Studies on the Artificial Insemination in the Domestic Fowl

II. Effects of the Dilution of Semen and the Insemination Interval on the Fertility of Pullets.

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Introduction

Effects of the dilution of fowl semen on its fertlity have been reported by several authors. Most of them, however, have been carried out either to investigate the relationship between the dilution rate of semen and the fertility level[1)~6)] or to determine the minimum number of the spermatozoa required for optimal fertility[7)~9)]. The effect of dilution per se, or the problem which is more effective, in inseminating the same number of spermatozoa, the undiluted semen or diluted one, has been left unclarified. Solution of this problem is one of the aims of this experiment.

Under the practical conditions, it is desirable to extend the insemination interval as long as possible without decreasing fertilty, in order to lighten the burden of the laborious work of insemination. Another aim of this experiment is to investigate whether the insemination interval may be extended from 7 days to 10 days without decreasing the fertility.

Experimental procedure

1. Stock used and its management ;

Cockerels : For semen collection, highly fecund S. C. White Leghorn Cockerels were used. When any cockerel began to show a poor fecundity, it was replaced by a cockerel with rich fecundity: total number of cockerels amounting to fourteen.

Pullets : S. C. White Leghorn pullets were used for insemination. As described in the previous paper, pullets of lowered fertility were removed from the experimental ones by the following method ; pullets were inseminated with 0.1 *ml.* of the diluted semen (about one million spermatozoa per cubic millimeter) four times at three day intervals, and the pullets with more than ninety percent fertility were used in this experiment.

All cockerels and pullets were kept in the individual cages and were fed laying mash.

2. Semen collection and the assessment of semen quality :

About 1*ml.* of dense semen without any contamination was collected from several cockerels by the selective collection technique. The pooled semen was mixed thoroughly and spermatozoal concentration was determined, as soon as possible, by the optical density method; 0.1 *ml.* of semen was diluted in 10 *ml.* of 3.6 percent sodium citrate solution.

The optical density of the mixture was determined at a wave length of 660 *mμ* using a photoelectric photometer, Hitachi EPO-3. The time needed for determination was no more than 3 minutes. And according to the experimental design, the pooled semen was divided into two or three parts and the pullets in each group were inseminated with very small doses of semen (undiluted semen) or with 0.1 *ml.* of the semen diluted with Ringer solution or physiological salt solution (0.9% NaCl solution) up to the expected number of spermatozoa.

The details of each experiment will be discribed in the "Result and Discussion".

After the insemination was completed, motility of spermatozoa and spermatozoal concentration of the inseminated semen were determined by the routine microscopical methods.

3. Artificial insemination and fertility determination:

Inseminations were carried out carefully and cleanly at 7 day or 10 day intervals by the methods described in the previous paper[10]. For insemination of the diluted semen, the same inseminating syringe as described in the previous paper was used in this experiment, too. It was, however, not possible to inseminate an exceedingly small dose of semen with the syrings, and so a micro-pipette with 0.001 *ml.* graduation was used to introduce undiluted semen. Eggs laid between the second day after first insemination and the seventh or the tenth day after last insemination, were collected and fertility was determined by the method described in the previous paper.

Results and Discussion

(1) Comparison between diluted semen and undiluted semen on the fertility of pullets:

Three trials were performed. In the first trial, the pullets of 2 groups (group R and S, 8 fowls in each) were inseminated with 0.1 *ml.* of the diluted semen with a concentration of 0.7 million spermatozoa per cubic millimeter. The semen to be inseminated into the pullets of group R and S was diluted with Ringer solution and physiological salt solution respectively. Eight pullets in the third group (O group) were inseminated with 0.01 *ml.* of undiluted semen. Inseminations were performed four times at one week intervals and eggs laid during the 4 week experimental period were collected for the fertility determination.

The results were shown in Table 1. The average number of the spermatozoa inseminated was significantly lesser in the group O than the other two groups ($P<.05$). On the other hand, fertilities in three groups were almost similar each other and there were no significant differences among them.

To compare more clearly the fertility of diluted semen with that of undiluted one, additional two experiments, trial 2 and 3, were performed. In the second trial, two groups (Group S and O) of pullets, 10 fowls in each were designed to be inseminated 100 million spermatozoa at 7 day intervals three times; in the group S, semen was designed to be diluted with physiological salt solution so as to contain one million spermatozoa per cubic millimeter and the pullets were inseminated with 0.1 *ml.* of the diluted semen. Those of the group O were inseminated with undiluted semen, and the volume to be inseminated was determined so as to contain 100 million spermatozoa. Eggs laid between the second day after first insemination and the seventh day after last insemination were

Table 1. Comparison of the effects on the fertility of pullets between diluted semen and undiluted semen.

Trial	Treatment	No. of fowls	Dilution rate	Semen dose	Total number of spermatozoa inseminated	Insemination Interval	Insemination Frequency	Laying rate	Fertility rate
				ml.	million	day	time	%	%
I	1. Ringer solution (R)	8	1 : 8.5	0.1	72	7	4	52.7	90.1
	2. Physiological salt solution (S)	8	1 : 8.5	0.1	71	7	4	57.1	86.9
	3. Undiluted semen (O)	8	0	0.01	58	7	4	61.6	87.5
II	1. Physiological salt solution (S)	10	1 : 6.1	0.1	105	7	3	72.5	95.7
	2. Undiluted semen (O)	10	0	0.01	101	7	3	80.0	98.1
III	1. Ringer solution (R)	9	1 : 6.5	0.1	98	10	3	52.2	77.3
	2. Undiluted semen (O)	10	0	0.015	92	10	3	52.0	81.7

collected for fertility determination. As shown in Table 1. the number of spermatozoa inseminated and the percentage of fertility in both groups, S and O, were similar each other and there were no significant differences between the two groups.

Similar comparision was made again in trial 3. The same methods as the trial 2 were used with the exception that Ringer solution was used for diluent instead of physiological salt solution, the insemination intervals were 10 days, the frequency of insemination was 3 times and the duration to collect the eggs after the last insemination was 10 days. The results obtained from trial 3 were similar to those from trial 2 ; there were no significant difierences between the two groups in the number of spermatozoa inseminated and in the fertility.

Taking a general view of the results obtained from the three trials, it was concluded that if the pullets were inseminated with the same number of spermatozoa, similar level of fertility would be observed irrespective of the dilution of semen. However, it appeared that the insemination of the undiluted semen was apt to result in higher fertility as compared with that of diluted semen. Recent reports revealed optimal fertility with very small doses of undiluted semen. Hence, so far as feritlity is concerned, undiluted semen may preferably be inseminated into the pullet. From the practical stand point, however, accurate insemination of very small doses of undiluted semen is rather difficult because of the present unavailability in Japan of the suitable apparatus to introduce the exceedingly minute dose of semen. The chore of diluting semen before insemination is not a laborious one, and the diluted semen can be inseminated accurately, safely and easily. Thus, we should like to recommend to dilute semen with proper diluent before insemination until the suitable and safe apparatus to introduce minute doses of semen become available.

As to the diluent for chicken spermatozoa, many investigations have been done and great variety of diluents have been developed. Among them, Ringer solution and physiological salt solntion are considered to be undesirable as diluents because chloride ions increase the development of abnormal spermatozoa after storage and decrease fertilizing capacity [11)12)]. The use of the stored semen for artificial insemination, however, has not yet been brought to the stage of practical application, since in case of storing semen for one or

two days, fertilizing capacity is rapidly depressed and the fertility decreases below the practical levels, and fresh semen has been commonly used for practical artificial insemination, although a sort of suitable diluent has been developed recently by Wambeke (1967)[13].

In this experiment, Ringer solution and physiological salt solution were used for the diluent of fresh semen, and high fertilities were observable. In comparing Ringer solution with physiological salt solution, the motility of spermatozoa of the semen diluted with physiological salt solution was apt to decrease more quickly with the lapse of time. Thus it may be concluded that Ringer solution will be better for diluent than the physiological salt solution, when sometime lag is inevitable between semen dilution and insemination.

In the previous paper[10], optimal fertility was maintained for one year (94.2 percent in average throughout the year) with the semen diluted with Ringer solution. Schindler et al. (1955)[14] also reported optimal fertility using Ringer diluent and Bonnier and Trulson (1939)[15] noted that fertility of hens inseminated with the semen diluted with Ringer solution was actually higher than the one obtained when undiluted semen was used. Optimal fertilities were also obtained by using the semen diluted with Tyrode solution[16] and with Lake's solution[17].

In the practical artificial insemination, saline solution such as Ringer solution and phosphate buffer may be suitable, because of the easiness in its making, long time-preservation of the solution, low cost and good fertility.

(2) The number of spermatozoa inseminated and the fertility

Whatever diluent may be used, fertility decreases with increasing dilution rate, and the decrease is not apparent until a certain dilution-rate has been reached. The principal cause of decreasing fertility with increasing dilution-rate is the decreasing number of live spermatozoa inseminated (Rowell and Cooper, 1957)[3].

Munro (1938)[8] reported that fertility was reduced when fewer than 100 million spermatozoa were inseminated, and fewer than one million spermatozoa resulted in complete infertility. Rowell and Cooper (1957)[3] noted that the minimum effective dose for fertility was 170 million live spermatozoa. Taneja and Gow (1961)[5] reported that optimal fertilities were obtained by insemination of about 50 to 120 million spermatozoa of undiluted semen. Further, Taneja and Gow (1962)[9] who inseminated a large number of doses of undiluted semen varying from 0.0002 to 0.15 *ml.* revealed that about 40 to 70 million spermatozoa were necessary for optimal fertility, depending on the strain.

Table 2. Effect of injection interval and sperm concentration on the fertility of pullets.

Experimental design	No. of fowls	Dilution* rate	Semen dose	Total number of spermatozoa inseminated	Insemination Interval	Insemination Frequency	Laying rate	Fertility rate
			ml.	milliom	day	time	%	%
70 million sperm, every week	6	1 : 7.9	0.1	73	7	4	57.7	79.6
100 million sperm, every week	6	1 : 5.6	0.1	101	7	4	46.6	92.5
100 million sperm, every 10 days	6	1 : 6.3	0.1	95	10	3	50.6	95.3

* Semen was diluted with Ringer solution.

In this experiment, 70 and 100 million spermatozoa were inseminated with diluted or undiluted semen at a week or 10 day intervals and all of the trials resulted in optimal fertility (Table 1). In the trial 2, 58 million spermatozoa that were inseminated with undiluted semen also resulted in optimal fertility. These results were similar to those of Taneja and Gow (1961, 1962)[5,9], and were fewer in spermatozoa number than that reported by Munro (1938)[8] and Rowell and Cooper (1957)[3].

(3) Insemination interval and fertility.

Eighteen pullets were divided into three groups, 6 pullets in each and the pullets in each group were designed to be inseminated by the following plan; the pullets of first group were designed to be inseminated 70 million spermatozoa at a week intervals (equivalent to 10 million spermatozoa per day), those of second group, 100 million spermatozoa at 7 day intervals (Standard insemination) and those of third group, 100 million spermatozoa at 10 day intervals (equivalent to 10 million spermatozoa per day). Semen was diluted with Ringer solution and each pullet of all groups was inseminated with 0.1 *ml.* of the diluted semen. In the group 1 and 3 inseminations were carried out four times and the eggs laid during the 28 day experimental period were collected for fertility determination. In the group 2, inseminations were carried out three times and the eggs were collected during 30 day period.

As to fertiility, there were no significant differences among three groups,though the average fertility in group 1 was slightly lower than that of the other two groups ; fertility of pullets remained unaltered though the extention of the insemination interval from 7 days to 10 days, when the same number of spermatozoa per day was inseminated or when 100 million spermatozoa were inseminated.

It may reasonably be said that the shorter the intervals of the insemination are, the higher fertility results in, by the following reasons ; 1) Moore and Byerly (1942)[18] reported that maximum fertility was attained on the third and fourth days after single artificial insemination and that fertility fell off sharply after the sixth day. 2) The decrease of fertility resulting from any failure of insemination may be made up for the shorter interval insemination. On the other hand, according to Taneja and Gow (1961,[5] 1961,[19] 1962),[9] the duration of fertility following a single insemination was related to the dose of semen or spermatozoa number inseminated, particularly when very small doses were employed. Hence, insemination interval should be considered with refernce to the number of spermatozoa to be inseminated.

In this experiment, it was possible to extend the insemination interval from 7 days to 10 days without decreasing fertility when 100 mllion spermatozoa were inseminated.

Summay

Using S. C. White Leghorn pullets and cockerels four trials were performed in order to investigate the following two problems ; 1) which is more effective for fertility, undiluted semen or diluted semen, in inseminating the same number of spermatozoa, 2) whether it is possible to extend the insemination interval from the period of 7 days to that of 10 days without decreasing fertility. Clear dense semen was collected from several cockerels by selective collection technique and the pullets in each group were inseminated with exceedingly small doses of undiluted semen or with 0.1 *ml.* of diluted semen,

semen being diluted with Ringer or physiological salt solution up to the appointed number of spermatozoa according to the experimental plans. Inseminations were carried out three or four times at 7 day or 10 day intervals.

Results obtained were as follows;

1. When 100 million spermatozoa were inseminated, there were no significant differences in fertility between diluted semen and undiluted semen. And also, there was no significant difference in fertility when pullets were inseminated with about 70 millions spermatozoa of diluted semen and 58 millions spermatozoa of undiluted semen. From these data, it was concluded that if pullets were inseminated with the same number of spermatozoa, similar level of fertility would be obtained. However, it appeared that the insemination of undiluted semen was apt to result in slightly higher fertility as compared with that of diluted semen.

2. Optimal fertility was attained from the insemination of 70 and 100 million spermatozoa.

3. Fertility of pullets remained unaltered even though the extention of the insemination interval from 7 days to 10 days when 100 million of spermatozoa were inseminated.

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