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FACTORS AFFECTING THE Artificial Insemination *OF TURKEYS*

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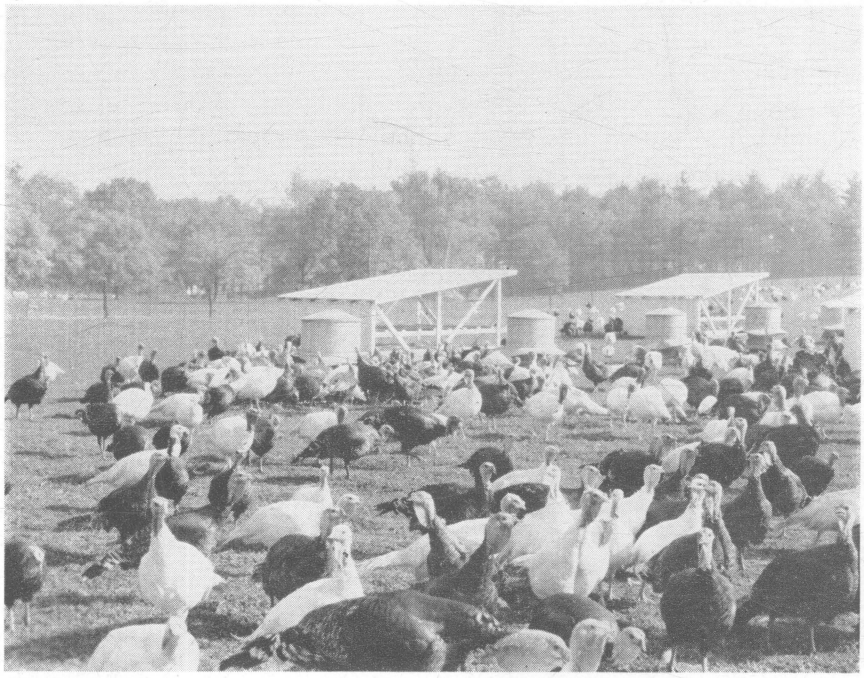

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INTRODUCTION

The practice of artificial insemination has recently become very common and widely accepted among turkey breeders as a method of improving reproductive performance in many low-fertility strains. The fertility problem is one of the most costly to the turkey industry and, therefore, any method of improving fertility will indirectly improve reproductive performance, including hatchability and poult production.

In order to increase the efficiency of artificial insemination for commercial application, there are several factors which must be determined before sound recommendations can be made to the turkey industry. Many insemination programs have been handicapped because there is limited factual experimental evidence concerning the maximum optimal interval between successive inseminations with doses of semen of varying amounts. Also, there is little evidence concerning satisfactory methods of holding turkey semen for any appreciable length of time. Likewise, it is not clearly understood what effect frequency of semen collection may have on resultant fertility and hatchability.

A clearer understanding of the effect of these factors on reproductive performance of artificially inseminated flocks is needed by the turkey industry.

Although, in practice, most flock owners are inseminating their turkeys with 0.05 to 0.025 cc. of undiluted pooled semen at intervals of two or three weeks, there is some evidence indicating that satisfactory fertility and hatchability can be obtained with smaller amounts of semen at less frequent intervals.

LITERATURE REVIEW

Burrows and Marsden (1) inseminated turkeys repeatedly at intervals of 1, 2, 3 and 4 weeks with 0.05 ml. of pooled semen and found no apparent tendency for fertility to decrease with increasing length of time between successive inseminations. Similarly, Parker (10) inseminated turkeys at 3-week intervals with amounts of semen varying from 0.005 ml. to 0.01 ml. and observed little effect of semen dosage on fertility.

Lorenz (4) mated turkeys artificially with single inseminations of 0.05, 0.025 and 0.0125 ml. of semen and suggested that the minimum optimal dose of semen was 0.025 ml. at 3-week intervals. On the other hand, McCartney (6) observed that about 90 percent fertility and 80 percent hatchability of fertile eggs was maintained for six weeks and seven weeks, respectively, following a single insemination of 0.05 ml. of semen and suggested that satisfactory fertility and hatchability might be obtained by inseminating turkeys once every six weeks with 0.05 ml. of semen.

Therefore, McCartney (7) inseminated turkeys with 0.05 ml. of semen and found that increasing the interval between successive inseminations from 2 to 4 or 6 weeks resulted in significantly lower fertility, hatchability and poult production. However, McCartney (8) inseminated turkeys with 0.05 and 0.025 ml. of semen at intervals of 2 and 3 weeks and noted no significant differences in reproductive performance attributable to frequency of insemination or semen dosage and suggested that turkey breeders should inseminate their flocks with 0.025 ml. of semen at intervals of 3-weeks for the most efficient use of semen and labor.

There is little information in the literature concerning the effects of holding time and temperature of turkey semen on fertilizing capacity. Harper (3) held turkey semen at 55° and 60° F. for periods up to four hours and observed that holding the semen for this period of time was not detrimental to fertility for hens inseminated during a 3-week period starting in February. However, inseminations later in the season showed a progressive decrease in fertility as affected by length of holding period. It was concluded that turkey semen should be used within one hour after collection for best fertility.

Since the volume of semen obtained from turkey males at any one time is relatively small, varying from barely measurable amounts to slightly more than 0.3 ml., Carson et al. (2) and McCartney (9), information is needed to determine the effect of collection frequency on quantity of semen and fertilizing capacity. Lorenz et al. (5) studied the effect of collection frequency on the quantity of semen produced by turkey males and found that the volume of semen decreased gradually during the first week of daily collections; however, this decrease in volume had no apparent effect on sperm concentration, except when daily collections were continued long enough to reduce volume drastically. Although these results suggest that daily collections of semen for 5 or 6 days may not have any adverse effects on the fertilizing capacity of the spermatozoa produced, there are apparently no reports in the literature concerning this important point.

RELATION OF FREQUENCY OF INSEMINATION AND SEMEN DOSAGE TO REPRODUCTIVE PERFORMANCE

EXPERIMENT 1. LARGE-TYPE WHITE TURKEYS

In this experiment, eight groups of 14 Large-type White turkey females each hatched on April 15, 1955, were selected at random and housed in 10′ × 20′ breeding pens on November 1, 1955. All pullets were exposed to lighting at 4 A.M. on and after February 3, 1956. On March 2, 1956, artificial insemination of undiluted pooled semen was started and continued for a 12-week period at intervals of 2 and 3 weeks as follows: Groups 1 and 2—0.05 cc. of semen; groups 3 and 4– 0.025 cc. of semen; groups 5 and 6—0.0125 cc. of semen and groups 7 and 8 —0.01 cc. of semen. All birds were trap-nested and starting on March 2, 1956, all eggs laid in the nests were collected and set bi-weekly for the 12-week period.

Fertility was determined by candling on the fourteenth day of incubation. All eggs removed were broken out after candling and examined macroscopically to identify any early-dead embryos that otherwise would have been classified as infertile. Hatchability was determined at the end of each hatch and average poult production at the conclusion of the experiment.

All groups of females were inseminated with undiluted pooled semen obtained from a group of males of the same variety, age and lighting history as the females. All inseminations were made immediately following the collection of semen, usually within 20 to 30 minutes.

The percentage fertility, hatchability and average poult production for groups of females inseminated with the four dosage levels of semen at intervals of two and three weeks are presented in Table 1, 2 and 3. These results indicate that there were differences for fertility among the groups of females inseminated with the four dosage levels of semen. However, the only large difference was for the females inseminated with 0.025 cc. of semen at the 2-week interval. Since females inseminated with 0.0125 and 0.01 cc. at intervals of 2-weeks had as good fertility as those inseminated with 0.05 cc. at the same interval, it appears that the lower fertility for the females inseminated with 0.025 cc. of semen every two weeks was a chance occurrence. Also, there was apparently no difference in fertility between the two insemination frequencies. Although there was considerable variation among groups for hatchability, it appears that none of these differences could be attributed directly to semen dosage or interval between inseminations.

TABLE 1.—Percent Fertility—Large Whites

Insemination interval (weeks)	Semen dosage (cc.)				Average all dosages
	0.05	0.025	0.0125	0.01	
2	96	84	92	95	92
3	95	87	91	87	90
Average	96	86	92	91	91

The over-all picture for average poult production reveals that differences in semen dosage and time interval had little or no affect on this variable. This is shown by the fact that no large differences were noted in average poult production per female among groups inseminated with 0.05 cc. and 0.01 cc. of semen at the two or three-week intervals between inseminations.

The results of this experiment indicate that for the most efficient use of semen and labor, poult producers can inseminate Large-type White turkeys with as little as 0.01 cc. of semen at intervals of three weeks. However, work with this type of turkey needs to be continued to determine if less frequent inseminations would produce satisfactory fertility and hatchability.

TABLE 2.—Percent Hatchability of Fertile Eggs—Large Whites

Insemination interval (weeks)	Semen dosage (cc.)				Average all dosages
	0.05	0.025	0.0125	0.01	
2	74	76	75	79	76
3	81	72	60	74	72
Average	78	74	68	76	74

TABLE 3.—Average Poult Production per Female—Large Whites*

Insemination interval (weeks)	Semen dosage (cc.)				Average all dosages
	0.05	0.025	0.0125	0.01	
2	24	21	23	25	23
3	26	21	18	22	22
Average	25	21	21	23	22

*Calculated from percent hatchability of all eggs set, using an average of 34 eggs set per female for all groups.

EXPERIMENT 2. SMALL-TYPE WHITE TURKEYS

In this experiment, twelve groups of 15 Small-type White turkey females each hatched on June 14, 1956, were selected at random and housed in 7.5′ × 20′ breeding pens on December 12, 1956. On February 11, 1957, artificial inseminations of undiluted pooled semen were started and continued for either a 15-week or 16-week period as follows: Groups 1 and 7—0.025 cc. of semen at 3-week intervals; groups 2 and 8—0.01 cc. of semen at 3-week intervals· groups 3 and 9—0.025 cc. of semen at 4-week intervals· groups 4 and 10—0.01 cc. of semen at 4-week intervals; groups 5 and 11—0.025 cc. of semen at 5-week intervals and groups 6 and 12—0.01 cc. of semen at 5-week intervals. All eggs were collected and set weekly for the 15 or 16-week breeding period.

All females were exposed to lighting at 4 A.M. on and after January 2, 1957. As in the case of Experiment 1, all groups of females were inseminated with semen obtained from a group of males of the same variety, age and lighting history as the females. However, in this experiment fertility was determined on the seventh day of incubation and then all eggs removed were broken out and examined macroscopically for early-dead embryos that would otherwise have been classified as infertile. Hatchability and average poult production were also determined following each hatch.

The percentage fertility, hatchability and average poult production for the six duplicate groups artificially inseminated with 0.025 cc. or 0.01 cc. of semen at intervals of 3, 4 and 5 weeks are presented in Tables 4, 5 and 6. Although the fertility, hatchability and average poult production of the duplicate groups of females inseminated with 0.01 cc. of semen at the 4-week interval was somewhat lower than those for the other groups, it was concluded that these variables were not affected by

TABLE 4.—Percent Fertility—Small Whites

Insemination interval (weeks)	Semen dosage (cc.)		Average both dosages
	0.025	0.01	
3	85	85	85
4	86	78	82
5	85	83	84
Average	85	82	84

the two levels of semen used in this experiment because semen dosage had no affect at the 3 or 5-week intervals. Similarly, fertility, hatchability and average poult production were apparently not influenced by the three intervals between inseminations, indicating that inseminations, at 5-week intervals provided as good reproductive performance as insemination at 3 or 4-week intervals in this variety of turkey. These results are not too surprising in view of the findings of McCartney (6), who found that the average duration of fertility following a single insemination was approximately 7 weeks. These results suggest that the duration of fertility may be longer for the Small-type White turkey than for other varieties of turkeys.

Figure 1 presents the fertility curve for the data summarized on a weekly basis for each insemination interval and both dosage levels of semen. This curve suggests a curvilinear relationship between weeks after insemination and fertility and indicates that fertility tends to

TABLE 5.—Percent Hatchability of Fertile Eggs—Small Whites

Insemination interval (weeks)	Semen dosage (cc.)		Average both dosages
	0.025	0.01	
3	78	74	76
4	76	71	74
5	79	76	78
Average	78	74	76

decrease very slightly between the first and second week after insemination (87 to 86%); but the rate of decrease accelerates between the second and third (86 to 82%) and subsequent weeks following insemination.

These results indicate that for the most efficient use of semen and labor that Small-type White turkeys can be inseminated with as little as 0.01 cc. of semen at intervals of 5 weeks. However, since fertility tends to decrease at a more rapid rate after the second week following insemination, it appears that maximum fertility would be obtained by inseminating every two weeks.

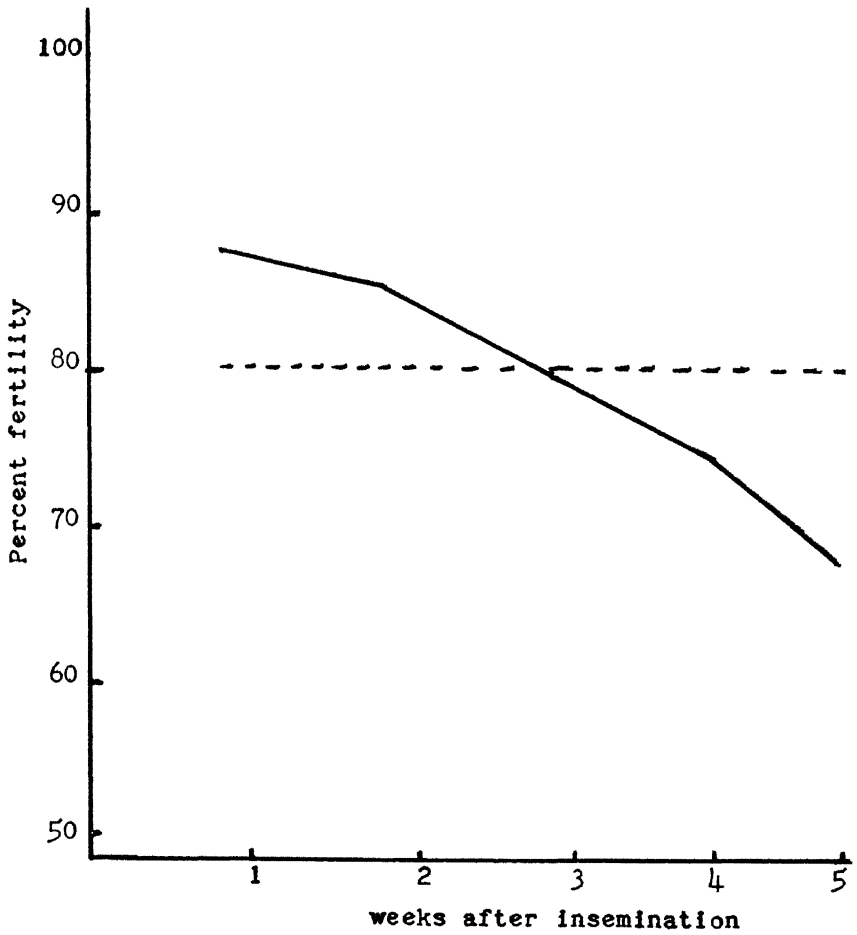

Fig. 1 - Fertility curve for Small-type Whites

TABLE 6.—Average Poult Production per Female—Small Whites*

Insemination interval (weeks)	Semen dosage (cc.)		Average both dosages
	0.025	0.01	
3	34	32	33
4	33	28	30
5	34	32	33
Average	34	31	32

*Calculated from percent hatchability of all eggs set, using an average of 51 eggs set per female for all groups.

EXPERIMENT 3. THREE VARIETIES OF TURKEYS

In this experiment, four duplicate groups of 15 females each of three varieties (Bronze, Large-type Whites and Small-type Whites) were inseminated with 0.01 cc. of semen at 2, 3, 4 and 5 weeks for a 15 or 16-week period, beginning on January 13, 1958. The Bronze, Large-type Whites and Small-type Whites were hatched on April 26, May 13 and May 6, 1957, respectively. All birds were randomly selected and housed in comparable sized pens (120 sq. ft.) on December 5, 1957.

All females were exposed to lighting at 4 A.M. on and after December 6, 1957. As in the case of Experiments 1 and 2, all groups were inseminated with semen obtained from a group of males of the same variety and age as the females. However, the males were stimulated with artificial lights at 4 A.M. on and after November 18, 1957. In this experiment fertility, hatchability and poult production were determined the same as for Experiment 2.

Tables 7, 8 and 9 present the percentage fertility, hatchability and average poult production for duplicate groups of females of three varieties of turkeys inseminated with 0.01 cc. of semen at 2, 3, 4 and 5-week intervals.

These results show that there were differences among the three varieties for the three characteristics under comparison. The Bronze variety had the poorest fertility, hatchability and poult production, while there was little or no difference between the other two varieties for these three reproductive characteristics. However, for frequency of insemination, the females of each variety inseminated at 2-week intervals were the most highly fertile, while there tended to be no great differences in fertility for the females inseminated at 3, 4 or 5-week

TABLE 7.—Percent Fertility in Three Varieties*

Insemination interval (weeks)	Bronze	Large White	Small White	Average all varieties
2	74	87	88	83
3	61	79	80	73
4	58	73	76	69
5	67	79	78	75
Average	65	80	81	75

*Semen dosage, 0.01 cc.

intervals. These results indicate that optimum fertility was obtained by inseminating every two weeks. However, reasonably good fertility was obtained even when the interval between inseminations was extended to as long as once every five weeks.

The results for hatchability of fertile eggs show the same varietal differences as obtained for fertility, with the Bronze being poorest and no differences between the other two varieties. There appeared to be small differences in hatchability of fertile eggs which are related to the frequency of insemination. The highest average hatchability value of 74 percent was obtained by inseminating every two weeks, with a small decrease in hatchability resulting when the frequency of insemination was decreased to once every 3, 4 or 5 weeks.

TABLE 8.—Percent Hatchability of Fertile Eggs in Three Varieties*

Insemination interval (weeks)	Bronze	Large White	Small White	Average all varieties
2	68	80	73	74
3	70	68	77	72
4	65	77	71	71
5	69	72	76	72
Average	68	74	74	72

*Semen dosage, 0.01 cc.

TABLE 9.—Average Poult Production per Female in Three Varieties*

Insemination interval (weeks)	Bronze	Large White	Small White	Average all varieties
2	26	36	34	32
3	23	28	32	28
4	20	30	29	26
5	24	30	31	28
Average	23	31	32	29

*Calculated from percent hatch of all eggs set, using an average of 52 eggs set per female for all groups.

The average poult production per female followed the same trend obtained for fertility and hatchability of fertile eggs and suggests very strongly that insemination at 2-week intervals would be an economic advantage from the standpoint of the additional numbers of poults produced by these females.

The curves presented in Figure 2 summarizes the fertility data on a weekly basis for the three varieties of turkeys inseminated at the four frequencies of insemination. Those curves are somewhat similar to those calculated for the Small-type Whites in Experiment 2. Except for the Bronze variety, fertility tended to be slightly higher for the eggs laid during the second week than for those laid during the first after insemination. However, after the second week fertility decreased quite rapidly from 85% to 64% for the Large-type Whites and from 86% to 66% for the Small-type Whites. The fertility curve for the Bronze variety indicates that slightly better fertility would be obtained by weekly inseminations, while that for the two White varieties indicate that there would be no advantage of inseminating them more often than once every two weeks. These results, in part, suggest that the difference in fertility level between the three varieties of turkeys may be due to a more rapid decrease in fertility following insemination in the Bronze variety. This somewhat more rapid decrease in fertility suggests that duration of fertility may be shorter in the Bronze than in the two White varieties.

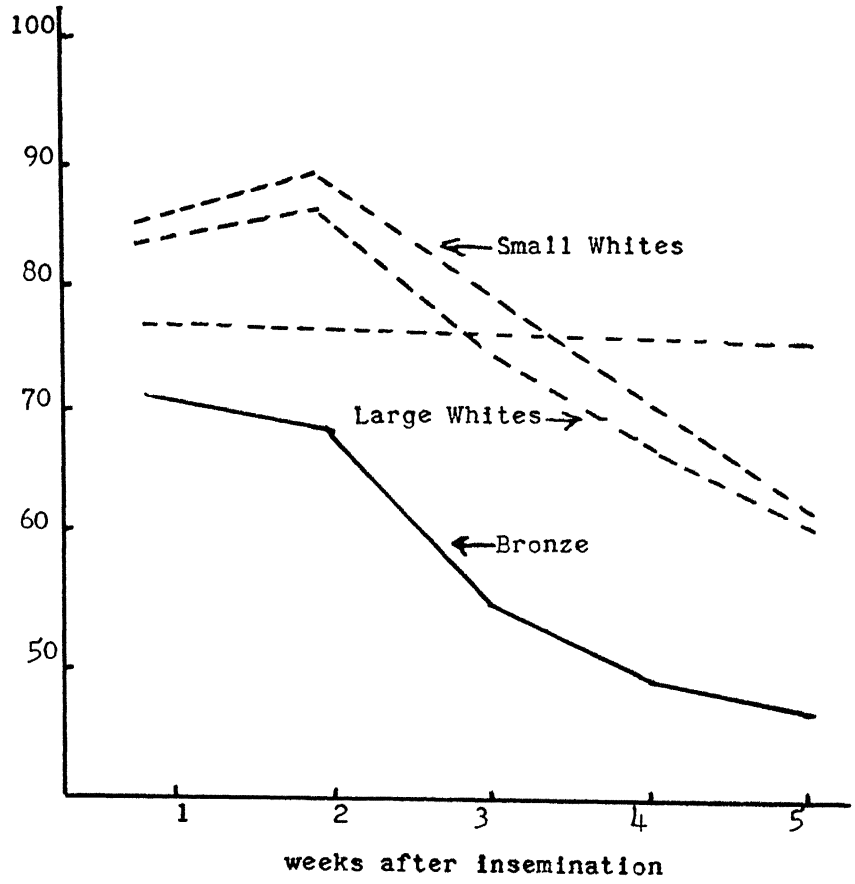

Fig. 2 - Fertility curve for three varieties of turkeys.

RELATION OF STORAGE TIME AND TEMPERATURE ON FERTILIZING CAPACITY OF SEMEN

Large-type White turkey pullets, hatched on April 15, 1955, were divided at random into 8 groups of 18 pullets each and housed intermingled in five 14′ × 18′ breeding pens. Each group of 18 consisted of sets of 3 or 4 pullets from each pen. All pullets were exposed to lights at 4 A.M. on and after February 14, 1956. On March 14, 1956, each group was artificially inseminated once with 0.05 cc. of undiluted pooled semen which had been stored under one of the following 8

conditions: 50° F. or 60° F. for periods of 0, 2, 4 or 6 hours. The semen used for this purpose was obtained from a group of 20 males of the same age, variety and lighting history as the females.

The pooled undiluted semen was divided into 1 cc. samples, one sample being used for immediate insemination and the remainder stored in stoppered glass tubes. The samples to be stored were placed in a thermos bottle maintained at either 50° F. or 60° F., until taken to the

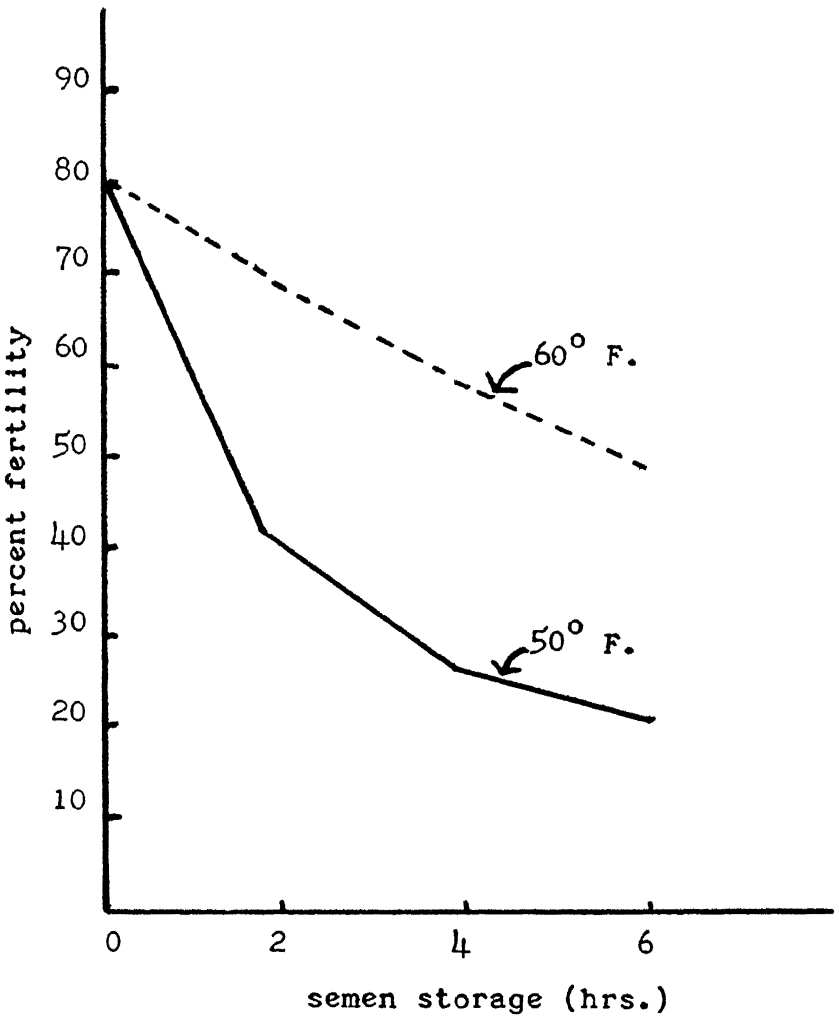

Fig. 3 - Effect of temperature and storage time on fertility.

laboratory where they were stored in constant temperature chambers maintained at these temperatures. At the proper time intervals, the semen samples were removed from the constant temperature chambers and females inseminated with it.

Eggs were set bi-weekly for a period of 6 weeks after the single insemination. All eggs were candled on the fourteenth day of incubation and those removed as infertiles were broken out and examined macroscopically for embryonic development. Hatchability was determined at the conclusion of each hatch.

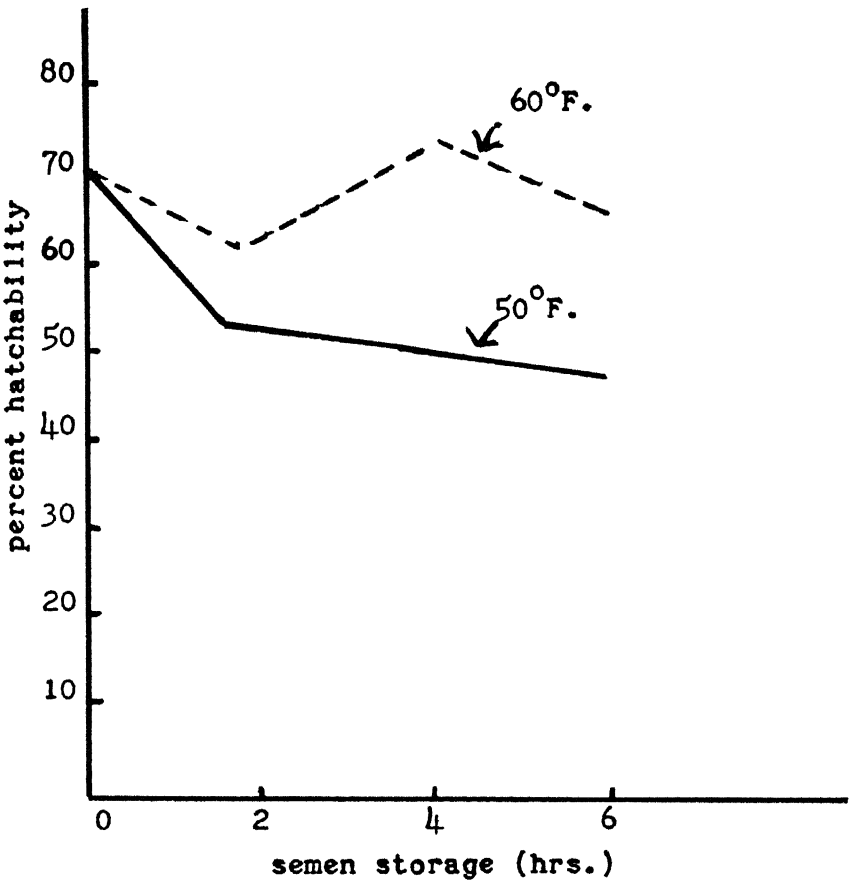

Fig. 4 - Effect of temperature and storage time on hatchability of fertile eggs.

The curves for fertility and hatchability of fertile eggs laid by the turkey females inseminated with a single dose of 0.05 cc. of pooled undiluted semen, which was stored under the various conditions, is presented in Figures 3 and 4. These curves show that the fertilizing capacity of turkey semen decreases as the storage period increases and that the rate of decline depends upon the storage temperature. The fertilizing capacity of semen stored for 6 hours at 60° F. decreased from 79 to 49 percent, while that stored at 50° F. for the same length of time decreased from 80 to 22 percent. Also, there was a considerable decrease in fertility at both temperatures even after a storage period of only two hours.

The curves for hatchability of fertile eggs indicate that storage at 50° F. may have a more deleterious effect on aging spermatozoa than storage at 60° F. Since it has been shown by Nalbandov and Card (11) and McCartney (6) that eggs fertilized by stale sperm are more apt to terminate their development prior to hatching than are embryos in eggs fertilized by fresh spermatozoa, it appears that storage temperature of semen prior to insemination may adversely affect hatchability by prematurely decreasing the viability of the spermatozoa.

Both the fertility and hatchability results obtained in this study indicate that turkey semen should be used for artificial insemination as soon as possible after collection, probably within 30 minutes, and that storage at 60° F. preserves fertilizing capacity of the semen and maintains the viability of the spermatozoa for hatchability better than storage at 50° F.

RELATION OF FREQUENCY OF SEMEN COLLECTION TO FERTILITY AND HATCHABILITY

Five groups of 30 Small-type White turkey pullets each hatched on June 14, 1956, were housed in 14′ × 18′ breeding pens on December 12, 1956. On and after January 2, 1957, all birds were exposed to lighting at 4 A.M. On February 11, 1957, artificial inseminations of 0.025 cc. of pooled undiluted semen were initiated and continued at 3-week intervals over a 12-week breeding period.

The semen used to inseminate these females was obtained from a group of 30 males of the same variety, age and lighting history as the females. Each male was ejaculated on the Friday preceding a series of five successive daily collections and the semen discarded. On the following Monday, the series of daily collections were initiated for insemination purposes and continued for five successive days. The semen for

each day's collection was pooled and used to inseminate a different group of females. This semen collection and insemination plan was repeated at 3-week intervals over a 12-week period.

All pullets were trap-nested and, starting on February 18, 1957, eggs for hatching were collected and set weekly through May 6, 1957. Fertility was determined by candling on the seventh day of incubation, all removed eggs being broken out to identify any early dead germs that would otherwise have been classified as infertile. Hatchability was determined at the conclusion of each hatch.

The percentage fertility and hatchability of fertile eggs and average number of poults produced per female of groups artificially inseminated with semen obtained from males on five successive daily collections are presented in Table 10. The results of this study indicate that frequency of semen collection had no effect on fertility, hatchability or poult production; although fertility tended to increase slightly as the frequency of collection increased, none of these differences were significant. Similarly, the differences in hatchability and average poult production between collection at the five different intervals were not significant.

Although total semen volume was not measured for each of the five successive daily collections, gross observations indicated that semen volume decreased as frequency of collection increased from 1 to 5 in succession. However, spermatozoa concentration was determined for each series of five daily collections. The mean number of spermatozoa as determined by the hemocytometer, using 1:500 dilution, was 5.8 $\pm$ 1.2 million per cubic millimeter. There was no indication that frequency of semen collection had any affect on spermatozoa concentration.

TABLE 10.—Effect of Frequency of Semen Collection on Reproductive Performance

Semen collection frequency (days)	Percent fertile	Percent hatchability fertile eggs	Average number poults per female*
1	86	75	28
2	89	80	30
3	89	77	29
4	91	76	29
5	92	75	29
Average	89	77	29

*Calculated from percent hatch of all eggs set, using an average of 43 eggs set per female for all groups.

SUMMARY

The relation of frequency of insemination and semen dosage to reproductive performance was studied over a three-year period, using three varieties of turkeys. In general, inseminations at 2-week intervals tended to result in better fertility, hatchability and average poult production than inseminations at less frequent intervals in all three varieties. In the third experiment, where all three varieties were studied simultaneously, fertility, hatchability and average poult production was 12, 2 and 15 percent less, respectively, when the interval between successive inseminations was reduced to once every 3, 4 or 5 weeks.

There was no evidence to indicate that fertility, hatchability and poult production were affected by variations in the dosage level of semen. As good reproductive performance was obtained with 0.01 cc. of undiluted pooled semen as with 0.05 cc. per insemination. The results indicate that optimum reproductive performance of turkeys can be obtained by insemination at 2-week intervals with as little as 0.01 cc. of undiluted pooled semen.

The Bronze variety had consistently poorer fertility, hatchability and poult production than the Large-type Whites and Small-type White varieties, while there were little or no differences between the two varieties of Whites for these reproductive characteristics. The lower level of fertility on the part of the Bronze variety is apparently due, in part, to a more rapid decrease in fertility following insemination than in the case of the other two varieties. This suggests that duration of fertility following insemination may be shorter for the Bronze than for the two White varieties.

Turkey semen stored at 60° F. had better fertilizing capacity than at 50° F., however, a sharp drop in fertility was experienced when semen was stored at either temperature for a period of only two hours. Hatchability of fertile eggs was more adversely affected by storage of semen at 50° F. than at 60° F., indicating that spermatozoa viability may be interferred with at the lower temperature.

Fertility, hatchability and poult production were not affected by increasing the frequency of semen collection from 1 to 5 successive days. Semen volume decreased gradually as the frequency of collection was increased from 1 to 5 days in succession. However, the spermatozoa concentration was not apparently affected by frequency of collection, averaging 5.8 $\pm$ 1.2 million per cubic millimeter of semen for Small-type White turkeys.

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