatozoa inseminated.

In the duration of fertility of this trial, it showed a tendency to be shorter in both of the trials than in normal insemination.

Takeda (1966)[17] reported that the duration of fertility following the infundibulal insemination ranged over 12 to 27 days, with a mean of 17.2 days, but when the insemination was made in the vagina, it ranged over 6 to 17 days, with a mean of 12.2 days. Moreover, other workers showed that there was no difference in percentages of fertile eggs following the infundibulal and vagina insemination. Although any distinct explanation was not vouchsafed about the differences between the results, one of the cause was assumed to be the technique by which the insemination was carried out with the tuberculin syringe.

Summary

To determine whether capacitation is necessary for fowl spermatozoa or not, two trials were conducted.

In the first trial, three cockerels and ten virgin pullets and in second trial five males and ten females were used.

Shortly after laying, the pullets in the first trial were inseminated with 0.1*ml.* of diluted semen (1 million spermatozoa/mm^3) into the the lower part of the infundibulum, and in the second trial they were inseminated with 0.1 *ml.* of undiluted semen (3.7 million sper-

matozoa/mm^3) into the same portion.

The fertility of eggs laid on the following day after insemination was determined to investigate capacitation, and the duration of fertility was observed, too.

The results obtained were as follows ;

1) It was presumed from the first trial that the spermatozoa introduced into the infundibulum had met the ovum during 11.5 to 28.5 minutes after insemination in case of the shortest time, and 31.5 to 48.5 minutes in case of the longest time. In the second trial the similar result was obtained, too.

2) The rates of fertility of the eggs laid on the next day after insemination were 77.7 % in the first trial and 87.5 % in the second trial.

Therefore it was assumed that the spermatozoa which had met the ovum in 11.5 to 48.5 minutes after infundibulal insemination could fertilize the ovum. These results mean that the spermatozoa which resided in the infundibulum for a very short time had already the fertilizing capacity.

Hence it was concluded that capacitation was scarcely necessary for fowl spermatozoa.

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