intraperitoneal insemination was, as far as could be ascertained, the sole unusual treatment received by the hens), showed results which did not vary from the gross results (see and *cf.* Graphs D and F).

The degree of the initial loss of eggs as far as the intraperitoneal method of insemination is concerned is illustrated in Graph G, but the data are not sufficient to gauge the extent of the compensatory increased production after the sixteenth day. The observations were not suitable for analysis of the production level beyond the third week after insemination. All the results show a tapering off in percentage production during the last three five-day periods, but a glance at table 15 shows that only a small number of experimental and control subjects were kept under observation during these periods, and that they were, as a rule, the poor producers.

Abnormal eggs are not an uncommon occurrence in fowls selected for high production. Previous experience had found the battery system of keeping fowls very useful for determining the laying qualities of hens in this respect. In the run or intensive fowl-house, premature eggs apparently rarely reach the trapnest, often being eaten before they dry up, and so pass unnoticed.

In the battery laying cage, broken or premature eggs never escape notice as they fall through the wire screen floor onto the dropping tray and can be recorded at daily cleaning. The records show that about 6 per cent. of eggs laid within 48 hours after intraperitoneal insemination were premature. No similar figures for other methods are available. Intraperitoneal inseminations did not affect the production of double yolked eggs. [Perhaps occasionally, *cf.* operation (v) part (2), page 53)].

In connection with the loss of health in birds subjected to intraperitoneal injections, the case of localized peritonitis in pigeon number 375 and the two cases of generalized peritonitis in hens numbers 42 and 50 are all of small significance. It is almost certain that such cases would not occur under proper routine methods with efficient sterilization of instruments. The small bore needle used in fowl hens has an obvious defect in its flexibility of the point which cannot fully be controlled by the operator and this may cause fatal damage to the blood vessels as happened in the case of hen number 31. The death of hen 19 (control) was apparently the result of liver rupture caused by pressure on the abdomen applied to obtain temporary prolapse of the vagina for direct insemination into the oviduct. Rupture of the liver in fowls is not often met with and is usually associated with a kick or fall and with a predisposing fatty condition of the organ. It was not previously experienced with artificial insemination in South Africa or elsewhere, but the case is a reminder that accidents can occur even with a proved technique. (Rupture of the liver is a common cause of internal haemorrhage in pigeons which collide with a wire during flight or in squeakers that fall from a high nest.)

(4) *The fertility resulting from intraperitoneal insemination of pigeons and fowls.*

(*a*) *Pigeons.*—The fertility to be expected in pigeons after intraperitoneal injections of semen was determined by recording the results of incubation of all eggs collected after the operations mentioned in part (3) (*a*). The technique of insemination was described in part (2) (*a*).

The results are summarized in table 19 from which can be seen that 11·3 per cent. of the total number of operations carried out were followed by a fertile egg, but not more than one fertile egg in each case. Fifty inseminations

failed to show any results, but thirty-four of these were executed outside the limits postulated by Owen (1941) for successful insemination *per vaginam;* viz.: one to six days before the laying of the first egg of a clutch.

In this series of intraperitoneal inseminations successes only occurred with operations performed between the times fifty hours before to two hours after the laying of the first egg. i.e. 46 to 98 hours before the laying of the fertile eggs. The details are listed in table 20.

TABLE 19.

The Fertility of Pigeon Eggs after Intraperitoneal Insemination.

Line.	Time of Insemination.		Number of Insemina-tions.	Number of First Eggs Laid.	Number of First Eggs Fertile.	Number of Second Eggs Laid.	Number of Second Eggs Fertile.
	Period.	Days and Hours.					
a	Before the lay-	7–18 days	10	10	0	8	0
b	ing of the first	120–140 hrs.	2	2	0	2	0
c	egg of a clutch	95–120 hrs.	1	1	0	1	0
d		72–96 hrs.	3	3	0	3	0
e		48–72 hrs.	3	3	0	3	0
f		24–48 hrs.	8	8	1*	6	3
g		12–24 hrs.	10	10	—	8	1
h		0–12 hrs.	1	1	—	1	0
i	After the laying	0–1 hrs.	16	16	—	14	4
j	of the first egg	1–2 hrs.	4	4	—	4	3
k	of a clutch	2–3 hrs.	1	1	—	1	0
l		3–4 hrs.	—	—	—	—	—
m	When no eggs were laid	—	3	0	—	0	—
	TOTALS.....	—	62	59	1	51	11

* Unfortunately not followed by a second egg.

TABLE 20.

The Pigeon Eggs Fertilized by Intraperitoneal Insemination.

Line.	Number of Egg.	Number of Pigeon.	Position of Egg in Clutch.	Interval between Insemina-tion and Laying.	Minimum Time for onset of Fertility.	Maximum Time for Duration of Fertility.
a.....	34	222	First.....	48 hrs.	—	—
b.....	61	37	Second....	78 ,,	—	—
c.....	63	304	Second....	46 ,,	46 hrs.	—
d.....	75	544	Second....	47 ,,	—	—
e.....	76	375	Second....	46·5 ,,	—	—
f.....	78	688	Second....	46 ,,	46 hrs.	—
g.....	84	244	Second....	46·5 ,,	—	—
h.....	96	222	Second....	46·5 ,,	—	—
i.....	106	37	Second....	98 ,,	—	98 hrs.
j.....	108	375	Second....	73 ,,	—	—
k.....	112	61	Second....	68 ,,	—	—
l.....	119	61	Second....	46 ,,	46 hrs.	—

The total eggs collected numbered 110; four were cracked and 106 were set in the fowl egg incubator [see part A. page 35 and Experiment (3), page 46]. Ninety-four failed to show fertility. Between the minimum time of onset and the maximum time for duration of fertility encountered in this series, forty-seven eggs were laid (i.e. between 46 and 98 hours after operation) of which two were cracked giving a percentage fertility of twenty-seven. Within the limits postulated by Owen (1941) for vaginal artificial insemination, fifty-six eggs were laid (i.e. the first eggs of a clutch laid two to six days after insemination and the second eggs laid two to eight days after insemination are all included). Five of these proved fertile.

Controls.—Some pigeons, naturally mated in the loft during the same season, were used for control purposes. A few cases in which the hens in cages were paired up with castrated males or homosexually mated, were also used for this purpose. The latter had their mate replaced by a fertile cock for an afternoon (i.e. a time period of four hours), at a time considered suitable for fertilization of the clutch expected to be laid within six days after. All eggs were collected and incubated in the fowl egg incubator. The result of intraperitoneal insemination in pigeons are compared with control results in table 21. The eggs laid after natural continuous mating and incubated by the parents in the nest were also considered but not listed. These hatched much better than the incubator eggs indicating that some eggs recorded as infertile may have contained embryos that died early and escaped notice.

TABLE 21.

The Fertility of Pigeon Eggs after intraperitoneal Insemination and Natural Coitus.

Line.	Method of Insemination.	Time of Insemination.	Number of Insemi-nations.	Number of Eggs Laid.	Number of Eggs Incu-bated.	Number of Eggs Proved Fertile.
a	Intraperitoneal insemination (hens paired to castrate or to other hen homosexually)	18 days before to 3 hours after laying first egg of clutch	59	110	106	12
b		44–144 hours before laying first egg of clutch	49	56	52	5
c		46–98 hours before any one egg was laid	44	47	45	12
d	Natural mating of 1 to 4 hours duration : (Hen paired with castrate or with other hen during the rest of the time)	44–144 hours before laying the first egg of the clutch	7	11	11	1
e		46–98 hours before any one egg was laid	4	8	8	1
f	Natural continuous mating	44 hours or more before laying any egg	14	28	28	21

(*b*) *Fowls.*—In fowl hens inseminated by intraperitoneal injection of semen the fertility to be expected was determined by analysis of the 117 serial repetitions of the operation described in part (2) (*b*) (i) i.e. the first operation on bird No. 9. This first series was carried out over a period of twelve months and out of the

117 cases of intraperitoneal insemination 90 were followed by the laying of eggs that could be tested for fertility. In 28 cases all eggs set were found infertile and in 15 cases the fertility could not be included because the eggs were fertilized by spermatozoa introduced with simultaneous, previous or subsequent inseminations or the result was doubtful. Thus 47 cases of proved fertility are presented.

The criteria by which the fertility was valued were:—

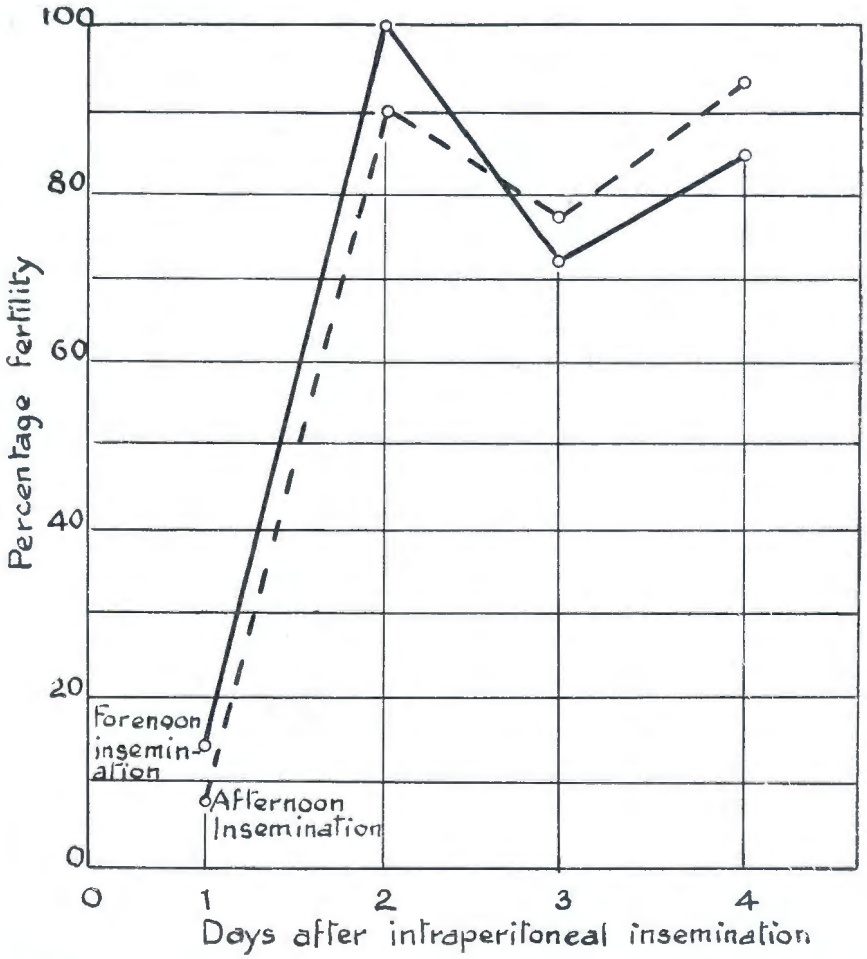
(*i*) The onset of fertility,

(*ii*) the duration of fertility,

(*iii*) the degree, intensity (Raimo, 1943) and consistency of fertility and

(*iv*) the percentage fertile inseminations.

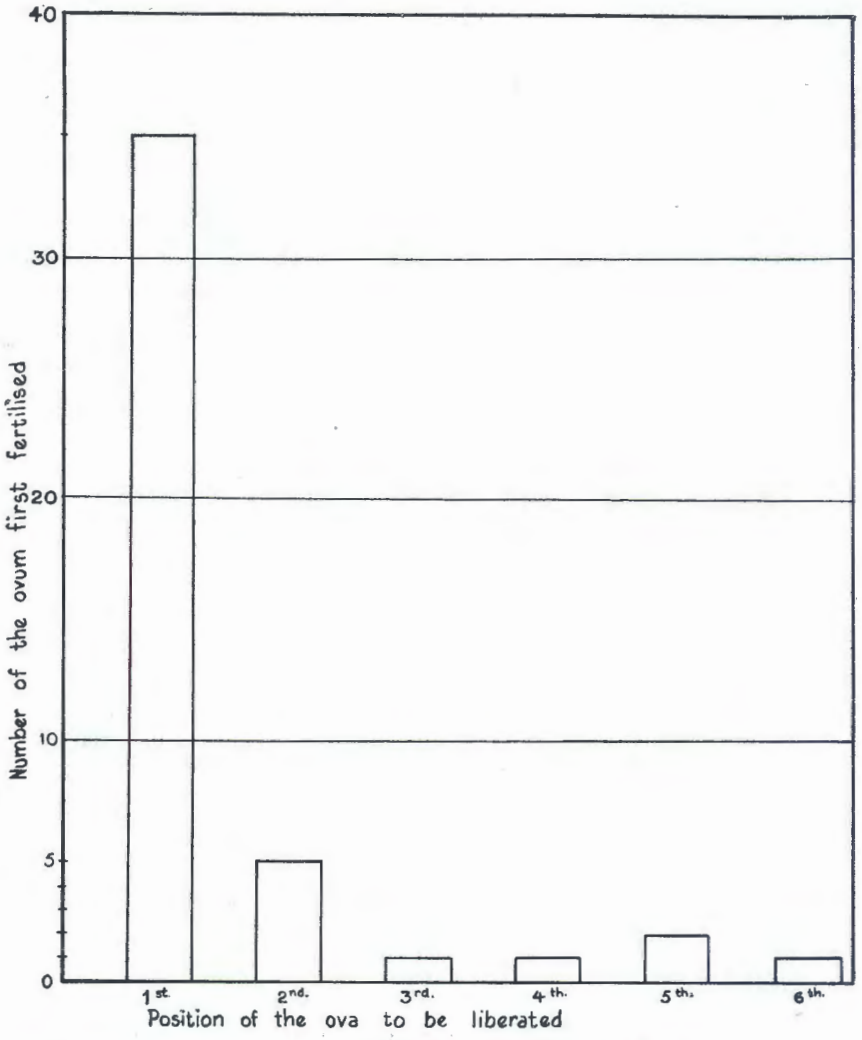
GRAPH H.—The percentage of eggs laid on each of the first four days after intraperitoneal insemination, that were proved to be fertile following the operations done in the forenoon, compared with the percentages after operations done in the afternoon.



Graph H shows that forenoon intraperitoneal insemination produced slightly higher fertility than afternoon insemination on the first two days following the operation; but on the third and the fourth day the results for afternoon insemination were better.

(*i*) *The onset of fertility.*—In the review of the literature the reasons why the time of insemination affects the lengths of the period between insemination and the laying of the first fertile egg have been mentioned. In table 22 a summary is given of the fertility of eggs laid on the four days following the day of intraperitoneal insemination performed at different times during the day. The percentage of eggs found fertile on the first four days after such insemination in the forenoon is compared with the percentage fertile after doing the operation in the afternoon in Graph H.

GRAPH I.—The frequency of being the first ovum to be fertilized, in each of the first six ova to be liberated after successful intraperitoneal insemination.



Graph I shows that in the case of intraperitoneal insemination the first ovum to be liberated after the operation, was successfully impregnated in the large majority of cases.

TABLE 22.

Fertility of Eggs laid on four days after Intraperitoneal insemination at the Different Times of the Day (All Cases with Fertile Eggs Laid within Four Days.

Line.	Time of Insemination.	Number of Inseminations.	Number of Eggs laid on the same Day.		Number of Eggs laid on the first Day.		Number of Eggs laid on the second Day.		Number of Eggs laid on the third Day.		Number of Eggs laid on the fourth Day.	
			Before Insemination.	After Insemination.	Total Number Laid.	Number or Percentage Fertile.	Total Number Laid.	Number or Percentage Fertile.	Total Number Laid.	Number or Percentage Fertile.	Total Number Laid.	Number or Percentage Fertile.
a	8–9.00 a.m.	4	1	3	1	0	2	2	1	1	3	3
b	9–10.00 a.m.	3	0	0	1	0	1	1	2	2	1	1
c	10–11.00 a.m.	7	3	1	4	1	6	6	4	2	4	3
d	11–12.00 a.m.	1	1	0	1	0	1	1	1	1	0	0
e	Forenoon..	15	5	4	7	14%	10	100%	8	72%	8	85%
f	12–1.00 p.m.	3	2	0	1	0	1	1	2	2	1	1
g	1–2.00 p.m.	3	1	0	2	0	3	3	0	0	2	2
h	2–3.00 p.m.	5	2	0	2	0	3	3	3	3	2	1
i	3–4.00 p.m.	2	1	0	2	0	0	0	2	1	1	1
j	4–5.00 p.m.	1	0	0	1	1	1	0	2	1	2	2
k	5–6.00 p.m.	3	2	0	2	0	1	1	2	2	3	3
l	6–7.00 p.m.	2	2	0	1	0	0	0	2	1	2	1
m	7–8.00 p.m.	2	0	0	2	0	1	1	1	1	1	1
n	Afternoon.	21	10	0	13	8%	10	90%	14	77%	14	93%

TABLE 23.

The First Ovum to be Fertilized, of those Liberated after Intraperitoneal Insemination.
(All the Cases with Fertile Ova Among the First Six.)

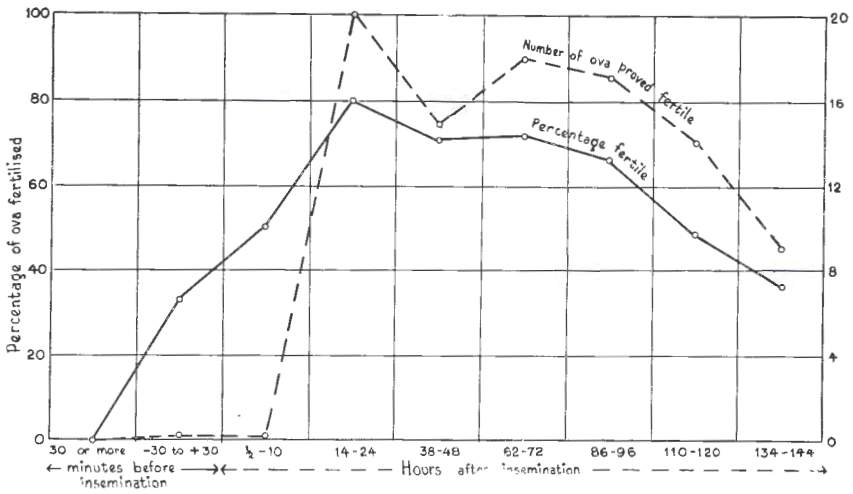
Line.	Time of Insemination.	Number of Fertile Inseminations.				First Ovum to be Fertile of those liberated after Insemination.					
		After Laying.	Before Laying.	On Egg-less Day.	Total.	First.	Second.	Third.	Fourth.	Fifth.	Sixth.
a	8.00– 9.00 a.m....	1	Nil	3	4	3	1	—	—	—	—
b	9.00–10.00 a.m....	1	1	3	5	3	—	1	—	1	—
c	10.00–11.00 a.m....	3	1	4	8	7	1	—	—	—	—
d	11.00–12.00 noon...	1	Nil	Nil	1	1	—	—	—	—	—
e	Forenoon.....	6	2	10	18	14	2	1	Nil	1	Nil
f	12.00–1.00 p.m.....	1	Nil	2	3	3	—	—	—	—	—
g	1.00–2,00 p.m.....	2	Nil	2	4	3	—	—	—	—	1
h	2.00–3.00 p.m.....	3	Nil	4	7	6	—	—	—	1	—
i	3.00–4.00 p.m.....	2	Nil	Nil	2	1	1	—	—	—	—
j	4.00–5.00 p.m.....	Nil	1	Nil	1	1	—	—	—	—	—
k	5.00–6.00 p.m.....	3	Nil	1	4	3	—	—	1	—	—
l	6.00–7.00 p.m.....	1	Nil	1	2	1	1	—	—	—	—
m	7.00–8.00 p.m.....	4	Nil	Nil	4	3	1	—	—	—	—
n	Afternoon.....	16	1	10	27	21	3	Nil	1	1	1
o	TOTAL.....	22	3	20	45	35	5	1	1	2	1

Graph H shows the percentage of eggs fertile after forenoon insemination compared with this percentage following inseminations done in the afternoon, on each of the first four days after the operation.

As the birds did not ovulate on every day, consideration was given to the position of the fertile eggs amongst the eggs laid after insemination and the position of the first ovum to be fertilized after insemination. (Table 23 and Graph I.)

Ovulation in the domestic hen, when occurring on the same day as oviposition, usually follows the act of laying within one hour (mean 32 minutes; Warren and Scott, 1935). If there is no ovulation on the same day, no normal egg can be laid on the next day (see literature: pp. 26 and 28). A number of periods were selected relative to the time of insemination. For all the ova liberated in each of these periods, the chances of being fertilized were examined This information is given in Graph J. Here, although the figures are small, percentages are given also for the purpose of easy comparison.

GRAPH J.—The percentage and number of ova proved to have been fertilized, out of all the ova liberated in each of the nine specified time-periods. The periods are specified in respect of the time intraperitoneal insemination was performed.



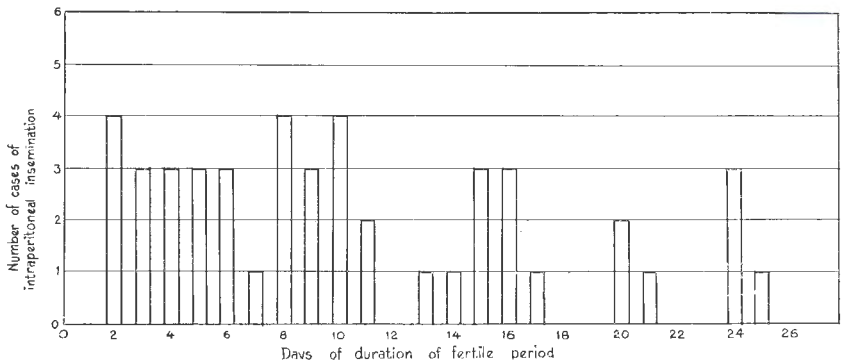
Graph J shows the increase in fertility in the ova liberated between thirty minutes before intraperitoneal insemination and twenty-four hours after; and a decline on the fifth and sixth day following the operation.

(*ii*) *The duration of fertility.*—The lengths of the periods between insemination and the laying of the last fertile eggs have been mentioned fully in the literature, reviewed on page 24.

The duration of this period may be sensitive to factors such as the route of introduction of the semen but also to changes in technique, etc. (see p. 106). In Graph K the incidence of the different periods in days between intraperitoneal inseminations in the first series and the laying of the last fertile egg is shown. Forty-six cases of insemination are represented in Graph K. Out of the 117

intraperitoneal inseminations performed, twenty eight showed no fertility i.e. a duration of nil (0) days. Forty-three could not be tested for duration of fertility, but had to be discarded because either no eggs were laid in these cases or the spermatozoa introduced were superseded (i.e. prevented from being able to fertilize the ova by other sperm, present in the same hen at the time, which fertilized all the available ova). In one case fertility was proved but the length of the fertile period not recorded (insemination No. 18). In Graph L the incidence of each of the various numbers of eggs fertilized from one intraperitoneal insemination for the same forty-six (46) cases is shown.

GRAPH K.—The incidence of the different periods of duration of fertility following intraperitoneal insemination of fowl hens.



Graph K shows that after intraperitoneal insemination, several periods of fertility extended into the fourth week, but the majority had a duration of ten days or less.

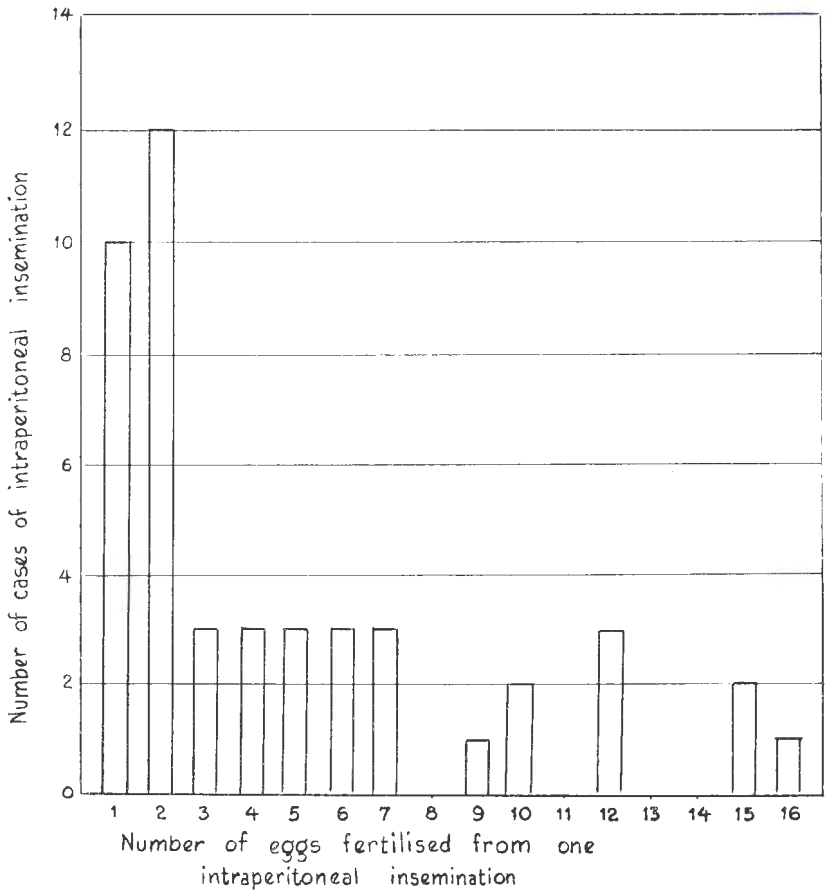
Controls.

N.B.—The environmental factors in all work of this nature, as a rule have a measure of influence on the results. This is often extremely difficult to assess unless some control data are available.

A few control inseminations with methods well established at experimental stations elsewhere, were carried out. Table 24 contains a summary of the results wherein all cases from the first series of consecutive inseminations are included except those cases in which no eggs were obtained or in which the sperms were superseded or replaced by other spermatozoa present in the same female (74 cases). The cases are of comparable distribution through the seasons and it should be kept in mind that the intraperitoneal method is a new experimental technique on which no previous experience was available. The maximum duration of fertility after control inseminations was 22 days (case record in Table 8) and the number of eleven fertile eggs was attained once after a single natural copulation and not exceeded by any other control insemination.

Although the hens were not laying with the same intensity of egg-production in the cases used for compilation of Graphs K and L, the number of eggs fertilized is of course highly correlated with the length of the fertile period. [$r = 0{\cdot}8622$, significant at the $P = 0{\cdot}001$ fiducial level:—(relation: $y = -1{\cdot}087 + 0{\cdot}5482x$; y = number of eggs fertilized and x = days of duration of fertile period)].

GRAPH L.—The incidence of each of the numbers of eggs fertilized by an intraperitoneal nsemination.



Graph L shows that up to 15 and 16 fertile eggs were sometimes obtained from one intraperitoneal insemination, but as a rule seven eggs or less were fertilized.

This is demonstrated in Graph M giving the number of times, that each of the fertile periods (No. of days), coincides with each of the numbers of eggs fertilized from an intraperitoneal insemination.

(*iii*) *The* (i) *degree,* (ii) *intensity or* (iii) *consistency of fertility.*—In the fowl it is necessary to consider (i) whether all the eggs laid between insemination and the laying of the last fertile egg are fertile, (ii) whether the egg laid during long periods of fertility are equally likely to be fertile as eggs laid during shorter periods, and (iii) whether the eggs laid later in this period are as likely to be fertilized as those laid earlier. The basis of the data collected is the number of eggs laid on each of the days in respect of the day of insemination and the number of those that were proved fertile. This information is set out in Graph N which gives the gross results in all 3421 hen-days observed before and after 117 intraperitoneal inseminations in the first series of inseminations. As has

been pointed out earlier the fertile eggs collected on the ten days before insemination are in many cases duplication of the last fertile eggs of previous inseminations but in other cases they are from inseminations by other methods. Infertile eggs laid during the period of fertility were found after intraperitoneal insemination as after other methods. Table 25 shows the number and percentage of eggs fertile in the 46 consecutive cases of intraperitoneal insemination that could be tested in different lengths of fertile periods.

TABLE 24.

The Duration and Percentage Fertility in the Fowl Hen after Intraperitoneal and other Methods of Insemination.

Line.	Method of Insemination.	Number of Cases of Insemination that could be tested for duration of Fertility.	Number of Cases proved to be Fertile.	Average duration of Fertility in Days.	Average number of Eggs laid in Fertile Period.	Percentage of Eggs laid in Fertile Period that were proved Fertile.
a	Artificial intraperitoneal insemination (EXPERIMENTAL)	74	46	10·78	5·78	82·70%
b	Artificial insemination *per vaginam*	13	7	14·43	8·57	73·00%
c	Natural one day matings.....	6	5	12·40	7·80	89·00%
d	Natural single copulations....	2	1	16·00	11·00	100·00%
e	Total inseminations via the oviduct (CONTROL)	21	13	13·85	8·15	82·08%

The number of eggs actually fertilized averaged 4·78 in the case of intraperitoneal inseminations proved fertile, and 6·69 fertile eggs for control inseminations.

To determine if the decline in the percentage of fertility in eggs laid towards the end of the fertile period, which has been noticed in natural fertility also occurs after intraperitoneal insemination, all cases from which infertile eggs were obtained during the period of fertility were summarized in table 26. The results of the first and second half of the fertile period are given in separate columns, all data being in respect of the cases tested for duration i.e. for the full length of the fertile period.

(iv) *The percentage of inseminations that could be proved to be fertile.*

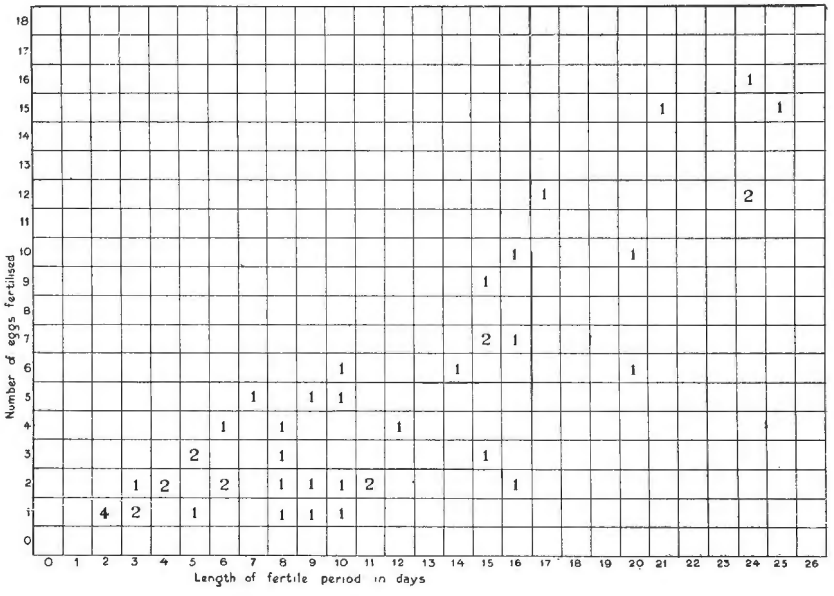
The chances of obtaining fertility in fowl hens by means of intraperitoneal insemination are compared with those of the control methods for obtaining fertility in table 27, in which all cases of proved fertility are included i.e. also insemination number 18 which had fertility that was not tested for duration on account of being superseded by a following insemination No. 19.

TABLE 25.

The Number and Percentage of Fertile Eggs laid during Fertile Periods of Different Lengths after Intraperitoneal Insemination.

Line.	Length of Fertile Period in Days.	Number of Cases of Insemination followed by Fertile Periods of such length.	Number of Eggs Incubated.			Number of Eggs not Set. (Cracked or Premature).	Percentage of Eggs that were proved fertile: Laid between 24 hours after Insemination and the day on which the last Fertile Egg was laid.
			Number Fertile.	Number Infertile.			
				Laid more than 24 hours after Insemination.	Laid within 24 hours after Insemination.		
a	1	—	—	—	—	—	—
b	2	4	4	0	3	—	100%
c	3	3	4	0	2	—	100%
d	4	3	6	1	2	—	86%
e	5	3	7	2	1	—	78%
f	6	3	8	1	2	—	89%
g	7	1	5	0	1	—	100%
h	8	4	10	6	1	1	60%
i	9	3	8	4	2	—	66%
j	10	4	14	8	1	—	63%
k	11	2	4	3	1	1	57%
l	12	—	—	—	—	—	—
m	13	1	4	2	1	—	66%
n	14	1	6	2	1	—	75%
o	15	3	23	7	1	2	76%
p	16	3	19	1	1	1	95%
q	17	1	12	0	0	2	100%
r	18	—	—	—	—	—	—
s	19	—	—	—	—	—	—
t	20	2	16	4	—	—	80%
u	21	1	15	2	—	2	89%
v	22	—	—	—	—	—	—
w	23	—	—	—	—	—	—
x	24	3	40	3	1	2	93%
y	25	1	15	0	0	1	100%

GRAPH M.—The number of occasions that each of the lengths of the fertile periods coincides with each of the numbers of eggs fertilized from an intraperitoneal insemination.



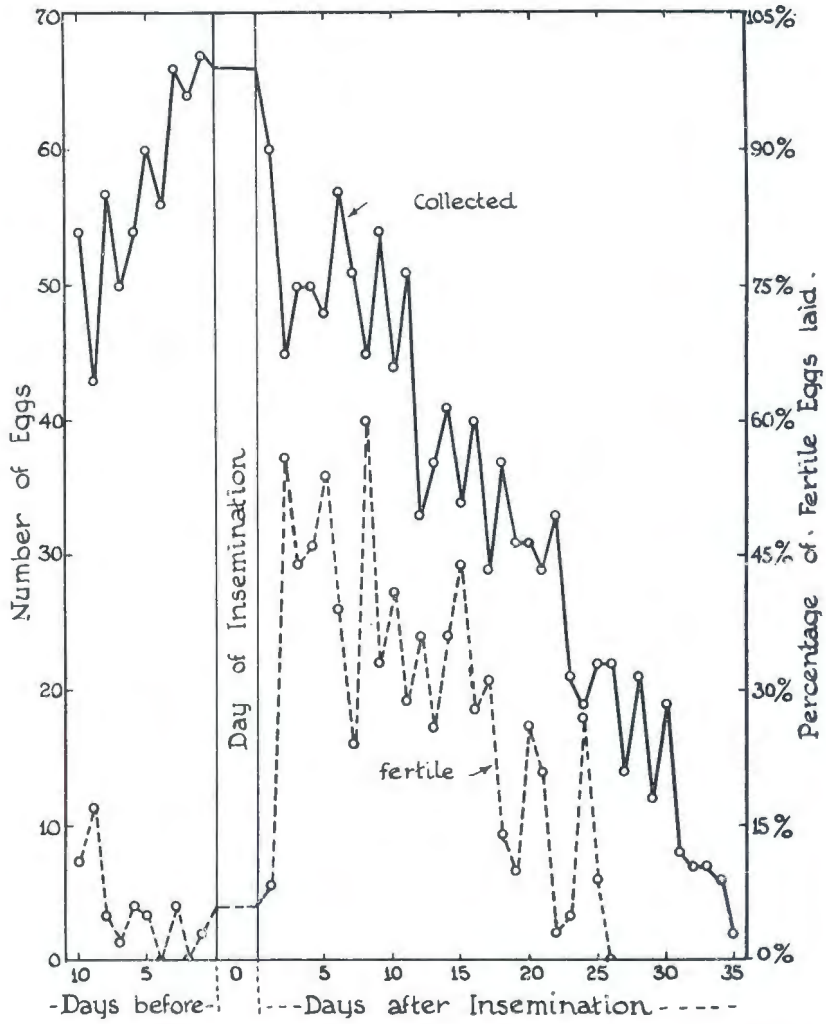
Graph M shows the correlation between numbers of eggs fertilized and the length of the period of fertility in days, which is expected to be more highly significant for intraperitoneal insemination, but control data could not be found.

TABLE 26.

The Percentage of Fertility in the First Half of the Fertile Period, Compared with that of the Second Half.

Line.	Method of Insemination.	Number of Cases with some Eggs Infertile.	Number of Eggs laid in First Half later than 28 hours after Insemination.	Percentage Fertile.	Number of Eggs laid in Second Half of Fertile Period.	Percentage Fertile.	Percentage reduction of Degree of Fertility.
a	Intraperitoneal inseminations	16	46	71%	65	64%	10%
b	Inseminations via the oviduct (total)	6	24	87%	27	44%	50%

GRAPH N.—The gross number of eggs collected and the percentage of eggs fertile before and after the 117 intraperitoneal inseminations of the *first series* of operations.



Graph N shows the decline in gross percentage fertility after the first week, but the number of eggs used for experimental purposes was also steadily reduced on account of the less numerous observations made on the days later after the operation; i.e., most hens were reinseminated before having been observed the full 35 days. Had they all been kept the full period, then the decline in percentage fertility would probably have been more rapid after the first week following insemination.

TABLE 27.

The Percentage and Number of Inseminations Followed by the Laying of Eggs which on Incubation Proved Fertile.

Line.	Method of Insemination.	Number of Inseminations.					Percentage of Testable Inseminations that were proved Fertile.
		Performed.	Superseded by other Inseminations or not Tested for other Reasons.	Which could be Tested for Fertility.	After which only Infertile Eggs were obtained.	After which one or more Fertile Eggs were obtained.	
a	Artificial intraperitoneal insemination	117	42	75	28	47	64%
b	Artificial insemination: *per vaginam*	22	9	13	6	7	54%
c	Natural one-day matings	9	3	6	1	5	83%
d	Natural single copulations	2	0	2	1	1	50%
e	Total inseminations via the oviduct	33	12	21	8	13	62%
	TOTAL.....	150	54	96	36	60	—

Discussion.—The results of intraperitoneal insemination in the pigeon under the circumstances reported merely show that it is possible to fertilize pigeon hens in this way. Riddle and Behre (1921) in work on ring doves obtained a fertile egg as early as 37·25 hours after placing the male with the hen and occasional fertile eggs were obtained more than eight days after insemination. Owen (1941) had fertile eggs two to eight days after artificial *per vaginam* insemination of pigeons. In the present work the limits were 46 to 98 hours. The excessive dilution reported in connection with experiment (3) in part (2), was later eliminated without greatly improving the percentage successes. The relatively good results of the first trials may be due to clumps of spermatozoa sticking together in the diluent and so being saved from dilution shock. When injected the amount of fluid present instead of being a danger to sperm-life, may, by affording a liquid current in the direction of the active ciliated mucosa of the oviduct, help in the conveyance of the sperm to the ova. This is a matter for investigation under more suitable conditions with carefully selected control birds. In the absence of any signs of detrimental effects on production and health of the pigeon hens used, it appears that fertility was influenced adversely by the unnatural methods of mating.

Intraperitoneal insemination in the fowl may have comparatively good results, as can be seen from the data. As expected, the onset of fertility was very rapid; maximum fertility was attained within 24 hours after insemination, which is

almost the minimum time of egg-formation since a fertile egg laid nineteen hours after intraperitoneal insemination, was observed. Although Nicolaides (1934) obtained a fertile egg 19·5 hours after natural mating, from which he deducted that impregnation could occur after the beginning of egg formation, most evidence available is in favour of the assumption that fertilization occurs shortly after ovulation in the fowl.

The duration of fertility after intraperitoneal insemination was on the average shorter than in control cases. The maximum length of the period of fertility can, however, be considered very encouraging indeed. Four cases of artificial intraperitoneal insemination showed results [i.e. a duration of 24 days thrice and one of 25 days once (Graph K)], which exceeded all local experience (22 days after insemination *per vaginam,* see Table 8.)

The results reported in the literature (Barfurth, 1896; Crew, 1926; and Nalbandov and Card, 1943) which exceeded the maximum obtained in the present work, were from females isolated after natural copulation. It is possible, indeed probable, that more than one coitus occurred before separation, so that these authors were dealing with multiple inseminations rather than single doses of semen.

The number of eggs fertilized (Graph L) by intraperitoneal insemination in three cases, viz.: sixteen eggs once and fifteen eggs twice, exceeded the maximum reported for natural mating (14 fertile eggs; Nicolaides, 1934). The average figure was 4·78, as against 6·69 fertile eggs for control inseminations. This difference between the maximum and average comparative results suggests that the range between good and bad technique in the intraperitoneal method is considerably greater than in the case of inseminations via the vagina. It thus appears that further research, aimed at selection of the most favourable method, apparatus and materials, would probably succeed in obtaining a level of efficiency supplying greater average fertility, than previously possible.

The percentage of fertile eggs laid between the time of insemination and collection of the last egg that proved to be fertile, (see table 24) agrees very closely for the different methods of insemination. The slight superiority of the results after the intraperitoneal method may be due to the shorter duration of fertility and fewer eggs obtained in these cases. This view is interesting in connection with what has been named the " consistency " of fertility. From general poultry experience it can be expected that during long periods of fertility in hens separated from the cock, infertile eggs would become more frequent as the time of separation from the male became longer. Different lengths of fertile periods after intraperitoneal insemination did, however, not show a different percentage of fertility which bore any relation to the length of the fertile period (Table 25). In fact the longer fertile periods showed percentages above the average (82·70 per cent.) Consequently the steady decline in the gross daily percentage fertility following on the first week after intraperitoneal insemination (Graph N) must be attributed solely to the reduction in the number of hens that remained fertile for periods up to each of the successive days after the operation. The difference between the percentage fertility in the first and the second half of the fertile period was markedly smaller in the case of intraperitoneal insemination than in the controls (Table 26). This result suggests that the sperm stored in the hen after insemination by the new method were more favourably situated, even if the average duration of their fertilizing capacity did not exceed that of the sperm in the controls

No difference in percentage of successful inseminations between the various methods was demonstrated (Table 27). In this connection it is interesting to draw attention to the fact that the experimental and control cases were laying at about the same rates i.e. with the same intensity. This is shown by the fact that the average duration of the fertile period bore almost the same relation to the number of eggs laid during that period in both groups. (viz. experimental: 1·0 to 0·55 and control group: 1·0 to 0·59).

(5) *The hatchability of eggs fertilized from semen injected into the peritoneal cavity of female pigeons and fowl hens.*

(*a*) *Pigeons.*—The hatchability to be expected in pigeon eggs fertilized by the new method of insemination was determined by incubating the eggs collected after the operations mentioned in part 3a i.e. the series of 62 intraperitoneal inseminations in pigeons, and also some eggs suitable for control purposes. The eggs were laid by birds confined in pairs to small cages or breeding pens. Some were outside and others in the loft. Unfortunately all the experimental birds were outside. The records are from the same years and seasons and as has been stated earlier, the artificially inseminated hens were paired to castrated birds, salpingectomised hens or hens laying eggs of different size and shape. The same procedure was adopted with hens mated one to four hours, the female mate (or castrate) being replaced by a male for that brief mating period. All eggs were incubated in a small still-air incubator with the fowls' eggs, and they were collected from the nests on the day of laying or on the following day. A summary of the results is given in table 28.

TABLE 28.

The Hatchability of Pigeon Eggs Fertilized by Intraperitoneal Insemination and by Natural Coitus.

Line.	Method of Insemination or Mating.	Number of Insemina-tions per-formed.	Number of Eggs incu-bated.	Number of Eggs Fertile.	Number of Eggs Hatched.	Number of Chicks dead in Shell.	Number of dead Em-bryo's.	Number not incu-bated (cracked)
a	Artificial intraperito-neal insemination	44	45	12	0	5	7	2
b	Natural mating for one to four hours at one to six days before laying	7	11	1	1	—	—	—
c	Natural continuous mating	Multiple (14 cases)	28	21	15	4	2	—
	TOTALS.....	65	84	34	16	9	9	2

The hatchability of fertile eggs from natural continuous matings was lower amongst those eggs set in the incubator than in the eggs incubated during the same year in the nests by the parent birds (94 per cent. of the eggs laid hatched).

Only fifteen out of twenty-one (21) naturally fertilized eggs hatched in the incubator. This is an indication that hatchability was affected by undetermined artificial conditions.

(*b*) *Fowls.*—The results of incubation of those fowls' eggs which were proved fertile after intraperitoneal insemination and after some control methods of obtaining fertility, were determined by setting all suitable eggs collected after the inseminations of the first series in the present work. This means that as far as the intraperitoneal method is concerned, the total results of the first 117 consecutive inseminations carried out, were all included. The data are set out in table 29. In respect of the number of fertile eggs (222), it must be noted that the single doubtful result (insemination No. 45) was omitted. In respect of the average duration of hatchability (8·07), the one case of fertility, superseded before the termination of the fertile period (insemination No. 18), was not used in the calculations. The criteria that were considered in connection with hatchability were (*i*) percentage of fertile eggs hatched; (*ii*) the duration of hatchability i.e. the duration of the period between insemination and the laying of the last fertile egg that hatched.

(*i*) *The percentage of fertile eggs that hatched* during the fertile period is given in the ninth column of table 29. The highest percentage was for the small number of 33 fertile eggs collected from hens separated after one-day mating with the males i.e. probably after multiple coitus. The lowest percentage, viz. 46 per cent., was obtained for 43 eggs found to be fertile after insemination *per vaginam*. The result from intraperitoneal insemination for 222 fertile eggs was intermediate: 52 per cent. of these eggs hatched.

The aspect of hatchability raised by Barfurth, (1896) Crew, (1926) and others i.e. an inconsistency in the hatchability potential of fertile eggs laid at different times after insemination of the hen, is considered in Table 29, where comparative figures are given for the respective percentages of fertile eggs hatched in the first half and second half of the fertile period. From these figures it will be seen that in almost all groups there is a decline in hatchability after the first half of the fertile period, and that this decline is greatest in artificial insemination via the oviduct (from 56 per cent. to 32 per cent.), very small in intraperitoneal inseminations (54 per cent. to 47 per cent.) and insignificant after one day natural matings (multiple matings?). Unfortunately the group of natural single matings where the reverse was obtained, is too small to justify consideration here.

However, some workers (e.g. Warren and Kilpatrick, 1929) held that hatchability was not affected by sperm aged in the oviduct for fourteen days or less. To see if this was the case after intraperitoneal insemination Graph O was compiled showing daily percentages of eggs hatched, chicks dead in shell and with dead embryos. The number of fertile eggs is given to explain why the graphs should be used only to about the twenty-first day after insemination (numbers too small).

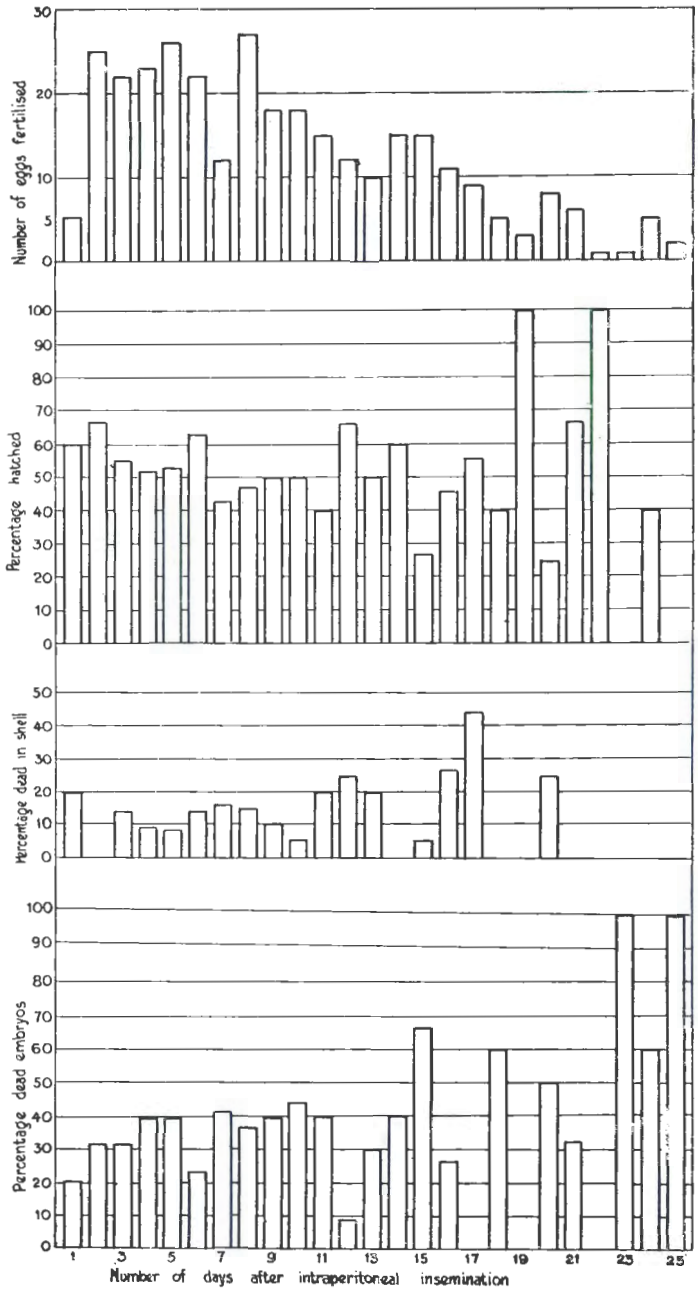
Chicks were hatched in 37 out of the 47 cases of intraperitoneal inseminations in respect of which observations on the hatchability of some fertile eggs could be made. The distribution of the percentages of the fertile eggs which hatched is interesting: out of the 37 cases a hundred percent hatching was observed in ten cases and another ten failed to show any eggs that completed their development. The remaining seventeen cases were followed by fertile eggs of which only a proportion hatched, as is shown in Graph P.

TABLE 29.

The Hatchability of Fowl Eggs Fertilized by Semen Introduced by Various Methods of Insemination.

Line	Method of Insemination.	Number of Insemi-tions Per-formed.	Number of Eggs proved Fertile.	Number of Eggs Hatched.	Number of Chicks dead in Shell.	Number of dead Embryos.	Percen-tage Hatched during the First Half of Fertile Period.	Percen-tage Hatched during the Second Half of Fertile Period.	Percen-tage Hatched during the Whole Fertile Period.	Average Duration of Hatcha-bility in Days.
a	Artificial intraperitoneal insemination.....	117	222	118	29	75	54%	47%	52%	8·07
b	Artificial insemination *per vaginam*	22	43	20	7	16	56%	32%	46%	8·14
c	Natural mating one-day period...	9	33	20	5	8	62%	59%	61%	10·8
d	Natural single copulation.....	2	11	6	5	—	40%	66%	55%	16·0
e	Total via the oviduct.....	33	87	46	17	24	57%	48%	52%	9·72
f	Total inseminations.....	150	309	164	47	99	—	—	—	—

GRAPH O.—The incubation results of fertile fowl eggs, collected on each of the first twenty-five days following intraperitoneal insemination.



Graph O shows that in the case of intraperitoneal insemination, a decline in hatchability and in age reached by the embryo in eggs laid longer after insemination, is very slight and not as much as reported in hens separated from the cock after natural mating.

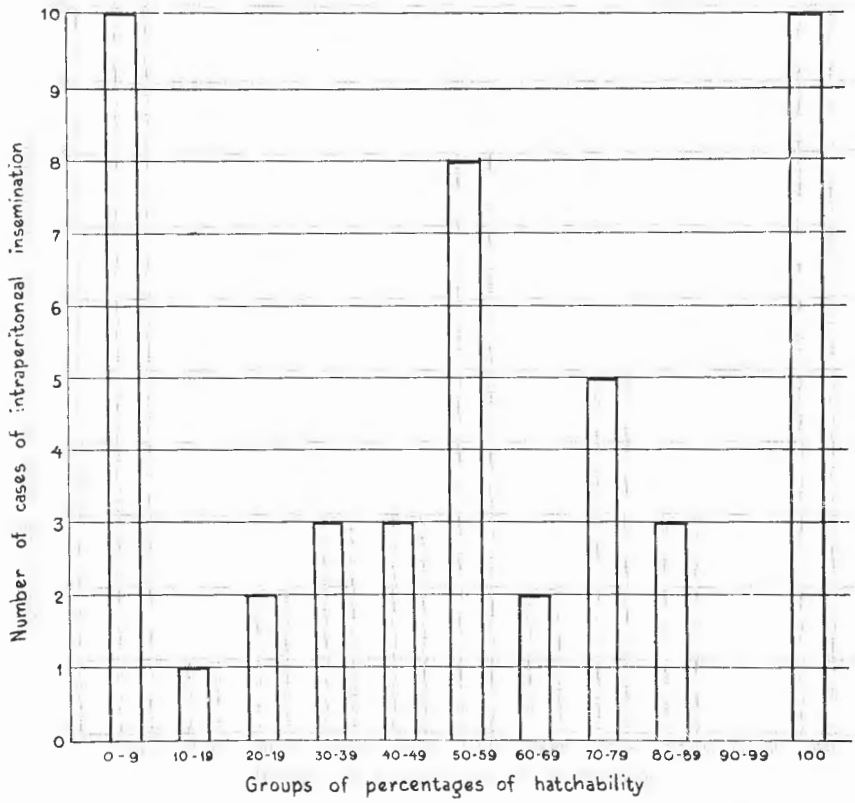
(*See Nalbandov and Card*, 1943).

(*ii*) *The duration of hatchability.*—The length of the period between insemination and the time of laying of the last egg that hatched, could be determined in 36 cases of artificial intraperitoneal insemination. Graph Q has been drawn to show how many cases of such periods of hatchability (in days) occurred.

(*iii*) *The number of chicks obtained.*—The number of chicks which hatched from each of the 36 cases is shown in Graph R which also gives the number of cases in which each of the different numbers of chicks were obtained from a single intraperitoneal insemination.

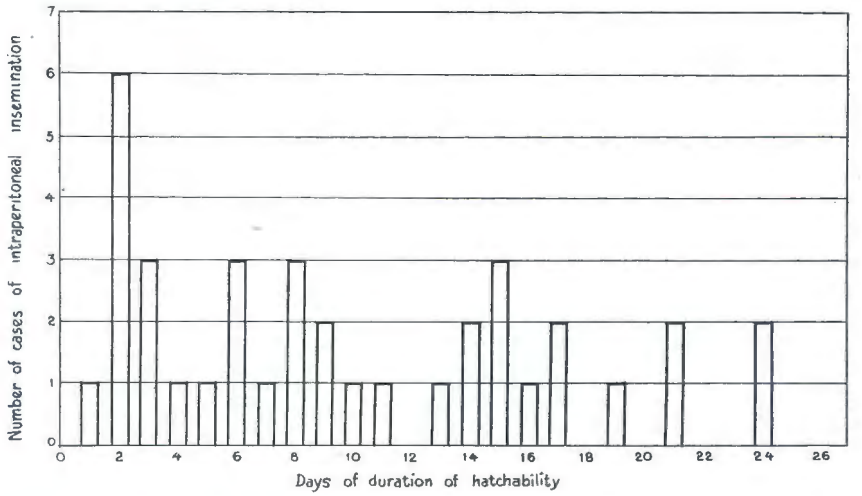
The correlation between the number of chicks and the number of eggs fertilized in the case of the intraperitoneal method is demonstrated in Graph S. ($r = 0 \cdot 8265$, significant at the $p = 0 \cdot 001$ fiducial level; relation: $y = 0 \cdot 054 + 0 \cdot 516\ x$; y = number of chicks obtained and x = number of eggs proved to have been fertile). Unfortunately control data for the other methods of insemination under the same conditions could not be obtained.

GRAPH P.—The incidence of the different percentages of hatchability observed after intraperitoneal insemination.



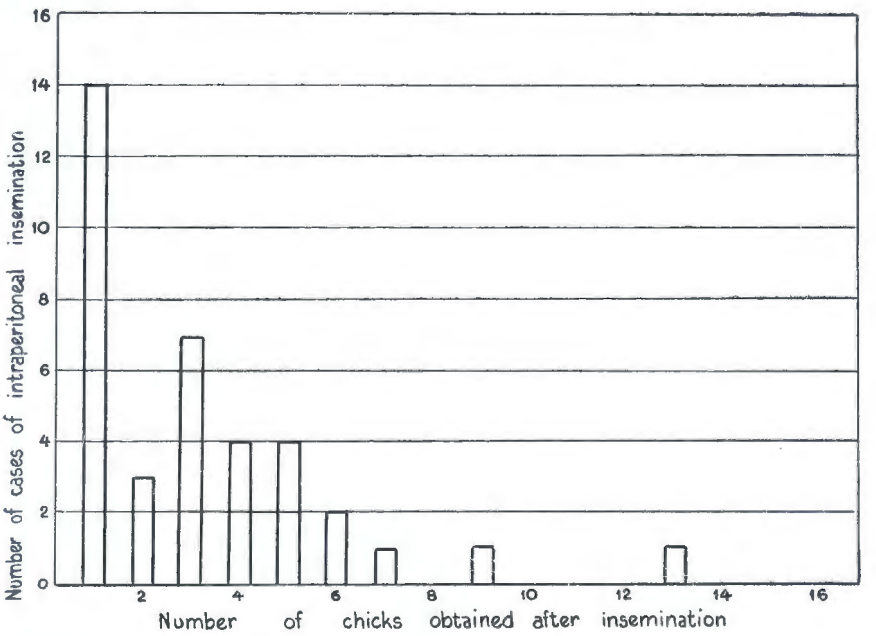
Graph P shows that in cases of intraperitoneal insemination, in which chicks were obtained either all or only about half of the fertile eggs hatched. Although the figures are small, they may suggest a tendency of the results from the operation to be either very good or fairly poor, pointing to a more favourable time or site of injection in the most successful cases.

GRAPH Q.—The incidence of the different periods of duration of hatchability in days after intraperitoneal insemination.



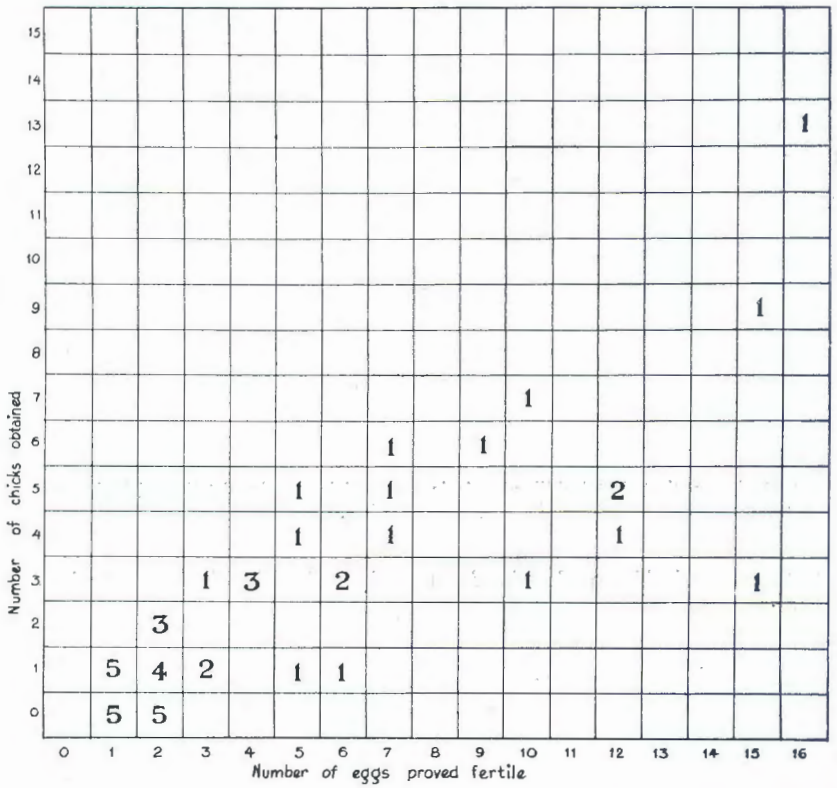
Graph Q shows that hatchability periods were fairly evenly distributed in lengths of one to twenty-four days following intraperitoneal insemination.

GRAPH R.—The incidence of each of the numbers of chicks obtained after intraperitoneal insemination.



Graph R shows that rarely more than six chicks were produced from one insemination. The one case of thirteen chicks is believed to constitute a maximum reported so far.

GRAPH S.—The number of occasions that each of the numbers of eggs fertilized from one intraperitoneal insemination, coincided with each of the numbers of chicks obtained from one operation.



Graph S shows the correlation between numbers of eggs fertilized and numbers of chicks obtained from the intraperitoneal method, which is believed to be more highly significant than for the older methods, but again no control data could be found in the literature.

Several exceptional results were encountered e.g. the maximum number of chicks that hatched from eggs fertilized by one intraperitoneal insemination, was 13 (Graph R) compared with a maximum of seven for the control inseminations. The average number of chicks for the new method was 3·3 per insemination as against 1·7 per control insemination.

Similarly an exceptional duration of hatchability of 24 days (graph Q) was recorded twice and twenty-one and twenty days once each. The average duration of hatchability was 8·07 days after intraperitoneal insemination and 8·14 days after insemination *per vaginam* (Table 29).

As in contrast to the usual experience, the first few eggs following some inseminations by the new method, failed to hatch although they were fertile, whereas fertile eggs laid later after the operation hatched perfectly. Other eggs were continually hatching in the incubator, proving that incubation was normal. A few examples of this type of result are given in Table 30 though no information on the causes responsible for such finding can be reported.

TABLE 30.

Some Cases of Irregular Hatchability.

Line.	Number of Operation.	Number of Hen.	Days on which Fertile Eggs were laid.	
			That Failed to Hatch.	That Hatched.
a	44	19	2, 5, 8, 13, 16, 17, 23, 24	14, 18, 19, 20
b	75	9	5, 8, 9, 11, 20	17
c	102	27	2	3
d	109	20	3, 4, 5, 7, 8, 9, 10, 11, 13, 15, 18	14, 16, 21
e	120	54	2, 5, 8	10, 12, 14, 16

Discussion.—Although the majority of the pigeon eggs taken from the loft and incubated artificially, hatched in the incubator, the percentage of failures was relatively high in comparison with the general experience on eggs naturally incubated in the loft even from the same birds. This can be a contributory cause of the failure of all the twelve experimentally fertilized eggs to hatch. Yet the fact that all the fertile eggs obtained after intraperitoneal insemination, failed to hatch, suggests the existence of other causes present in the egg at collection, that may have been responsible for the early death of the embryo. For instance, the injection of foreign bodies into the abdominal cavity; or the effect of abnormal mating on the condition of the oviduct; or the adverse condition of confinement of the experimental birds, can be considered.

Fowl chicks hatched after intraperitoneal insemination in a way which showed no full conformation with rules established for naturally mated hens, e.g.: the slight decrease in percentage hatchability towards the end of the period of fertility (Crew, 1926; Barfurth, 1896; Nalbandov and Card, 1942). The results in natural single copulation controls is not supported by sufficient data to permit consideration and the one-day mated controls probably show a result based on multiple copulations. The controls inseminated via the oviduct show a decidedly greater decline than in the case of intraperitoneal insemination. As in connection with the similar feature observed with the fertile period in part 4, this finding suggests that the sperms after artificial intraperitoneal insemination gained a better storage position in the organs of the hen than other methods of artificial insemination. There was, however, no indication that in respect of hatchability the sperms were more favourably situated after intraperitoneal insemination than after natural copulation (multiple).

Another finding of Nalbandov and Card (1943) is the decrease in the age reached by the embryo in eggs laid longer after separation from the males. This was also the case after intraperitoneal insemination (Graph O) but to a very much smaller degree than in the case reported by the authors mentioned.

Features of the hatchability produced after intraperitoneal insemination in the present work were—(i) the lower average duration of hatchability was slightly shorter than in the case of controls. (ii) The maximum duration of the period of hatchability was exceptionally long, viz.: 24 days in two cases and 20 and 21 days in one case each. (iii) The average number of chicks obtained per insemination

was about twice as large as in the case of controls (3·3 and 1·7 chicks respectively). (iv) The maximum number of chicks obtained from one insemination was also twice as large (13 chicks) as for the controls (seven chicks) and as far as can be ascertained the highest on record. (v) The distribution of the cases with different percentages of hatchability was irregular in that the number of cases of percentages of less than one, fifty to fifty-nine, and 100 per cent., numbered three fifths of the total, i.e. when leaving the cases with no hatchability out of consideration there were more cases with 100 per cent. and 50 per cent. hatchability than with all other percentages together.

These features when taken together suggest that undetermined factors exist in connection with the technique of intraperitoneal insemination by which the results are segregated into two groups, the larger of which produces an average hatchability of mediocre quality, and the smaller group producing a hatchability of an exceptionally high standard. This points to the occurrence of a particularly favourable physiological *moment for the discharge* of semen in the region of the ovary, e.g. at a time of great activity of the funnel such as is the case before ovulation; or of a very favourable *locality for discharge* of the injected semen, e.g. in the ostium abdominale of the oviduct. Experiments to test this view would require suitable laboratory facilities.

(6) *The effect of environmental factors on the results of intraperitoneal insemination of pigeons and fowls.*

The reduction of reproductive ability in birds kept under adverse conditions is an accepted fact. Weather changes, starvation, thirst and agitation are usually avoided as much as possible in poultry plants. The description of conditions of work in section A of this chapter makes it clear that the pigeons in the loft had good accommodation, but the birds in the cages were subjected to excessive exposure. The fowls were either in outdoor laying batteries without protection against the weather or indoors with insufficient sunlight and lack of fresh air and green feed. Significant changes in attendance did not occur though there were no regular feeding times. Handling was by no means routine, and in the case of pigeons different methods of mating to stimulate laying were employed as described.

For these reasons it was decided to compare the results of several groups of intraperitoneal inseminations.

(*a*) *Pigeons.*—The comparison of results with pigeons in different methods of mating is given in Table 32.*

(*b*) *Fowls.*—Data from the 117 intraperitoneal inseminations carried out on fowl hens in the first series, are analysed in respect of the internal influences from exposure and the season of the year, in Tables 32 to 34. The lines have been marked by letters a, b, c, etc., to facilitate reference in the discussion.

* One of the salpingectomised hens (bird number 362) was found to carry a cluster of three fully developed yolks in her ovary when she was eventually slaughtered at the conclusion of the experiment. At the time she was in the " sitting " stage of the sexual cycle, i.e., she was incubating the eggs of her female cage mate. More than two large follicles were never seen before in post-mortem examinations of pigeon hens, neither before laying nor during incubating of their eggs.

TABLE 31.

The Effect of Mating Methods and Season on the Results of Intraperitoneal Insemination in the Pigeon.

Line	Description of Condition.		Number of Inseminations Performed.	Number of Inseminations followed by One or Two Eggs.	Number of Eggs Laid 46 to 98 Hours after insemination.					Remarks.
	Quality.	Particulars.			Laid.	Set.	Fertile.	Dead in Shell.	Dead Embryos.	
a	Season	July to December	43	43	32	31	10	4	6	Breeding season.
b	Season	January to June	19	17	15	15	2	1	1	Moulting season.
c	Method of Pairing	One hen with castrated male	5	5	4	4	1	1	0	Unnatural pairs.
d	Method of Pairing	Hen paired with salpingectomised hen	7	7	5	5	2	1	1	Unnatural pairs.
e	Method of Pairing	Two hens homosexually paired	50	48	38	37	9	3	6	Unnatural pairs.

Table 32 provides for a comparison of the egg-production in fowl hens after intraperitoneal insemination, with some reference to previous production and with some control data. The groups considered are the breeding season [July to December, (line a)] and the moulting season [January to June (line b)], also the results in birds kept outdoors (line c) and in those kept indoors (line d.). Lines e and f supply the totals for intraperitoneal insemination and control methods which can be referred to when comparing the figures from the different groups. Short terms indicate these groups.

In Table 33 the effect of external influences on fertility is set out on the same lines as in Table 32.

Table 34 shows the hatching results of the groups mentioned in the two previous tables.

TABLE 32.—(See Appendix A, Table 32.)

The Effect of Season and Exposure on Egg-production in the Fowl after Intraperitoneal Insemination.

Line.	Description of Environmental Factors ; Particulars of Groups.	Production Percentage after Insemination.	Production Records for the period from the Tenth Day prior to Insemination to the Tenth Day following Insemination.		
			Number of Cases with an equal number of Eggs before and after.	Number of Cases with more Eggs before than than after.	Number of Cases with more Eggs after than than before.
	Season.				
a	July to December.....	54%	12	27	12
b	January to December.....	39%	5	16	5
	Exposure.				
c	Hens outdoors.....	50%	11	34	10
d	Hens indoors.....	42%	6	8	7
	Totals.				
e	Intraperitoneal insemination (first series).....	48%	17	42	17
f	Control inseminations *per vaginam* and natural mating....	42%	3	12	6

Discussion.—The data submitted on pigeon eggs fertilized by semen injected into the peritoneal cavity, supply a small amount of evidence indicating that the breeding season was more suitable for attempts with the new method, than the moulting season, i.e. 32 per cent. fertility in eggs laid 46 to 98 hours after insemination during July to December against 7 per cent. fertility in similar eggs laid during January to June. There was no demonstrable difference between the results of pigeon hens paired for egg-production by different types of mates, but the results were so markedly inferior to natural fertility and hatchability that it was considered probable that undetermined influences had

affected fertilization and development in the case of these pigeons. From circumstantial evidence on the conditions of work, and the hatchability of eggs in the loft, these influences are suspected to be environmental.

TABLE 33.—(See Appendix A, Table 33.)

The Effect of Season and Exposure on the Fertility Following Intraperitoneal Insemination of Fowls.

Line.	Description of Environmental Factor ; Particulars of Groups.	Percentage Fertility in Total Number of Eggs Set.	Percentage Fertility in Eggs laid 24 Hours to Ten Days after Insemination.	Average duration of Fertility in Days.
	Season.			
a	July to December.....	27%	38%	10·4
b	January to June.....	28%	37%	10·9
	Exposure.			
c	Hens outdoors.....	29%	40%	12·1
d	Hens indoors.....	22%	33%	6·7
	Totals.			
e	Intraperitoneal insemination (first series).....	27%	38%	10·8
f	Control inseminations *per vaginam* and natural mating....	82%	89%	13·9

TABLE 34.—(See Appendix A, Table 34.)

The Effect of Season and Exposure on the Hatchability of Fertile Eggs Obtained after Intraperitoneal Insemination of Fowls.

Line.	Description of Environmental Factor : Particulars of Groups.	Percentage hatchability in total number of Fertile Eggs Set.	Percentage hatchability in Fertile Eggs laid 24 Hours to Ten Days after Insemination.	Average duration of hatchability in Days.
	Season.			
a	July to December.....	53%	55%	8·2
b	January to June.....	53%	60%	7·2
	Exposure.			
c	Hens outdoors.....	50%	54%	9·0
d	Hens indoors.....	68%	64%	5·1
	Totals.			
e	Intraperitoneal insemination (first series).....	53%	56%	8·1
f	Control inseminations per *vaginam* and natural mating....	53%	52%	9·7

The results in fowl hens showed only a slight advantage in the effect on production (Table 32) with hens kept indoors (line d: 38 per cent. of cases with a decrease and 33 per cent. of cases with an increase), as against those kept outdoors (line c: 61 per cent. with a decrease and 10 per cent. with an increase) which suggests a better resistance (indoors) to inhibiting environmental factors after the operation. The overall production from outdoor hens was however better. [Appendix A Table 32 columns (3) and (4)]. The other figures provide evidence that environmental factors had no unusual influence on the egg-production following intraperitoneal insemination in the fowl. In line f it is shown that the control results were on the whole slightly inferior to those of intraperitoneal insemination as regards the interference with egg-production.

With regard to fertility (Table 33) there was no difference in results due to seasonal influences (lines a and b) but the birds kept outdoors (line c) performed decidedly better than those indoors (line d). The control results in this respect were also markedly better than those from intraperitoneal insemination.

The duration of hatchability (Table 34) was slightly longer during the breeding season (line b) and markedly longer for hens kept outdoors (line c) than for indoor birds (line d). The slight differences in percentage should not merely be explained by the differences in duration of hatchability or in duration of the fertile period, as it was a consistent experience right through the work, that in the consideration of factors *affecting the fowl hen only,* groups with lower fertility percentages showed a compensatory higher hatchability percentage. Effect of temperature, bad weather, etc. on the eggs after laying (e.g. outdoors group) cannot be responsible, as the work of Lamoreux, (1904), Phillips, (1945) and Heywang (1945) sufficiently proves that temperature between 32° F. and 109° F. hardly affect the incubation results of fertile eggs, if exposure is limited to the first few hours after laying.

The hatchability percentage of eggs from groups with lower percentages of fertile eggs, must here be due to qualities present in the egg at oviposition. No explanation is offered, but this experimental result suggests, that in the fowl, when fertility is reduced by influences which affect the *female only,* such eggs as still become fertilized possess a higher potential hatchability than the eggs that become fertilized when the adverse effects on the hen are absent. This is contrary to the experience with the male and with spermatozoa, where adverse effects on fertility are accompanied by even greater reductions in hatchability, (Nalbandov and Card 1943).

(7) *The effect of some qualities of the subject used for intraperitoneal insemination on the results obtained.*

The observation in the present work are confined to domestic pigeons and fowls, and the following qualities have been considered:—

(*a*) *Pigeons.*

(*i*) Species.

(*ii*) Age.

(i) Young birds.

(ii) Yearlings.

(iii) Old birds.

(*iii*) Production Record:—Interval since last previous oviposition.
- (i) 0—10 days.
- (ii) 11—18 days.
- (iii) 19—30 days.

(*iv*) Record of previous inseminations to which the birds had been subjected:—The number of previous intraperitoneal inseminations experienced:
- (i) Nil.
- (ii) One.
- (iii) Two.
- (iv) Three to twelve.
- (v) Thirteen.

(*b*) *Fowls.*

(*i*) Species.

(*ii*) Breed.
- (i) Heavy breeds.
- (ii) Light breeds.

(*iii*) Age.
- (i) Pullets.
- (ii) Second season hens.
- (iii) Old birds.

(*iv*) Production record:—The percentage of production (i.e. the number of eggs expressed as a percentage of the number of days in the period during which the eggs were collected) for the ten days prior to the day of insemination:
- (i) 60 per cent. and under.
- (ii) Over 60 per cent.

(*v*) Record of previous insemination to which the birds had been subjected:—The interval since the last previous insemination performed on the birds:—
- (i) Intraperitoneal insemination within 48 hours.
- (ii) Other methods of insemination performed within 48 hours.
- (iii) Intraperitoneal insemination ten to two days previously.
- (iv) Other methods of insemination performed ten to two days previously.
- (v) Insemination (all methods) more than ten days previously.
- (vi) No previous insemination (virgin hens).

(*a*) *Pigeons.*

The racing homers on which the work was done, showed some variability in respect of the factors indicated and the operations could be classified for analysis as set out in table 35. An accident occurred with hen number 375 after her fifth insemination, when she had laid within ten days previously. She was then an "old bird". A metal needle pass-through had inadvertently been left in the needle and this was found in the peritoneal cavity, after chronic peritonitis had set in. (See page 59).

TABLE 35.—(See Appendix A, Table 35.)

The Effect of Age, Egg-production and Previous Inseminations on the results of Intraperitoneal Insemination in Pigeons.

Line.	Description of Quality.	Particulars of Groups.	Number of Inseminations.	Number of Eggs Laid 46 to 98 Hours after Insemination.		Remarks.
				Percentage Fertile.	Percentage Hatched.	
a	Age	Young birds	1	0%	0%	Moult caused.
b		Yearlings	12	25%	0%	—
c		Old birds	49	27%	0%	—
b	Egg-production: (Period of time after the last previous egg was laid)	Ten days or less	9	0%	0%	—
d		11 to 18 days	22	33%	0%	—
e		19 to 30 days	31	29%	0%	—
f						
g	Previous inseminations performed	Nil	15	17%	0%	—
h		One	14	40%	0%	—
i		Two	11	25%	0%	—
j		Three to twelve	21	21%	0%	—
k		Thirteen	1	100%	0%	—

TABLE 36.—(See Appendix A, Table 36.)

The Effect of some Qualities of the Subject on the Egg-production of Fowl Hens Following Intraperitoneal Insemination.

Line.	Description of Quality : Particulars of Groups.	Production Percentage after Insemination.	Number of Cases of Insemination followed by :		
			Unchanged Production.	Reduced Production.	Increased Production.
	Breed,				
a	Heavy breeds.....	48%	10	28	8
b	Light breeds.....	48%	7	14	9
	Age.				
c	Pullets.....	49%	12	35	14
d	One year olds.....	53%	4	5	3
e	Old birds.....	—	1	2	0
	Production.—(Egg-production during the ten days prior to insemination in 108 cases in which the records were complete).				
f	60 per cent. and under.....	37%	11	12	13
g	Over 60 per cent.....	50%	6	30	4
	Previous Insemination.—(Intervals of time since the last previous insemination and the method used for these previous inseminations).				
h	Intraperitoneal within 48 hours before.....	59%	6	9	4
i	Other methods within 48 hours before.....	44%	1	7	0
j	Intraperitoneal ten to two days before.....	55%	0	4	1
k	Other methods ten to two days before.....	51%	0	1	1
l	All methods more than ten days before.....	47%	8	18	9
m	Not previously inseminated....	39%	2	3	2

(*b*) *Fowls.*

Birds possessing the qualities shown in the list and indicated in the first column of Table 36 are compared as regards egg-production in this table. The columns of table 36 are identical with those of table 32 and reference should be made to the last two lines of Table 32, viz.: totals e and controls f when the figures are considered. Accidents occurred in three pullets, one heavy and two of light breeds. The first case was peritonitis. following the sixth intraperitoneal insemination after 10 days during which she laid six eggs. The light breed pullets showed one case of direct haemorrhage and one of peritonitis after the 5th intraperitoneal insemination. A fatal haemorrhage also followed a control insemination *per vaginam* (see part 3).

The effect on fertility following intraperitoneal insemination due to the same qualities of the hen, is shown in the figures set out in Table 37. Again reference should be made to the corresponding table in part 6. (Table 33).

Table 38 gives the comparable figures for the groups of qualities of the hen with regard to hatchability (c.f. Table 34).

Discussion.—The ages, recent previous production of eggs and previous inseminations in pigeons, showed no demonstrable effects on the results of intraperitoneal insemination (Table 35), but in fowls some differences were found:—

Breed.—Birds of the "heavy" breeds gave better percentage fertility [Table 37 (33 per cent.)] and longer duration of fertile period (12·7 days) and of hatchability, (9·4 days), but the lower fertility in the hens of "light" breeds was compensated to some extent by a larger number of chicks obtained per dozen fertile eggs. [71 per cent. (light breeds) against 43 per cent. (heavy breeds) hatchability of eggs laid within ten days after insemination].

TABLE 37. (See Appendix A, Table 37.)

The Effect of some Qualities of the Subject Inseminated on the Fertility Following Intraperitoneal Insemination of Fowls.

Line.	Description of Quality : Particulars of Groups.	Percentage Fertility in Total Number of Eggs Set.	Percentage Fertility in Eggs laid 24 Hours to Ten Days after Insemination.	Average duration of Fertility in Days.
	Breed.			
a	Heavy breeds	33%	47%	12·7
b	Light breeds	18%	29%	7·4
	Age.			
c	Pullets	27%	38%	10·8
d	One year olds	27%	45%	7·4
e	Old birds	—	—	—
	Production.—(Egg-production during the ten days prior to insemination in 108 cases in which the records were complete).			
f	60 per cent. and under	16%	22%	8·6
g	Over 60 per cent.	37%	54%	11·9
	Previous Insemination.—(Intervals of time since the last previous insemination and the method used for these previous inseminations).			
h	Intraperitoneal within 48 hours before	34%	41%	13·1
i	Other methods within 48 hours before	56%	76%	16·0
j	Intraperitoneal ten to two days before	40%	51%	8·8
k	Other methods ten to two days before	15%	27%	3·0
l	All methods more than ten days before	22%	35%	9·7
m	Not previously inseminated	15%	20%	8·2

TABLE 38. (See Appendix A, Table 38.)

The Effect of some Qualities of the Fowl Hens Inseminated on the Hatchability Following Intraperitoneal Insemination.

Line.	Description of Quality and Particulars of Groups.	Percentage hatchability in Total Number of Fertile Eggs	Percentage hatchability in Fertile Eggs laid 24 Hours to Ten Days after Insemination.	Average duration of hatchability in Days.
	Breed.			
a	Heavy breeds	48%	47%	9·4
b	Light breeds	69%	71%	5·6
	Age.			
c	Pullets	55%	59%	8·1
d	One year olds	40%	33%	6·7
e	Old birds	—	—	—
	Production.—(Egg-production during the ten days prior to insemination in 108 cases in which the records were complete).			
f	60 per cent. and under	55%	60%	4·6
g	Over 60 per cent.	52%	56%	9·3
	Previous Insemination.—(Intervals of time since the last previous insemination and the method used for these previous inseminations).			
h	Intraperitoneal within 48 hours before	49%	52%	12·6
i	Other methods within 48 hours before	49%	42%	12·8
j	Intraperitoneal ten to two days before	79%	70%	7·0
k	Other methods ten to two days before	33%	33%	1·5
l	All methods more than ten days before	53%	58%	6·4
m	Not previously inseminated	39%	50%	4·6

Age.—2nd year hens performed better than pullets in respect of the number of intraperitoneal inseminations followed by fertility (12 out of 16) as well as showing a higher percentage fertility [Table 37 (45 per cent.)], but pullets showed a better percentage hatchability [Table 38, (59 per cent.)] and a longer average fertile period (10·8 days) and hatchability (8·1 days). This is in full agreement with general poultry experience.

Egg-production.—Birds with an egg-production of over 60 per cent. during the ten days prior to intraperitoneal insemination showed the greatest adverse effect on production (Table 36, line g), but these birds also showed the highest percentage fertility and longest duration of the periods of fertility and hatchability.

Previous inseminations.—These did not influence the results of intraperitoneal insemination in the fowl hen, except that the duration of fertility and hatchability was longest in the groups subjected to other

operations within 48 hours. In the majority of these cases the operations were done almost simultaneously and large amounts of semen were used in several instances. The groups with other intraperitoneal operations done within 48 hours (line h) had not as good fertility and hatchability percentages as the comparable group with such previous operations done two to ten days before (line j). However, in the case of intraperitoneal operations with concurrent inseminations by other methods (line i), better percentages fertility and hatchability were recorded than with such inseminations done ten to two days previously (line k).

(8) *The influence of technique, equipment and materials used, on the results of intraperitoneal insemination.*

In view of the small amount of data obtained in the work on pigeons, only the observations made on fowls will be included in this analysis. All the samples of semen from fowl cocks used for the 117 intraperitoneal inseminations in the first series from which the data were taken, were collected in sterile hard glass receptacles as described for the first operation in experiment (4) part (2) (*b*). This was therefore entirely uniform except for the fact that the temperature was not controlled, but all the collections were done at room temperature which may have varied from day to day. The following aspects can be considered:—

(*a*) The size, shape and construction of the instruments of insemination.

(*b*) The materials in contact with the semen, in the instruments used.

(*c*) The locality for entry of the insemination instruments into the abdominal cavity.

(*d*) The depth of penetration of the insemination instruments into the abdominal cavity.

(*e*) The direction of penetration of the instruments of insemination.

These aspects will now be considered in sequence:

(*a*) *The size, shape and construction of the instruments used in* 117 *consecutive cases of intraperitoneal insemination.*—Insemination operations were executed with a variety of instruments including four different commercial syringes and a specially constructed all-glass insemination syringe, plus a number of different metal needles:—

Syringes:—

(i) A glass syringe with metal nozzle:—size: No. 13; makers: Pretzel and Schultz, Hamburg; Capacity: 5 c.c.

(ii) An all-glass, small insulin syringe:—"Superward", Becton and Dickenson, 1 c.c.

(iii) A small record syringe with metal nozzle and metal piston:—capacity: 1 c.c.

(iv) An all-glass plain hypodermic syringe with metal piston:—capacity: 1 c.c.

(v) A specially made all-glass syringe with long glass nozzle, composed of a 6-8 cm. thick-glass capillary tube, joined (sweated in flame) to a 10-12 cm. long glass tube of about 3 mm. inner diameter, and fitted with a waxed cotton-wool plug on a glass or wooden rod for piston, with the inner surface of the glass tube and the piston well lubricated with liquid paraffin. (When used for insemination applied with trocar and canula).

Trocar and Canula:—

(vi) A small very sharp trocar of just over 3 mm. thickness with a stainless metal canula to admit with ease the nozzle of the special syringe(v).

Needles:—

(vii) "Westprod" exploring needles:— lengths: 110 mm. and 80 mm., diameters: 1·8 mm. and 1·5 mm.

(viii) "Westprod" fine anaesthetic needle blunted at tip:—length: 100 mm., diameter: 0·9 mm.

(ix) "Westprod" fine anaesthetic needle blunted at tip and with two extra openings cut 3 mm. and 6 mm. from the opening at the tip, length: 100 mm., diameter: 0·9 mm.

(x) "Westprod" superfine anaesthetic needle:—length: 100 mm., diameter: 0·6 mm.

(xi) "Westprod" veterinary needle, size 14, length: 2 cm., diameter: 1·2 mm. [See figure (iv)].

FIGURE (vi).—The Instruments suitable for Intraperitoneal Insemination.

Photograph of selected instruments employed in experimental and routine intraperitoneal insemination of fowls and other birds. (See legend).

(*Photo by Mr. R. H. Brinkman, for the Director of Veterinary Services, Onderstepoort*).

LEGEND TO FIGURE (vi).

1. Forceps used in sterilization.
2. Trocar and 3. Canula. [type (vi)].
4. All-glass insulin syringe [type (ii)].
5. Holborn sheep insemination instrument.
6. Glass file.
7. Rule in centimetres.
8. Glass pipette graduated to measure 0·01 ml. for measuring fowl semen volumes and for insemination *per vaginam*.
9. Glass pipette graduated to measure 0·001 ml. for collection of pigeon semen and for measuring the volumes.
10. Thick-glass capillary tube attached to all-glass syringe for artificial insemination of pigeons *per vaginam* with accurate doses (liquid paraffin between piston and semen).
11. Fine metal serum needle for intraperitoneal insemination of pigeons.
12. Needle adapter for use with insulin syringe.
13. Coarse, sharp, long, metal needle for intraperitoneal insemination of fowls [type (vii)].
14. Coarse, sharp, short, metal needle [type (xi)].
15. Fine, blunt, triple aperture, metal needle for use with short, sharp needle (*see* Number 14) [type (ix) = 15].
16. Superfine, sharp, metal needle for intraperitoneal insemination of fowls [type (x)].
17. Modified " Holborn " inseminator, adapted to fit insulin syringe, and applied through canula (*see* Numbers 2–4).
18. Glass pipette with piston made of wood, cotton wool and wax, and filled with liquid paraffin, used with canula.
19. Specially constructed all-glass syringe, made from thick-glass capillary joined (sweated in flame) to wider glass tube and fitted with piston made of wood, cotton wool and wax ; used with canula (*see* Number 3).
20. Thick-glass capillary adapted to fit nozzle of insulin syringe [type (ii)] and used through canula (*see* Number 3).
21. Very long, fine, blunt, metal needle for intraperitoneal insemination of large avian species, e.g., the turkey hen.

In performing the operation for intraperitoneal insemination all sharp needles were inserted direct through the abdominal wall as described in part (2) (*b*). Blunt needles were applied by passing the tip through the hole or lumen of a previously inserted veterinary needle [Type (xi)]. Similarly the glass nozzle of the special syringe [Type (v)] was admitted into the abdominal cavity through the canula [Type (vi)].

The data are set out in three tables as in parts (6) and (7) viz.: Tables 39 to 41:— the effect of type and material of instruments used for intraperitoneal insemination on the results of the operation. The data were segregated in several groups and the types considered here in lines a to d are sharp instruments, blunt instruments, coarse instruments and fine instruments. [Type (vii), (viii), (ix) and (x) needles.] The columns in these tables are similar to those of tables 32 to 34 given in part (6) (*b*).

Table 39 shows the effect of these four qualities on the egg-production of the hens.

The effect of the same qualities of the instruments on fertility is illustrated in table 40 with the same columns as described for table 33 of which the lines e and f should also be reread for comparison.

Similarly table 41 shows the influence on hatchability for the groups in columns identical with table 34.