Studies on the Artificial Insemination in the Domestic Fowl

I. On the influence of the long period artificial insemination on the fertility of pullets*

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Among the poultrymen in Japan a belief prevails that the fertility of eggs falls down suddenly after the execution of approximately 3 to 5 months of continuous artificial insemination. The same was reported by Iwaya et al. (1963)[1], Odera et al. (1963)[2] and Enya et al. (1964)[3]. A similar trend was noted by Schindler and Bornstein (1962)[4] in Israel.

Some spermatozoal antibodies in the blood stream brought forth by the artificial insemination were assumed to be the main cause for the decline in fertility both by poultrymen and investigators, and several immunological studies have been carried out[5)-11)].

The problem of the decline of fertility in the course of continuous insemination is serious from the practical standpoints and the clarification of the cause is a matter of importance from the academic standpoints. The clarification of the time and degree of the fertility-declining in the pullets put under a long period insemination was aimed in this study.

Experimental procedures

In this experiment were used the crossbred pullets (S. C. White Leghorn×Barred Plymouth Rock) in the first laying year. These pullets, fed laying mash, were kept in the individual cages.

It was in December 1961 that they began to produce eggs, the experiments were carried out for full one year, from the 2nd of February 1962 to the 31st of January 1963.

In a common flock there usually are to be found some females, sterile or of a lowered fertility[14)-17)]; and, in a study referring to fertility, some inaccuracies come to be inevitable; especially in the experiments using a small number of samples.

Therefore, 35 pullets were inseminated with 0.1 *ml* of semen which had been diluted three times with Ringer's solution. From the inseminated ones, 5 pullets with somewhat lowered fertilities were culled, and the rest (30 fertile pullets) were divided into three groups; 1) Insemination group (group I), 2) Sham insemination group (group S) and 3) Control group (group C), ten pullets in each.

* The contents of this paper were delivered at the 49th annual meeting of the Japanese Society of Zootechnical Science (1963)[12]. A part of this study was presented at the 13th annual meeting of the West Japan branch, Jap. Soc. Zootech. Sci. (1962)[13], previously.

Every three days for one year, the pullets of the group I were inseminated artificially with 0.1 *ml.* of semen previously diluted with Ringer's solution three times.

At three day intervals, with the exception that the insemination was executed only once a month, the injection of 0.1 *ml.* of Ringer's solution was made to the pullets belonging to the group S in the same manner as the artificial insemination. In group C, pullets were inseminated once a month with the same diluted semen as group I and group S.

The details of the techniques are as follows: Artificial insemination: Artificial insemination was carried out by Burrows and Quinn's method[18)19)], with a few modifications.

About 0.5 *ml.* of semen was collected (1.5 *ml.* once a month) from the highly fecund White Leghorn cockerels.

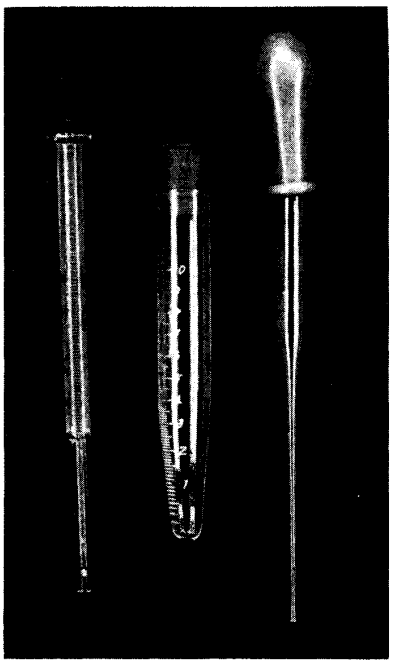

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Fig. 1. Equipments for the artificial insemination used in this experiment.
1. Inseminating syringe 2. Graduated test tube
3. Semen collecting pipette

When any cockerel began to show a poor fecundity it was replaced by a cockerel with rich fecundity: the total number of cockerels used for semen collection amounting to eighteen.

Attention was given to the execution of the collection of dense semen without any contamination; previous to the collection, the anus was cleaned by wiping and drying, a piece of cotton being inserted into the cloaca of the cockerel. Before dropping out from the anus, semen was collected on the upper-side of the phallus with a pipette. With the skillful use of the pipette, it was possible to collect selectively sperm-rich fraction out of an ejaculate to obtain dense semen. The semen collected out of 4 to 5 cockerels was

pooled, diluted and inseminated as soon as possible. After the completion of the insemination, the motility and the number of the spermatozoa of the diluted semen were determined by routine methods. The scoring of the motility of spermatozoa was carried out on a basis of 0 to 5[25]. The time when the semen collection and the insemination were performed was between 2 and 3 p.m., for the reason that high fertilization of eggs might be got when the insemination was performed in the afternoon[20]-[21].

Attention was also paid for the careful and clean operation of the insemination; this coupled with the gentle and careful operation of the oviducal eversion and the insertion of the inseminating syringe. After each insemination, the inseminating syringe was cleaned with fresh cotton.

Whenever cloacal fluid or faeces flowed out from the anus of the pullet under the oviducal eversion, they were removed through the tapping of the everted oviduct with a piece of cotton.

By applying pressure on the abdominal region, the eversion of the oviduct of the laying pullets can be done easily, but the intermission of her laying should naturally lead to the involution of the oviduct, which renders the eversion of the oviduct extremely difficult. Even in case of such a pullet, inseminations (or sham inseminations) were carried out continuously at regular intervals by the method mentioned below in order to keep on the influence of the insemination; namely, the location of the oviduct was made by the inseminator with a forefinger, an inseminating syringe being inserted along with it. Then, he withdrew the finger from the oviduct and the semen (or Ringer's solution) was deposited into the vagina.

Fertility determination: All the eggs laid in group I, and the ones laid during 7 days or between the 2nd and the 8th day after the insemination in group S and C were incubated for 3 days, and at the third day they were broken open and examined macroscopically. The fertilization was determined by the vascularization of the embryo, and the eggs in which blood vessels were observed around the blastoderm were grouped into the fertilized ones even if the embryo had died during the incubation for three days.

Group	month*	1 (Feb.)	2 (Mar.)	3 (Apr.)	4 (May)	5 (Jun.)	6 (Jul.)	7 (Aug.)	8 (Sep.)	9 (Oct.)	10 (Nov.)	11 (Dec.)	12 (Jan.)	Sum
I	D	0	0	1	0	0	0	0	1	0	0	0	0	2
	P	10	10	9	9	9	9	9	8	8	8	8	8	
S	D	0	1	0	0	0	0	0	1	0	0	0	0	2
	P	10	9	9	9	9	9	9	8	8	8	8	8	
C	D	0	1	0	0	0	0	0	0	0	0	0	0	1
	P	10	9	9	9	9	9	9	9	9	9	9	9	

Table 1. Number of pullets died and the number used for calculation of fertility.
I : Insemination group, S : Sham insemination group, C : Control group.
D : No. of pullets died, P : No. of pullets used for calculation.
* No. of month from start of the experiment.

In the course of this experiment, several pullets died, and the fertility of each group

was computed from the remaining ones, counting by the monthly unit (Table 1).

Data obtained were analyzed statistically after the methods of Snedecor (1956)[22].

Results and Discussion

1. The quality of the semen used for artificial insemination.

The concentration of the diluted semen inseminated was shown in Fig. 2, the mean value during one year being 1.499 millions per cubic millimeter. Judging from the Fig. 2, no seasonal variation was observable in the number of the spermatozoa inseminated. The whole semen had been diluted previously, three times, with Ringer's solution, the average concentration of the original semen was estimated to be 4.497 milions per cubic millimeter, and the semen collected from cocks was usually noted to be 2.63 millions per cubic millimeter on an average, as reviewed by Nishiyama (1961)[23], accordingly, these semen might be regarded as fairly conc ones.

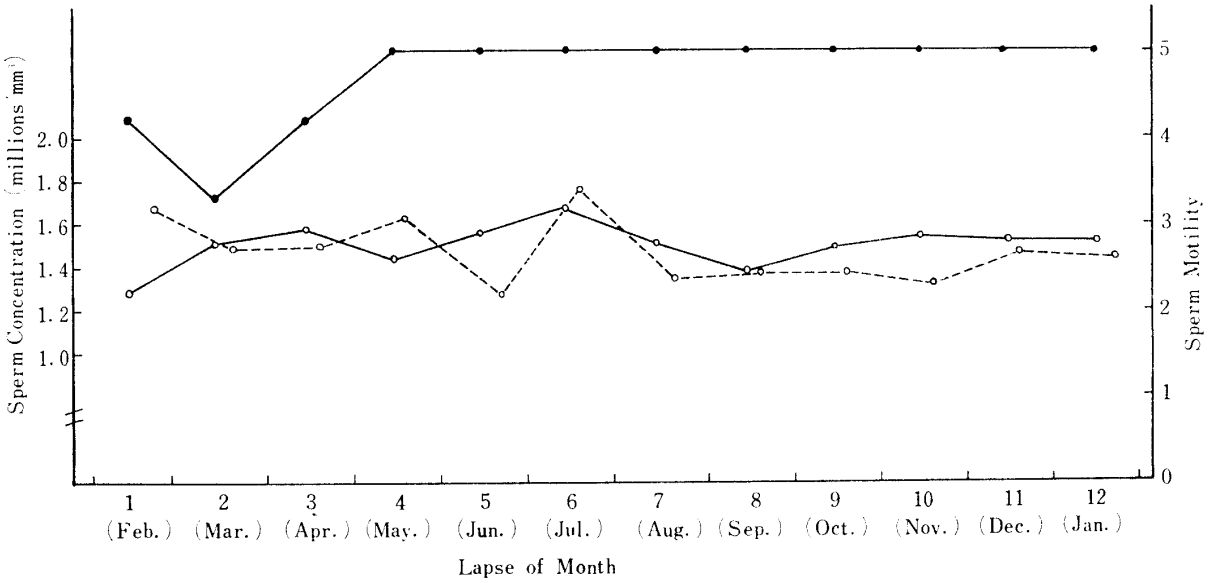

Fig. 2. Number and motility of spermatozoa of the diluted semen inseminated.
●——● monthly moving averages in motility.
○——○ monthly moving averages in number of speamatozoa.
○----○ No. of spermatozoa in each insemination in groups S and C.

Sperm motilities during the first three months rated as 3 to 4, thereafter all the samples scoring 5 (Fig. 2); the regression line, therefore, rises, with a gentle slope, toward the end of the experiments (Fig. 4).

From the comparatively high concentration and motility of the spermatozoa proved above, it may reasonably be said that the semen inseminated was kept in high quality throughout the experiment.

It is needless to say that this can not be regarded as an evidence for denying the seasonal variations in the semen quality reported by several authors[24)-28], but it may be said that this may be due to the selective collection technique of the semen, and to the proper replacement of the cockerels described in the paragraph of the experimental pro-

cedures.

2. Fertility during the extended artificial insemination.

In group I, the fertility in the respective month was beyond 92 per cent, excepting that of the 7th month (83 per cent in August), the average fertility through the whole year being 94.2 per cent.

The average fertilities in group S and group C were 94.6 and 95.6 per cent respectively, basing on the eggs laid during the three days or between the 2nd and the 4th day after the insemination, and were 92.6 and 90.2 per cent basing on the eggs laid between the 2nd and the 8th day (Table 2).

Group	Egg-laying (%)	Fertility (%)	Correlation Coefficient	
I	50.2	94.2	0.213	N. S.
S	49.4	94.6 (92.6)*	0.132	N. S.
C	51.2	95.9 (90.2)*	−0.350	N. S.

Table 2. Egg-laying, fertility and correlation coefficient
* based on the eggs laid during 3 days after insemination, numerals in parentheses are based on the eggs laid during 7 days after insemination.

In comparing the fertilities among the three groups, the former values may be preferable to the other ones, as the pullets in the group I were inseminated every three days.

The variations in fertility between the months in group S and group C were comparatively larger than those of group I, which might be attributed to a single monthly insemination in the former against the every 3rd day's one in the latter; which may be due to the following fact that in case of the single insemination, any failure or inadequacy of operation may result in bringing few or none of the fertile eggs (zero per cent fertility), which lowers the level of fertility in a group. On the other hand, when pullets are inseminated every three days, the decrease in the fertility through a failure in the insemination may probably be prevented by the preceding successful one, as a fair fertility usually lasts for a week after the insemination[29].

There are many factors influencing the fertility of eggs, among which the qualities of the semen play not a small part in the influence upon the degree of fertility. Hence the necessity of keeping the qualities of semen in a possible uniform condition in such an experiment as this. As was described in the preceding paragraph, the qualities of semen were kept in a high and uniform conditions throughout the present experiment.

The purpose of setting up 'group S' was to elucidate the adverse mechanical effects of the artificial insemination caused by the eversion of the oviduct and/or the insertion of the inseminating syringe into the vagina; that of setting up 'group I' was to clear up the adverse effects of an extended artificial insemination, especially, those of the semen injected repeatedly for a long period. If the spermatozoal antibodies caused by artificial insemination were one of the factors causing the declining of fertility, a dropping in the fertility of group I would have to be brought forth during the course of the experiment.

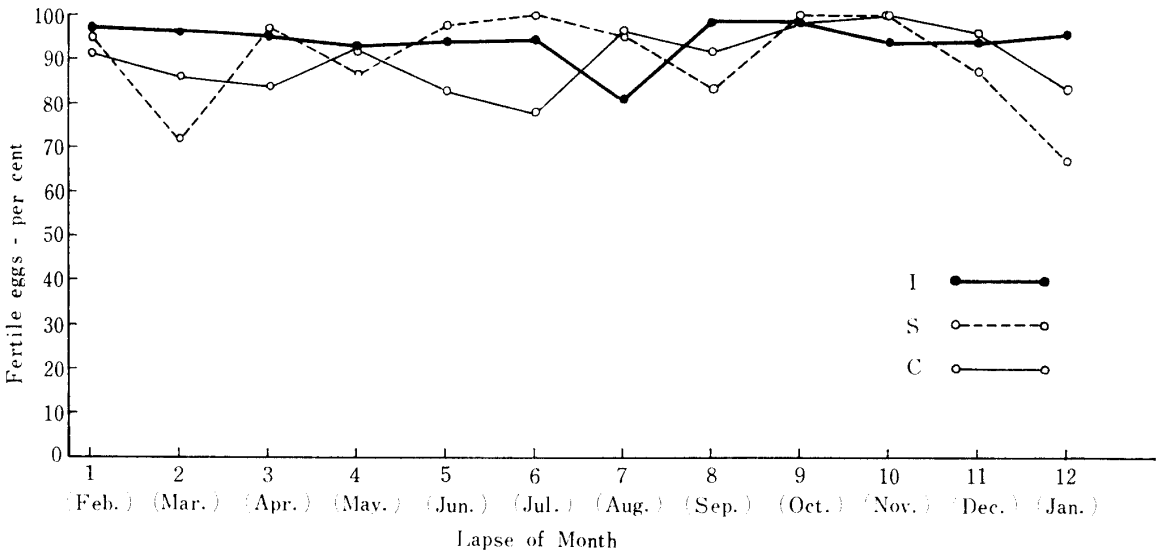

Fig. 3. Fertility of pullets during the experiment.
I : monthly moving averages of group I.
S and C : fertility based on the eggs laid during 3 days after insemination in groups S and C respectively.

In order to elucidate the cause of this sudden decline more clearly, pullets in group I were inseminated over the period of a year with one and a half times as many spermatozoa as usually used for the artificial insemination and at shorter time-intervals (every 3rd day insemination) than the normal intervals of 7 days.

The fertilities in both group I and group S, however, turned out to be very high, similarly in case with the control group, and no decreasing tendencies in fertility were to be found through the three groups (Fig. 3). And in fertility, no statistical differences were found among the three groups (Table 3). This is to say that even if the pullets were inseminated at 3 day intervals over a period of one year no decline would be seen in the fertility, and that if artificial insemination were made carefully no adverse mechanical effects could be found in the fertility.

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	F
Total	25	2465. 80		
Group	2	123. 22	61. 61	0. 60
Error	23	2342. 58	101. 85	

Table 3. Analysis of variance of the fertility of pullets in three groups (Arc sine transformation)

The results, presented here, contradicted the conclusion obtained by Iwaya (1963)[1], Odera et al. (1963)[2], and Enya et al. (1964)[3] and were similar to the results obtained by Schindler and Bornstein (1962)[4] and Saeki et al. (1965)[6],[30].

Schindler and Bornstein (1962)[4] inseminated a hen continuously for 5 to 6 months, with the confirmation that if the inseminations were performed by an experienced opera-

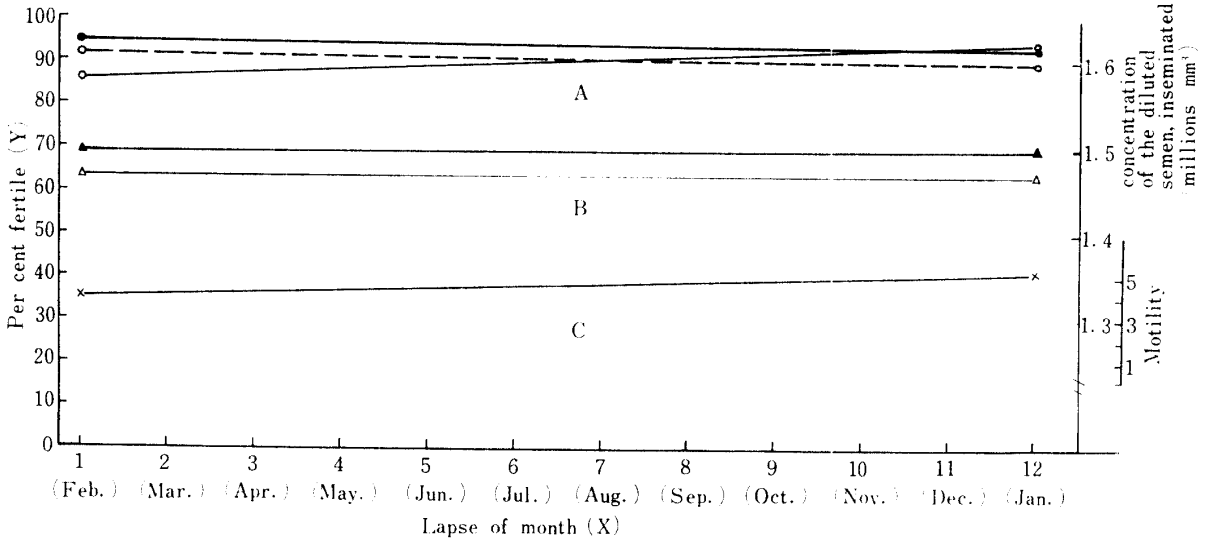

Fig. 4. Regression lines of number and motility of spermatozoa and of fertility of pullets.

A. Fertility
- ●——● group I ($\hat{Y}$=94.68−0.102X)
- ○----○ group S ($\hat{Y}$=91.91−0.215X)
- ○——○ group C ($\hat{Y}$=85.32+0.742X)

B. Concentration of semen inseminated
- ▲——▲ monthly average in groug I ($\hat{Y}$=1498900+0.00000881X)
- △——△ S and C group ($\hat{Y}$=1468800−0.00001097X)

C. Motility of spermatozoa
- ×——× ($\hat{Y}$=4.025+0.10385X)

tor with high cleanliness throughout the operation, only a slight decline in fertility occurred at the end of a prolonged breeding period, proving that the decline was much less than those reported to have happened usually in the field. Similar results were noted by Saeki et al. too (1965)[6),30)].

No decline was observed in our experiment, and the performance of the inseminations at shorter time-intervals than those in case of Schindler and Bornstein or Saeki et al. was expected to make up for the declining tendency in fertility. The posssibility of recompensing the reduced fertility through the aid of more frequently performed inseminations was noted by Schindler and Bornstein in their studies.

On the other hand, by observating the result of this experiment, it may be said that fertility in the control (group C) shows a slight tendency of improvement toward the end of the experiment as shown in the regression line of fertility on lapse of month, in accordance with a slight rise in motility of spermatozoa inseminated (Fig. 4). Hence, from the plateau in the level of fertility in group I, it might be possible to judge that the fertility in group I declines only slightly during a prolonged period of insemination.

As described previously, the chief cause for the decline of the fertility has been assumed, by poultrymen, to be the spermatozoal antibodies in the blood of the hen, endorsed with McCartney's assumption (1923)[31)], from the experiment using rats, that the infertility is caused by the antibodies.

Contrary to this, Lamoreux (1940)[32)] confirmed in the fowl that no fertility was to be

prevented when the formation of such antibodies was induced by the injection of semen. Recently, Itagaki et al. (1966)[10] got a similar conclusion from the results of the continuous inseminations. Then there appeared the report by Saeki et al. (1965)[6] and Abe et al. (1966)[9] that a slight effect might be brought forth upon the fertility by spermagglutinin, but the agglutinin was not considered to be strong enough to cause the lowering of the fertility following the successive inseminations. Moreover, Wentworth and Mellen (1964)[11] reported that, in the hens inseminated repeatedly via various routes, serum antispermatozoa titers increased, and the duration of the fertility decreased with the increase of the number of the inseminations. This decrease in the duration of fertility, however, may have had nothing to do with the level of fertility in the experiment like ours in which the inseminations were made at three day intervals, because, according to the investigation, it was confirmed that the duration of fertility was the period of 7 days or more, the mean duration being about 13 days.

Referring to the causes of severe decline in the fertility appearing only in the common poultry-breeding-farms, Schindler and Bornstein (1962)[4] postulated that the semen sample and the inseminating canula which were contaminated might be the possible cause of the chronic irritation and slowly brought forth inflammation of the vaginal mucosa, which, in turn, seemed to have had a detrimental effects on the sperm inseminated into this passage.

The inflammation of the vaginal mucosa caused by contamination, the injury of the vagina by careless operation, or by the inadequate inseminating syringes, used, or the decline in the quality of semen caused by the seasonal variation or by the careless collection of them; all these combined together or independently might result in a severe decline in fertility. Paradoxical as this may sound, it may be said that this assumption is to be endorsed by another one that careless inseminations have usually been performed in the common poultry breeding farms.

After all, from the results of this experiment it may reasonably be asserted that no or little decline in fertility is to be secured by the operation of the clean and careful artificial insemination, even if it continues for a year.

3. Fertility and the rate of egg production.

In natural mating, a positive correlation between the rate of egg laying and the percentage of fertile eggs was noted by Lamoreux (1940)[33] and Funk (1939)[34]. The results got by Parker and McSpadden (1943)[24] assumed a correlation between the decline of fertility in a hen and the extended term of the egg production; namely, according to their experiments, the fertility lowered gradually and finally almost vanished toward the end of the laying year, but the pullets starting production were made highly fertile with same dosage of semen from the same males. In another experiment, it was confirmed by us that a decreasing tendency in the fertility began to appear immediately before the hens paused to lay eggs.

In this experiment, considerable fluctuations in the egg laying from season to season, similar in patterns through the three groups, were observed as shown in Fig. 5, but no noticeable changes were observable in the fertility, and the high fertilities were maintained for one year, regardless of the rate of egg-production or of the time of the laying

year (Fig. 3, and 4). The effective prevention against the decline of fertility may be due

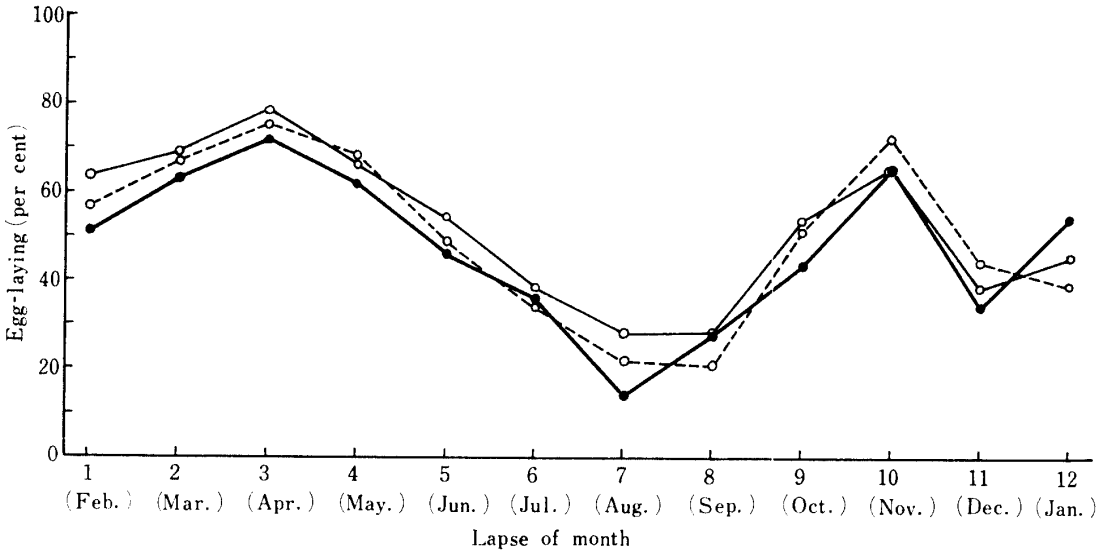

Fig. 5. Seasonal variation in egg-laying.
●——● group I, ○——○ group S, ○----○ group C.

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	F
Total	25	455.99		
Group	2	38.15	19.07	1.05
Error	23	417.85	18.17	

Table 4. Analysis of variance of the rate of laying.
There are no statistical differences among three groups.

to the insemination of the abundant number of active spermatozoa and the shorter time intervals of insemination.

Hard-shelled egg presenting in the uterus at the time of artificial insemination has been regarded as a factor in the decline in fertility, but no adverse effects on fertility were observed in this experiment, which may be attributed to the same reason mentioned above.

In short, provided that the artificial inseminations are to be performed with full amount of active spermatozoa at three day intervals, several adverse factors for fertility of eggs may be made up for, and a high fertility will be maintained.

Summary

This experiment was carried out in order to clear up the period and degree of the decline in fertility observable usually in the common breeding farms 3 to 5 months after the beginning of the artificial insemination.

Thirty crossbred pullets in the first laying year were divided into three groups, 1) In-

semination group (group I), 2) Sham insemination group (group S), and control group (group C), ten pullets in each.

The pullets belonging to group I were inseminated artificially with 0.1 *ml.* of diluted semen at three day intervals for one year. Those belonging to group S were injected Ringer's, solution in the same manner, with the same volume and at the same intervals as those belonging to group I, excepting the fact that they are inseminated only once a month. In group C, the pullets were inseminated once a month with the same semen as group I and group S. Attentions were paid especially to collect the dense semen not contaminated and to carry out the insemination as cleanly and carefully as possible.

The results obtained are as follows:

1. When the quality of the semen was evaluated on the basis of their concentration and the motilities of spermatozoa, the semen inseminated maintained high quality throughout the whole experimental process.

2. Fertilities in all the three groups were very high; 94.2, 94.6 and 95.9 per cent in the three groups I, S, and C respectively, no decreasing tendencies being perceived through all of them.

This implies that, even when the pullets are inseminated for a prolonged period, no or little decline occurs in fertility, and that if artificial inseminations are operated carefully, no adverse mechanical effects are to be brought forth on fertility.

3. Egg-laying rates fluctuated considerably from season to season, but no noticeable changes were observable in fertility; high fertilities were maintained for a year, regardless of the egg production rate or the length of the laying year. And if artificial inseminations are made with enough number of active spermatozoa at shorter intervals, several adverse factors influencing the fertility may be made up for.

Acknowledgments

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