TABLE 39.—(See Appendix A, Table 39.)

The Influence of Size, Shape and Materials of Construction of the Instruments used on the Egg-production after Intraperitoneal Insemination in Fowls.

Line.	Description of Type and Materials of Instruments: Particulars of Groups.	Production Percentage after Insemination.	Number of Cases of Insemination followed by:—		
			Unchanged Production.	Reduced Production.	Increased Production.
	Type, Shape.				
a	Sharp needles	46%	4	23	7
b	Blunt needles	54%	2	19	10
	Type, Size.				
c	Coarse needles	45%	5	24	8
d	Fine needles	57%	1	18	9
	Materials.				
e	Metal nozzle, piston and needles	54%	6	8	5
f	Metal piston and needles	32%	4	8	3
g	Metal needles but all-glass syringe	56%	3	12	4
h	Wax-coated metal needles and all-glass syringe	59%	2	12	4
i	Special all-glass syringe with long nozzle used without needle	36%	1	2	1

TABLE 40.—(See Appendix A, Table 40.)

The Influence of Size, Shape and Materials of Construction of the Instruments used, on the Fertility Following Intraperitoneal Insemination of Fowls.

Line.	Description of Type and Materials of Instruments: 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.
	Type, Shape.			
a	Sharp needles	30%	43%	8·4
b	Blunt needles	22%	31%	7·4
	Type, Size.			
c	Coarse needles	35%	44%	8·5
d	Fine needles	17%	29%	10·0
	Materials.			
e	Metal nozzle, piston and needles	17%	26%	9·3
f	Metal piston and needles	22%	30%	9·3
g	Metal needles but all-glass syringe	32%	51%	12·6
h	Wax-coated metal needles and all-glass syringe	32%	44%	11·1
i	Special all-glass syringe with long nozzle used without needle	26%	43%	10·1

TABLE 41.—(See Appendix A, Table 41.)

The Influence of Size, Shape and Materials of Construction of the Instruments used, on the Hatchability Following Intraperitoneal Insemination of Fowls.

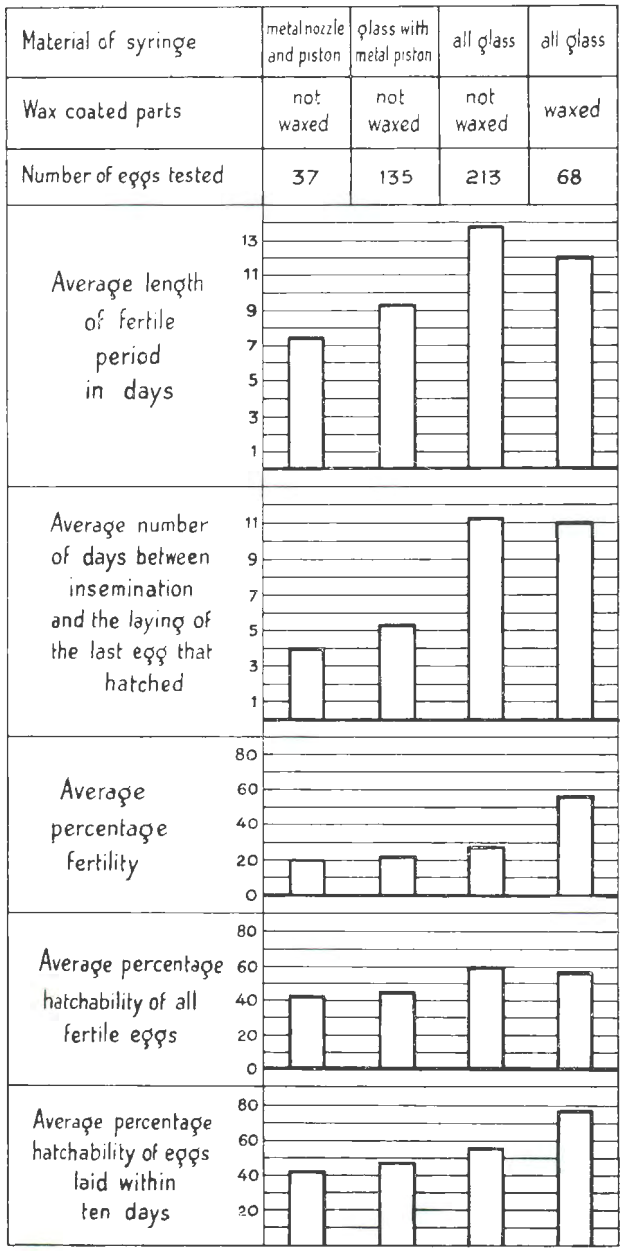
Line.	Description of Type and Materials of Instruments : Particulars of Groups.	Percentage hatchability in Total Number of Fertile Eggs Set.	Percentage hatchability in Fertile Eggs laid 24 Hours to Ten Days after Insemination.	Average duration of hatchability in Days.
	Type, Shape.			
a	Sharp needle	56%	64%	8·2
b	Blunt needle	49%	42%	7·4
	Type, Size.			
c	Coarse needles	56%	61%	8·2
d	Fine needles	48%	47%	7·1
	Materials.			
e	Metal nozzle, piston and needles	57%	53%	5·6
f	Metal piston and needles	44%	49%	5·3
g	Metal needles but all-glass syringe	60%	69%	10·1
h	Wax-coated metal needles and all-glass syringe	44%	50%	9·3
i	Special all-glass syringe with long nozzle used without needle	60%	33%	9·0

(*b*) *The influence of different materials of construction of the instruments used for intraperitoneal insemination.*—The detrimental effect of contact between semen and metals on the life duration of spermatozoa, is well known. This fact was considered in the development of the technique for intraperitoneal insemination. The receptacles used for semen collection were all made from hard glass, but for insemination four different types [(i) to (iv)] of commercial syringes were applied and also a special all-glass syringe (v) and a variety of metal needles [Types (vii) to (xi)]. The results are grouped in lines e, f, g, h, and i, in tables 39, 40 and 41. Lines e show all the data recorded for syringes of type (iii) which included the use of needles of types (vii), (viii) and (ix). Lines f refer to the 25 operations with type (iv) syringes and type (vii) needles. Lines g provide the details from inseminations with type (ii) syringe and needles of types (vii), (ix) and (x).

In order to avoid contact with metal altogether a system of wax-coating was attempted, with the intention to provide a continuous thin film of beeswax along the inner surface of all metal needles. The data from these attempts are given in line h and type (ii) syringes with type (vii), (xi) and (ix) needles were employed here. In seven operations with the special all-glass syringe [type (v)] no metal parts were used at all.

It is obvious that there is considerable variation in these groups apart from the factor of contact between metal and semen. In order to show the effect of this factor more clearly, all the operations done with one type of needle, type (vii), were segregated and grouped according to degree of metal contact observed in the use of different syringes. The semen was contained in the syringes sometimes for several minutes, but only passed through the needle at the moment of insemination. The results are demonstrated in Graph T.

GRAPH T.—The effect of contact between the semen used for intraperitoneal insemination and the metal parts of some of the syringes employed for the operation.



Graph T shows the marked detrimental effect of the contact between the semen and syringes with metal parts. Wax-coating of the needle (metal) further assisted in raising the fertility obtained even with an all-glass syringe.

(*c*) *The locality for entry of the instruments of insemination into the abdominal cavity.*—The site of penetration at first selected in the fowl for injection of semen into the " cavum peritoneii intestinale ", which contains the genital organs, was described in part (2) (page 47).

The anatomical grounds for this selection have been stated in connection with the first intraperitoneal inseminations [see page 47 and van Drimmelen, 1945 (*a*)]. Variation in his aspect of the technique was considered but it was clear that the possible variations were limited to the lateral and posterior abdominal surface as the ventral, anterior and dorsal sides of the ovarian region are obstructed by vital organs.

To recapitulate, the landmarks used for the operation in the fowl were: (i) the anterior border of the left pubic bone of which the process extending in postero-ventral direction is fused to the ischium; (ii) the ventral border of the superficial muscles to be seen extending from the costal to the pelvic region under the left abdominal skin. The sides remaining for consideration were:—

(i) The left abdominal surface, anterior to the usual site of penetration.

(ii) The posterior abdominal wall, caudal to the usual site of penetration.

(iii) The right abdominal wall.

Four birds were killed and several needles inserted into sites in these three areas. By means of careful dissection the position of the points of the needles were determined and the following findings recorded:—

(i) Penetration to the region of the ovary through the left anterior abdominal surface succeeded in three out of ten attempts, the needle passing through the left abdominal airsac and causing damage to ova in all three cases, whilst the seven failures were due to the point of the needle remaining in the airsac.

(ii) Penetration from behind succeeded in entering the peritoneal cavity in five out of five cases.

(iii) Penetration into the cavity to the ovarian region from the right side succeeded when attempted through the airsac eight out of eight times but failed to reach the ovary from the site at the pubis as the point of the needle was here obstructed by the gizzard and intestines in four out of four trials.

As the result of these findings it was decided to attempt the intraperitoneal insemination operation from the post-pubic site [see (ii)] in live birds, as the operations involving the airsacs and intestines were not considered practical. Accordingly a blunt, fine needle was inserted through the lumen of a short sharp needle passed through the abdominal wall posterior to the pelvic bones in hen No. 43 (insemination 65) and semen was injected. The technique was difficult and damage to abdominal organs was considered likely, so that no further attempts were made. The results were negative but inconclusive.

(*d*) *The depth of penetration with instruments for intraperitoneal insemination.*—Four inseminations were done with a short wax-coated veterinary needle after which the incubation results of three could be tested. The operations, viz.: inseminations number 101, 112, 106*a* and 107*b*, were undertaken to obtain information on the existence of limits, if any, in the distance between the site of discharge of semen and the locality of impregnation, permissible for successful intraperitoneal insemination. This appears to be important in view of the variation in size and conformation of different birds, which may be desired to

be inseminated with the same instruments. In the case of insemination number 101, hen number 24 was injected with 0·3 c.c. of 2 hours old mixed semen after she had laid 18 infertile eggs over an infertile period of 28 days. The operation was done at 11.00 hours after she had laid before (earlier than at 08·00 hours on the same morning). The egg laid at 08·30 the next day was infertile, but on the second day at 08·00 hours the hen laid another egg which proved to be fertile and developed up to the tenth day of incubation, when the embryo died. Insemination number 112 was followed by peritonitis of hen number 50 (killed after nine days, during which no egg was laid).

Insemination number 106 a. was followed immediately by another with a long needle and number 107 b. was preceded by one with a long needle on the same hen. The results of these four operations are set out in Table 42.

This Table provides a clear case showing that penetration to the region of the ovary has decided advantages over mere deposition of semen somewhere in the peritoneal cavity of the hen.

(*e*) *The direction of penetration of the instruments of intraperitoneal insemination.*—A number of fowl hens were sacrificed, after numerous intraperitoneal inseminations had been carried out successfully, in order to obtain factual information on the position of the instruments in the body of the bird at the moment of discharge of the semen. These post mortem investigations were done during the first, third and fourth series of inseminations and the first six will be considered separately. The seventh post mortem examination on this problem was repeated many times, with slight variations in the interval between the operation and death, on subjects, some of which were used for other work as well, and these can be considered together in one group (g).

(i) *Actual comparison of the courses followed by a sharp, coarse, long and a blunt, fine, long needle in the operation for intraperitoneal insemination.*—Hen number 25 was slaughtered with the following needles inserted into the body to a depth of 8·0 cm. in the direction and at the site described for operation (i) in part B. (2) (*b*) of this chapter—(i) type (vii) a coarse, long, sharp metal needle, with single aperture, and (ii) type (viii), a fine, long, blunt, metal needle, with single aperture, passed through a short coarse veterinary needle [type (xi) in the abdominal wall].

Results.—On opening the abdomen, the terminal apertures at the points of the needles were both found located in the region of the ovary, amongst several large follicles. The coarse needle had penetrated the peritoneum with its sharp point and taken a course dorsal to the intestines, gizzard and proventriculus and ventral to the coils of the oviduct. The point of the blunt and fine needle had emerged from the opening of the sharp veterinary needle in the peritoneal cavity and followed the identical course described for the other needle.

(ii) *Duplication of number* (i).—Hen number 52 was subjected to the same operations as hen number 25.

Results.—The position of both needles was found similar to that seen in hen number 25 but the blunt and fine needle [type (viii)] had penetrated a large follicle to a depth of 8 mm.

(iii) *The distribution of iodine-stained starch, in the abdominal cavity of a fowl hen after injection by the method of intraperitoneal insemination.*—Hen number 30 was slaughtered five minutes after injection into the abdomen 0·75 c.c. of a watery suspension of starch, stained darkblue with iodine solution by the technique described in part B. (2) (*b*), [operation (i) experiment (4)]. The needle used was type (vii), a coarse, sharp, long metal needle with single aperture.

TABLE 42.

The Results of Intraperitoneal Insemination on the same Hen with Long and Short Needles Separately at the Same Time.

Number of bird.....	No. 9	No. 9	No. 16	No. 16
Number of insemination..	106a	106b	107a	107b
Depth of discharge.....	2·0 cm.	6·0 cm.	6·0 cm.	2·0 cm.
Type of needle (wax-coated)	Type (xi).—Coarse, sharp, short	Type (ix).—Fine, blunt, triple aperture, long	Type (vii).—Coarse, sharp, long	Type (xi).—Coarse, sharp, short
Type of syringe.....	Type (ii).—Glass.....	Type (ii).—Glass.....	Type (ii).—Glass.....	Type (ii).—Glass.
Donor of semen and amount of semen	No. 6.—White Leghorn, 0·1 c.c.	No. 44.—Barred P. Rock, 0·3 c.c.	No. 6.—White Leghorn, 0·1 c.c.	No. 44.—Barred P. Rock, 0·3 c.c.
Duration of hatchability..	—	8 days	6 days	—
Number of chicks.....	—	One	Three	—

Results.—The starch was found in the peritoneal cavity, mostly on the left of the anterior coils of the oviduct and on the loops of the intestines ventral to the infundibulum. About one-third of the starch particles was spread amongst the smaller ova and on the fimbriae of the oviduct but no starch was found inside the oviduct.

(iv) *Comparison in the distribution of materials injected by the technique for intraperitoneal insemination with the special glass syringe* [*Type* (v)] *and with the metal needle* [*Type* (vii)].—Hen number 27 was slaughtered within five minutes after injecting 0·1 c.c. iodine-stained starch in watery suspension 6·0 cm. deep into the peritoneal cavity with the long glass nozzle of the special all-glass insemination syringe [Type (v)] inserted through the canula. The canula was in the usual pre-pubic position and the direction of the nozzle was antero-medial after entering through the canula. This operation was followed immediately by an intraperitoneal injection of 0·25 c.c. iodoform in water, given through a long sharp, coarse metal needle [Type (vii)] into the same locality about 6·5 cm. deep. The instruments were removed.

Result.—The starch was found to the left and ventral to the anterior part of the albumen region of the oviduct and not amongst the ova or intestines; the iodoform was found in the lumen of the middle albumen region, distributed over an area of about 3 cm. long.

(v) *The position of a thin glass capillary tube inserted into the abdomen through the short, coarse needle* [*Type* (xi)] *at the site and in the direction described for intraperitoneal insemination.*—Hen number 28, killed after introducing the instruments as indicated, was kept undisturbed in the operating position on her right side on the post mortem table. The position was explored through a 3 cm.-long tranverse cut through the left ribs and lateral abdominal airsac.

Result.—The end of the glass capillary tube was found amongst the follicles in contact with the numerous smaller follicles.

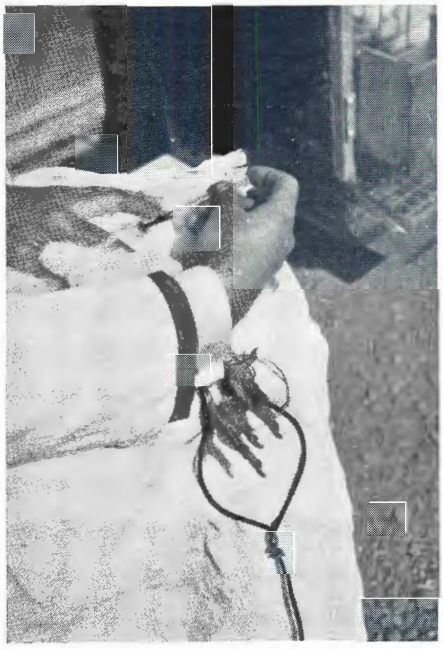
(vi) *The position of the nozzle of the specially made all-glass insemination syringe* [*Type* (v)] *in the body of a very large hen during the operation for intraperitoneal insemination compared with that of the long needle* [*Type* (vii)].—Hen number 16, a heavy-framed two-year-old Rhode Island Red bird, was slaughtered with the said instruments inserted into the body cavity as for the usual operation.

Results.—The glass nozzle tip was found among a cluster of yolks and the point of the metal needle had entered a yolk or large follicle to a depth of 13 mm.

(vii) *The location and photographical recording of a modified canula as described for the nozzle of the special all-glass syringe* [*type* (v)].—During the third series of inseminations a new instrument was adopted, in view of the fragility of the special all-glass insemination syringe [Type (v)]. This was made from the tip of a "Holborn" plastic sheep inseminator, by sawing off the end of the third portion of the tube and widening the lumen to admit the nozzle of an all-glass syringe. The thin tip which had to be passed through the canula when carrying out the operation had to be about 8 cm. long, [see figures (vii) and (viii)]. Hen number 58 was dissected after introduction of the modified "Holborn" sheep inseminator, having been killed in the position of the operation, i.e. lying on her right side. All the structures ventral to the inseminator

tip and the canula were removed and the end of the inseminator located among the large follicles in the ovary. A photograph of this specimen was made but this was not suitable for reproduction. This process was repeated during the fourth series of inseminations on many birds anaesthetised for investigations into the location of living sperm in fowl hens (van Drimmelen, 1945b) when the operation was done just before killing the bird (i.e. after the cranial parts of the genital organs had been searched through an opening in the abdominal wall under the left thigh, just ventral to the acetabular joint, as described in the article indicated). Twenty-one post mortem examinations were done in this way, but no photograph suitable for reproduction obtained. Eight further birds were injected with a suspension of powdered carbon by means of the same instruments and operative procedure, and the carbon located after killing them five minutes to 24 hours after this injection. Four carcasses were frozen in distilled water with the instruments in position, three hens having been killed immediately after the injection. Sections through the solid ice were sawn in a horizontal plane just ventral to the instruments in the case of the three and transverse, vertical sections through the ovary of the carcass of the fourth fowl killed 24 hours after injection of 1 c.c. thick watery carbon suspension. The cut surfaces were trimmed down to the instruments by means of a coarse file and cleared with a steam jet.

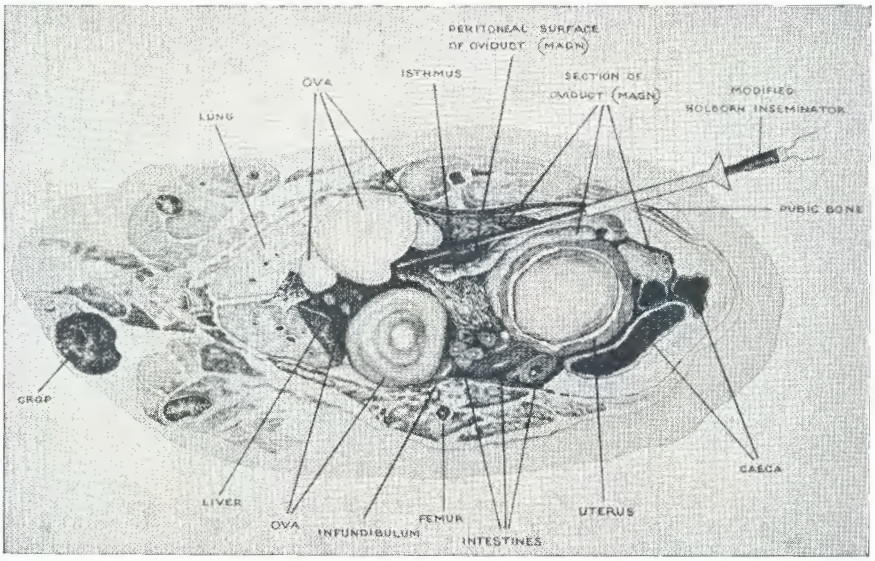
FIGURE (vii).—The Application of the Modified " Holborn " Sheep-inseminator for Intraperitoneal Insemination.



Photograph showing the position of the canula and the inseminator fitted to an all-glass insulin syringe. [*cf.* Figure (v)].

(*Photo by Mr. Th. Meyer, for the Director of Veterinary Services, Onderstepoort*).

FIGURE (viii).—The Position of the Instruments in the Body of the Hen.



Photograph showing the position of the instruments in the body of the fowl hen, at the moment of insemination by the intraperitoneal method (van Drimmelen, 1945a).

(*Drawing by Maj. C. G. Walker from a photograph of a frozen specimen by Mr. Th. Meyer for the Director of Veterinary Services, Onderstepoort*).

Useful photographs were obtained, one of which was redrawn for clarity and reproduced [see figure (viii)].

Results.—The tip of the inseminator was consistently found in the region of the ovary in the cases where the organs were removed before the instruments were taken out of the carcasses. In the other cases the carbon particles were found in the region of the ovary.

Table 43 gives a summary of the cases in which the direction of the instruments, that penetrated to the ovary, and the position of material injected into the region of the ovary, were investigated.

The *dose of semen* delivered in the operation for intraperitoneal insemination *and the dilution* of the semen used, will be referred to in part (9).

Discussion.—In the work on developing the technique for artificial intraperitoneal insemination of fowls (van Drimmelen 1945 a) a great variety of instruments for penetration of the abdominal wall was used. There was no marked difference in effect on egg-production of the hens after the operations with different instruments, but some indication was found (table 39) that " blunt " and " fine " needles (lines b and d) had less inhibitory effect on egg-production than " sharp " and " coarse " needles (lines a and c), being followed by relatively fewer cases of reduced production and relatively more cases of increased output. " Fine " needles also showed a longer duration of the fertile period after insemination.

However, the percentage fertility and the percentage hatchability were markedly better for the operations with " sharp " and " coarse " needles and the average duration of hatchability was somewhat longer than in the case of " fine " and " blunt " instruments.

TABLE 43.

The Direction of Penetration and the Position Reached by the Apertures of the Different Instruments used for Intraperitoneal Insemination.

Method of Investigation.	Type of Instrument used.	Number of Post Mortem Examinations.	Number of Cases in which the Ovary was Reached.	Number of Cases in which Organs were Damaged.	Kind of Organs Damaged.
Instruments examined *in Situ*	Type (vii) needle.....	3	3	1	Large follicles.
	Type (viii) needle.....	3	3	1	Large follicles.
	Type (v) all-glass syringe....	1	1	—	—
	Glass capillary tube.....	1	1	—	—
	Modified Holborn inseminator	25	25	—	—
Insoluble materials injected into the peritoneal cavity	Type (vii) needle.....	2	1	(1)	Oviduct once penetrated.
	Type (v) all-glass syringe....	1	1	—	—
	Modified "Holborn" Inseminator.....	5	5	—	—

In this connection a most interesting comparison must be made with the results reported in parts (6) and (7) where it was mentioned that available evidence indicated that a negative correlation between fertility and hatchability could exist, if influences affecting only the female were considered. In Tables 33, 34, 37 and 38 such results were found in respect of exposure of the hens, breeds of the hens, age of the hens and production rate of the hens.

No trace of such " compensatory hatchability " could be found in the groups examined for effects of the different instruments with which the semen was injected, suggesting that in matters affecting the semen a positive correlation between fertility and hatchability of eggs would be found. Munro (1938) mentioned the general poultry experience, which associated low fertility with high embryo mortality (see page 33) and argued that this was more likely to be due to factors affecting the ovum. It appears that this important aspect merits further investigation very urgently.

The figures on the different degrees of contact between semen and metal parts of instruments appears somewhat irregular in Tables 39, 48 and 41 (lines e, f, g, h and i) though the use of syringes with metal parts (i.e. metal in the instrument in which the semen is held for some time during the operation) shows decidedly inferior results as regards incubation. The group showing up best in this examination was the simplest technique (lines g), viz.: an ordinary all-glass

syringe with plain, clean metal needles. (Note: a modified "Holborn" inseminator was constructed at a later stage to replace the needle.) The effect of metal parts in the syringe was very clearly illustrated in Graph t, in which the superiority of the all-glass syringe is obvious.

Thus it is concluded that the best apparatus for intraperitoneal insemination would consist: (1) Solely of glass (or non-metal) parts, (2) of parts with a relatively large bore for rapid passage of the semen, (3) of blunt pointed penetrating parts to minimise the risk of injury.

The site of injection and the landmarks mentioned in part (2) were found to be the best possible in the fowl. The depth of discharge of the dose of semen obviously should be the distance between the ova and the site of injection. It was considered possible, however, that the movement of intestines and oviduct might facilitate the transfer of the spermatozoa from the posterior part of the cavity to the ovary; or alternatively that a natural flow of fluid from all parts of the peritoneal cavity into the mouth of the oviduct occurred and that this could assist sperm travel. If either were the case and if the peritoneal cavity provided a suitable habitat for the sperm then the deep penetration would be superfluous and dispensable. The data collected in the present work (Table 42) provide a small amount of evidence, that the region of the ovary was, as initially believed, the best site for discharge of the semen in the operation for intraperitoneal insemination.

The direction of penetration to reach this locality was considered from many aspects including the type of instrument used, the conformation of the bird inseminated and the distribution of the injected material in the body of the bird after discharge. The experience acquired during the actual execution of the work, led to the construction of the special all-glass inseminator syringe [Type (v)] and the modified "Holborn" sheep inseminator used with the all-glass insulin syringe. For best results with least danger to the subject this instrument is indicated, used with a small trocar and canula. Penetration to a depth of 6·0 to 8·0 cm. in an antero-medial direction (2·0 cm. forward for every 1·0 cm. inward), is shown to be sufficient. The coarse metal needle is nevertheless a very useful instrument for the intraperitoneal insemination operation, especially if more suitable substances than paraffin wax for coating the metal were to be evolved e.g. collodion, cellulose etc. (Pullinger, 1945.)

(9) *The examination of the semen used for intraperitoneal insemination in the present work.*

The inseminations reported in the present work were carried out with a number of semen samples very different from each other.

(*a*) *Pigeons.*

The samples taken from pigeons were in such very minute quantities that it was necessary to use mixed semen from several cocks for one injection in many cases. Records were kept of the volume [see part (1)] estimated density and morphology of some, and this data are presented in Table 44 [see figure (ix)].

(*b*) *Fowls.*

The semen from the fowl cocks was collected and examined as summaried in Table 45. For detail see appendix B, Table 45, where the collections have been grouped in separate lines in respect of age of the bird and season during which collected, as they were not all made at the same times.

TABLE 44.

The Samples of Semen Obtained from Some of the best Semen Producers amongst the Pigeon Males Used.

Line.	Number of Pigeon Cock.	Age of Pigeon Cock.	Number of Collections attempted.	Percentage successful.	Average Volume of Semen per successful collection in ml.	Number of Smears from Fresh Samples Examined.	Average Estimated Density (10^6 p.c.mm.)	Microscopical Findings.
a	125.....	Old bird.....	55	41%	0·005 ml.	4	3·0	Nothing unusual.
b	136.....	Young bird.....	18	50%	0·011 ml.	0	—	—
c		Yearling.....	20	95%	0·013 ml.	5	3·0	Nothing unusual.
d		Two-year old.....	12	84%	0·012 ml.	2	3·0	Nothing unusual.
e		Old bird.....	5	100%	0·012 ml.	0	—	—
f	P. 53.....	Yearling.....	10	90%	0·007 ml.	4	1·0	m. curled forms.*
g		Two-year old.....	10	90%	0·015 ml.	5	2·0	o. curled forms.†
h		Old bird.....	5	60%	0·004 ml.	0	—	—
i	716.....	Young bird.....	5	100%	0·010 ml.	0	—	—
j		Yearling.....	12	100%	0·016 ml.	3	2·5	Nothing unusual.
k		Old bird.....	5	80%	0·003 ml.	0	—	—

* Many abnormal curled heads were seen on the sperm in all the smears. [See figure (ix)a].

† Curled up heads were seen once early in the season. Apparently these were very vigorously motile sperm, but the other sperm showed nothing unusual.

The methods of testing used in one or more instances were:—

(i) Measurement of volume.

(ii) Estimation of viscosity.

(iii) Description of colour.

(iv) Description of purity or cleanliness.

(v) Examination of motility of sperm.

(vi) Estimation of density of semen.

(vii) Measurement of pH value.

(viii) Examination of morphology.

(ix) Testing of fertilizing power.

These will be shortly described:—

(i) *The volume* was measured in each case with a sterile dry glass pipette graduated to indicate 0·01 c.c.

(ii) *The viscosity* was estimated by comparison of the flow of semen into the pipette with the rate of the flow of water drawn up with suction from the same rubber teat at the same temperature.

(iii) *Colour* and (iv) *Purity* were recorded from visual examination.

(v) *Motility* was estimated by microscopical examination of hanging drop preparations and recorded as recommended by Milovanov, (1940) on the basis of progressive motility only, using the unit 1·0 for 100 per cent. progressive motility (see lit.).

(vi) *Density* was estimated from haemocytometer counts, from dry films on clean glass slides, and from appearance.

(vii) *The pH* was measured by Beckman's pH-meter and by B.D.H. capillator apparatus.

(viii) *Morphology* of the spermatozoa was examined in wet and dry preparations under the microscope, using various staining methods. [See figures (*x*), (*a*) to (*e*)].

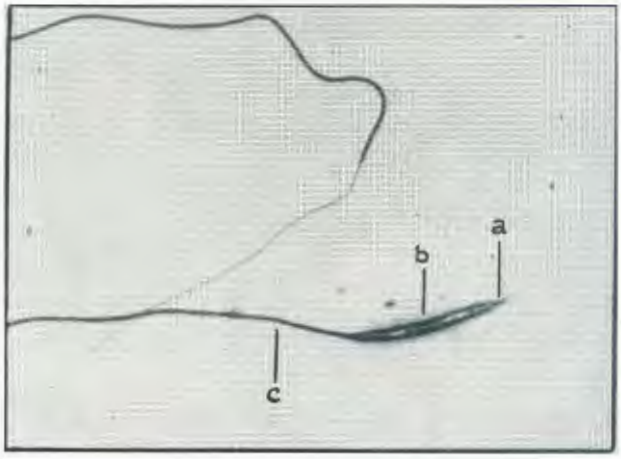
(ix) *The fertilizing power* of semen was tested by intraperitoneal and other methods of insemination in fowls.

FIGURE (ix) (*a*).—Pigeon Semen (Pathological).

Microphotograph of part of a dry stained smear from semen of the male pigeon: Number P 53. (*See* Table 44), showing spermatozoa with curled up heads. (X 300); (Giemsa's stain).

(*Photo by Mr. Th. Meyer for the Director of Veterinary Services, Onderstepoort.*)

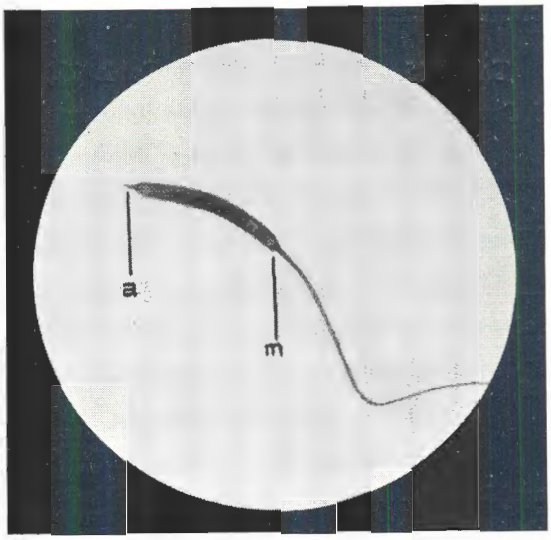
FIGURE (ix) (*b*).—The Pigeon Spermatozoon.



Microphotograph of the spermatozoon head of the pigeon in a dry stained film of pigeon semen, showing the acrosome (*a*), head proper (*b*), and part of the long tail with thin end (*c*). (X 1400) ; (Stain : Williams' method after Lagerlöf).

(*Photo by Mr. Th. Meyer for the Director of Veterinary Services, Onderstepoort.*)

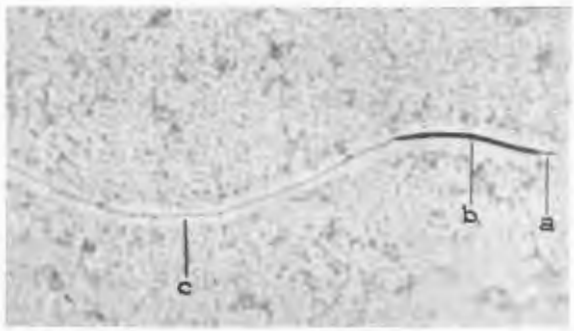
FIGURE (ix) (*c*).—Pigeon Spermatozoa.



Microphotograph of acrosome (*a*) and middlepiece (*m*) of some pigeon sperm cells, showing the very small size of the middlepiece in the pigeon. (X 1400) ; (Giemsa's stain, partly decolorized to remove nuclear stain in order to photograph adjoining acrosome and middlepiece).

(*Photo by Mr. Th. Meyer, for the Director of Veterinary Services, Onderstepoort.*)

FIGURE (ix) (*d*).—Pigeon Spermatozoa.



Microphotograph of pigeon sperm heads showing head proper (nucleus) (*b*), acrosome (*a*) and part of tail (*c*). (X 1400) ; (Stain : Giemsa's, followed by carbol-fuchsin to show up tail).

(*Photo by Mr. Th. Meyer, for the Director of Veterinary Services, Onderstepoort.*)

Fertilizing power of semen.—The data on fertilizing qualities of the semen samples tested in the *first* series of inseminations are submitted in Tables 46 to 48, (see also Appendix B). In these Tables the results from individual males are compared, without consideration of the dose of semen, ageing of the semen *in vitro,* or time of collection of the semen, (season and age of cock) and the

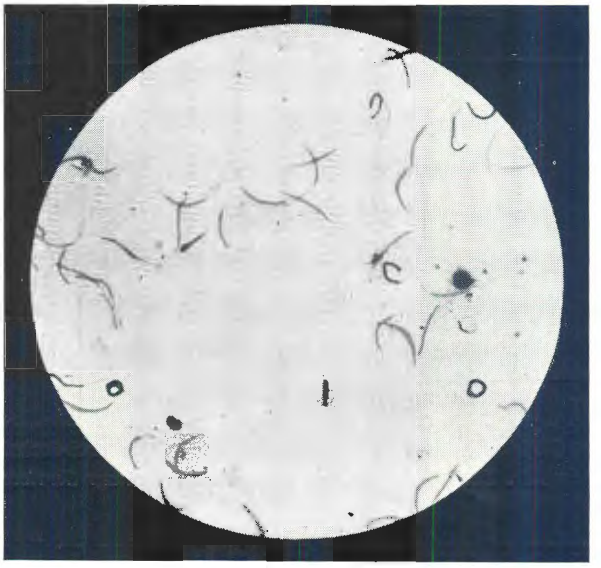
figures are not large enough to draw final conclusions; but these results are given here, as they form the only information available on the new intraperitoneal technique of artificial insemination of fowls.

TABLE 45.—(See Appendix B, Table 45.)

Summary of Fowl Semen Examinations Applied to Samples Collected by the Author's Method.

Line.	Method of Examination.	Number of Males Examined.	Number of Tests Performed.	Remarks on the Results Obtained.
a	Measurement of Volume.....	25	371	(*See* Table 5).
b	Estimation of Viscosity.....	21	292	Marked individual variation.
c	Description of Colour.....	21	292	Almost consistently ivory white.
d	Examination of Motility.....	9	61	When fresh, 100% motile or slightly less; but rapidly reduced when kept *in vitro* and all motility lost after approximately 96 hours
e	Determination of Density....	21	292	Marked individual variation.
f	Determination of pH.....	7	20	Range 7·00 to 7·74.
g	Examination of Morphology..	14	253	Abnormalities very rarely marked (5 cases).
h	Tests for Fertility.....	17	196	Marked individual and case variation.

FIGURE (x) (*a*).—Fowl Semen (Pathological).



Microphotograph of part of a dry stained film from semen of the fowl cock ; Number 44, showing spermatozoa with curled up heads. (X 300) ; (Giemsa's stain). (*See* Table 45, Appendix).

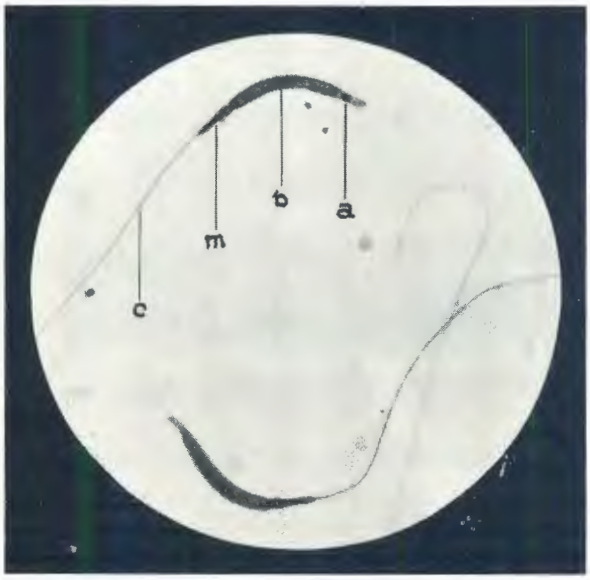
(*Photo by Mr. Th. Meyer, for the Director of Veterinary Services, Onderstepoort.*)

FIGURE (x) (*b*).—Spermatozoon of the Fowl.

Microphotograph of unstained fowl spermatozoon in isotonic Opal Blue medium (dry film). Note the wavy appearance of the central, longitudinal structure in the spermhead. (X 1400) ; (unstained, dry).

(*Photo by Mr. Th. Meyer, for the Director of Veterinary Services, Onderstepoort.*)

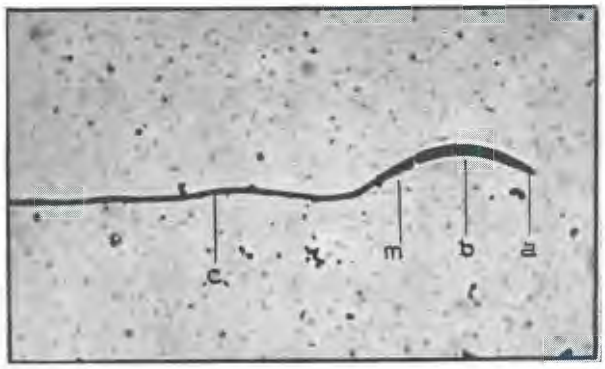
FIGURE (x) (*c*).—Spermatozoa of the Fowl.



Microphotograph of the head portion of a spermatozoa in a dry stained film of fowl semen. Note the acrosome (*a*), head proper (*b*), very distinct and prominent middlepiece (*m*) and part of the tail, (*c*). (X 1400) ; (Stain : Williams' after Lagerlöf).

(*Photo by Mr. Th. Meyer, for the Director of Veterinary Services, Onderstepoort.*)

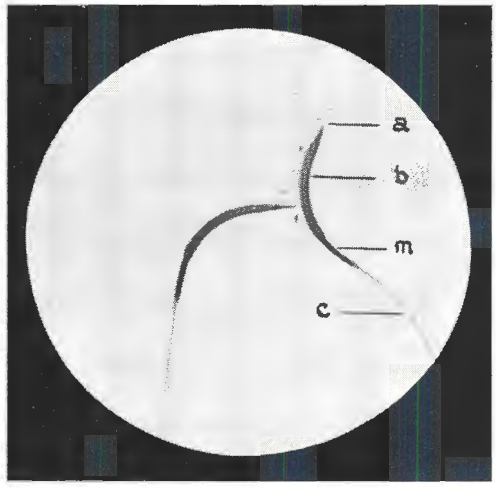
FIGURE (x) (*d*).—Spermatozoon of the Fowl.



Microphotograph of a dry stained film of fowl semen, with sperm cell showing acrosome (*a*) head proper (*b*), very distinct middlepiece (*m*) and tail (*c*). (X 1400) ; (Giemsa's stain).

(*Photo by Mr. Th. Meyer, for the Director of Veterinary Services, Onderstepoort.*)

FIGURE (x) (*e*).—The Spermatozoa of the Fowl.



Microphotograph of the head portion of fowl spermatozoa, showing acrosome (*a*), head proper (*b*), middlepiece (*m*) and part of the tail (*c*). (X 1400) ; (Stain : Carbol-acid-fuchsin, with some apparent adsorption).

(*Photo by Mr. Th. Meyer, for the Director of Veterinary Services, Onderstepoort.*)

Table 46 shows merely the number of failures and successes of the sperm of each male, when in competition with others [columns (2) and (3)], and also the number of fertile intraperitoneal and *per vaginam* inseminations with semen from the given cock only [column (4)]. The sum of columns (3) and (4), makes up the total number of cases of which the fertility and hatching results are set out in Tables 47 and 48, in the same way as in Tables 33 (37, 40) and 34 (38, 41) respectively, [see parts (6), (7) and (8)]. For details see Appendix B, Tables 46 to 48.

TABLE 46.—(See Appendix B, Table 46.)

The Samples of Semen from Different Donors used in the First Series of Intraperitoneal Inseminations.

Line.	Number given to each Cock.	Number of Cases followed by Eggs Fertilized by Sperm from other Semen present in the Hen at the same time.	Number of Cases followed by Eggs Fertilized by own Sperm when other Semen was introduced at the same time.	Number of Cases followed by Fertile Eggs when no other Semen was introduced at the same time.
		Intraperitoneal Insemination.		
	Column (1)	Column (2)	Column (3)	Column (4)
a.....	6	6	7	10
b.....	18	0	0	1
c.....	23	0	0	1
d.....	33	2	0	0
e.....	38	1	3	2
f.....	44	1	1	3
g.....	47	3	0	0
h.....	53	1	0	1
		Insemination *per vaginam.*		
i.....	6	0	3	1
j.....	33	2	0	0
k.....	34	1	0	0
l.....	34	0	0	1
m.....	47	0	0	1
n.....	53	1	0	0

TABLE 47.—(See Appendix B, Table 47.)

The Fertility Obtained with Samples of Semen from Different Donors used in the First Series of Intraperitoneal Insemination.

Line.	Number given to each Cock.	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.
		Intraperitoneal Insemination.		
	Column (1)	Column (2)	Column (3)	Column (4)
a.....	6	29%	41%	13
b.....	18	21%	75%	5
c.....	23	17%	20%	2
d.....	34	15%	24%	7
e.....	38	28%	26%	14
f.....	44	11%	19%	10
g.....	47	12%	18%	8
h.....	53	22%	100%	11
		Insemination *per vaginam.*		
i.....	6	39%	76%	16
j.....	44	39%	54%	13
k.....	47	37%	75%	9

TABLE 48. (See Appendix B, Table 48.

The Hatchability of Fertile Eggs Obtained with the Semen from Different Donors used in the First Series of Intraperitoneal Insemination.

Line.	Number given to each Cock.	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.
		Intraperitoneal Insemination.		
	Column (1)	Column (2)	Column (3)	Column (4)
a.....	6	56%	59%	11
b.....	18	100%	100%	5
c.....	23	100%	100%	2
d.....	34	83%	83%	7
e.....	38	40%	30%	9
f.....	44	38%	39%	9
g.....	47	42%	50%	3
h.....	53	100%	100%	11
		Insemination *per vaginam*.		
i.....	6	38%	48%	9
j.....	44	78%	86%	13
k.....	47	100%	100%	4

Dosage of semen.—The dose of semen given was not varied markedly during the *first* series of inseminations, as the experience showed [described in part (2)] that the minimal fully effective dose stipulated by Burrows and Quinn (1937,1939), i.e. 0·1 ml. was also fully effective with intraperitoneal insemination. (See and compare Tables 7 and 13.) In the *third* series of inseminations, some very large doses of semen were given, and in Tables 49 and 50 the results of both the *first* and *third* series are collected and arranged to show the effect of size of dose on fertility and hatchability in the same way as before (Tables 33, 37, 40, 47 and 34, 38, 41, 48). Lines a to d contain the information on the plain, simple operations, whereas all operation with additional complications such as admixture and multiplicity of injections are included in lines e to i. (For basic figures and details see Appendix B, Tables 49 and 50.)

Dilution of semen.—A few samples of semen were used after dilution with various media, some of which were only used after storage. A record of the work done in this connection is shown in Table 51. The diluent was mixed with

the semen by adding it to the semen drop by drop, stirring all the time. Very little data were collected as the work was only of a subsidiary nature, the purpose being to examine further avenues of experimentation i.e.—

(i) to see if dilution of fowl sperm injected by the intraperitoneal method was likely to be successful;

(ii) to see if better results could be obtained as regards fertility;

(iii) to see if any of the diluents had any useful qualities.

Storage of semen.—As not all samples of semen used were injected immediately after collection, and as careful records of the delay in each case are available, an analysis of the results of intraperitoneal insemination with stored semen is given in Table 52. The following were the methods employed:—

(*a*) Pure semen not mixed with other samples was collected in clean, hard-glass test tubes and immediately covered with liquid paraffin and then slowly cooled to room temperature: this was used up to four hours old.

(*b*) Diluted semen was used in a few instances after a lapse of time following collection and dilution, viz.:—

(i) Samples used 5 to 30 minutes after collection and dilution: Fifteen inseminations with diluted semen were done without delay, the diluents being fresh egg-white, Ringer's solution, physiological saline and Winter's buffer solution and egg-yolk. The diluent was mixed with the semen by adding it drop by drop. (Line c, Table 52).

(ii) Samples used one hour after collection and dilution: Two inseminations were done with semen mixed as before with Lardy and Phillip's diluent for bull semen, (i.e. 0·2 gm. KH_2PO_4 + 20 gm. NaH_2PO_4. 12 H_2O dissolved in 100 c.c. triple glass-distilled water and mixed before use with fresh egg-yolk;—pH: 6·75) and then covered with a layer of sterile liquid paraffin and allowed to cool to room temperature. (Line f, Table 52.)

(*c*) Samples used five hours after collection and diluted when used for insemination: Four inseminations were done with unmixed semen diluted with Winter's buffer and fresh egg-yolk (Winter's buffer = 15·4 gram $Na_2H\ PO_4\ 12H_2O$, 43·2 grams KH_2PO_4, dissolved in 1,000 c.c. triple-glass-distilled water and $CaSO_4$ added to the solution to saturation; pH—7) [Winter's (1939), sheep semen diluter]. These were also cooled to room-temperature and stored under liquid paraffin protection (line h Table 52).

(*d*) Samples used 22 hours after collection and dilution: Two inseminations were done with semen diluted as in (*b*) and stored in the frigidaire overnight (line i, Table 52).

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TABLE 49.—(See Appendix B, Table 49.)

The Fertility Resulting from Intraperitoneal Insemination with Different Amounts of Fowl Semen.

Line.	Size of Dose of Semen introduced.	Percentage of Cases followed by Fertility.	Percentage of Fertility in Eggs laid 24 Hours to Ten Days after Insemination.	Average duration of Fertility in Days after Insemination.
Pure, fresh semen, not mixed and given in a single dose ;				
	Column (1)	Column (2)	Column (3)	Column (4)
a.....	0·01–0·09 ml.	60%	30%	3
b.....	0·1 ml.	46%	29%	8·7
c.....	0·15–0·25 ml.	77%	56%	10·6
d.....	0·3–0·5 ml.	100%	53%	8·0
Pure, fresh semen, mixed or given in multiple doses :				
e.....	0·1 ml.	12%	20%	9
f.....	0·15–0·25 ml.	75%	50%	11·5
g.....	0·3–0·5 ml.	92%	41%	9
h.....	0·6–0·9 ml.	100%	100%	13
i.....	1·0–1·5 ml.	72%	100%	21

TABLE 50.—(See Appendix B, Table 50.)

The Hatchability of Fowl Eggs Fertilized from Different Amounts of Semen Introduced by Intraperitoneal Insemination.

Line.	Size of Dose of Semen introduced.	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 after Insemination.
Pure fresh semen, not mixed and given in a single dose :				
	Column (1)	Column (2)	Column (3)	Column (4)
a.....	0·01–0·09 ml.	67%	67%	2·5
b.....	0·1 ml.	50%	61%	6·0
c.....	0·15–0·25 ml.	78%	75%	8·1
d.....	0·3–0·5 ml.	48%	59%	4·6
Pure, fresh semen, mixed or given in multiple doses :				
e.....	0·1 ml.	20%	20%	9
f.....	0·15–0·25 ml.	42%	42%	6·5
g.....	0·3–0·5 ml.	60%	67%	6·7
h.....	0·6–0·9 ml.	36%	50%	8
i.....	1·0–1·5 ml.	33%	27%	21

TABLE 51.

The Effect of Dilution of the Semen used in the Results of Intraperitoneal Insemination in the Fowl.

Line.	Description of Group.	Number of Cases of Insemination performed.	Number of Observations in 'Hen-days' i.e., as many times as One Hen was seen One Day.	Number of Cases of Insemination followed by Eggs which were set.	Number of Cases followed by Fertility.	Number of Eggs laid 24 Hours to Ten Days after Insemination that were set.	Percentage of Eggs laid 24 Hours to Ten Days after Insemination that were proved Fertile.	Percentage of Fertile Eggs laid 24 Hours to Ten Days after Insemination that Hatched.	Average duration of Fertile Period in Days.	Average duration of Hatchability in Days.
a	Fresh Egg-Albumen : Diluted 1 : 1	1	21	1	1	8	100%	62%	15	15
	Ringer's Solution—									
b	Diluted 1 : 2	1	32	1	0	0	—	—	—	—
c	Diluted 1 : 10	1	17	1	0	5	0	—	—	—
	Physiological Saline—									
d	Diluted 1 : 1	2	22	2	0	8	0	—	—	—
e	Diluted 1 : 8	1	6	1	0	4	0	—	—	—
f	Diluted 1 : 20	1	13	0	—	—	—	—	—	—
	Lardy & Phillip's dilutor—									
g	Diluted 1 : 4	4	68	4	1	8	12%	100%	2	2
	Winter's dilutor and egg-yolk—									
h	Diluted 1 : 1	5	32	4	0	11	0	—	—	—
i	Diluted 1 : 2	5	81	2	0	4	0	—	—	—
j	Diluted 1 : 5	2	14	2	0	9	0	—	—	—
k	Diluted 1 : 9	1	20	1	0	5	0	—	—	—
l	Total	24	326	19	2	38	27%	67%	8·5	8·5
	Total undiluted control	142	2,789	95	62	350	45%	53%	10·3	7·0

FIGURE (xi).—Equipment for Artificial Intraperitoneal Insemination.

Improvised Insemination Equipment.—A box container with temperature control from body-heat to room-temperature. The spirits lamp in the bottom partition, when burning, creates a hot air current, which passes through the tin chimney in the rear wall of the upper chamber behind the thermometer. Semen containers, diluents, washing fluids, liquid paraffin and instruments can easily be reached through the sliding doors on both sides, and clear visibility is provided by the sliding glass window in front.

(*Photo by Mr. Th. Meyer, for the Director of Veterinary Services, Onderstepoort.*)

TABLE 52.

The Effect of Storage of Fowl Semen on the Results of Intraperitoneal Insemination.

Line.	Description of Group.	Number of Cases of Insemination performed.	Number of Observations in 'Hen-days', i.e., as many times as One Hen was seen One Day.	Number of Cases of Insemination followed by Eggs which were set.	Number of Cases followed by Fertility.	Number of Eggs laid 24 Hours to Ten Days after Insemination that were set.	Percentage of Fertile Eggs laid 24 Hours to Ten Days after Insemination that were proved Fertile.	Percentage of Fertile Eggs laid 24 Hours to Ten Days after Insemination that Hatched.	Average duration of Fertile Period. in Days.	Average duration of Hatchability in Days.
	Pure semen:—									
a	Stored 5–30 minutes.....	67	1,546	45	28	186	30%	50%	9·7	5·6
b	Stored 30–60 minutes.....	19	357	11	10	36	67%	42%	12·9	11·6
c	Stored 1–2 hours.....	11	232	8	7	34	48%	56%	7·0	5·0
d	Stored 2–4 hours.....	1	6	1	0	5	—	—	—	—
	Diluted semen:—									
e	Stored 5–30 minutes.....	15	186	12	1	26	31%	62%	15	15
f	Stored 30–60 minutes.....	2	40	2	1	8	12%	100%	2	2
g	Stored 1–2 hours.....	0	—	—	—	—	—	—	—	—
h	Stored 2–4 hours.....	5	72	3	0	21	—	—	—	—
i	Stored 4–24 hours.....	2	28	2	0	14	—	—	—	—
j	Control: Pure semen, stored less than five minutes.....	44	648	30	17	94	59%	60%	11·2	8·0

The purpose of these trials was simply to find out if the keeping qualities of fowl sperm in these diluents were such as to encourage proper investigation. In the absence of suitable facilities little could be done in this direction.

Discussion.—The examination of semen samples reported in part (9) was of a subsidiary nature as the conditions of work prevented planned experiments in this field. As a matter of fact experiments in storage and dilution of fowl semen were planned and preliminary tests made in the *second series* of inseminations on fowls, but this part of the work broke down on account of lack of facilities and war conditions. Such information as was obtained is presented clearly in the Tables (44 to 52).

Pigeon semen rarely averaged over 1 c. mm. in amount, was white in colour when pure, the estimated density of sperm cells, per c.mm. was one to three millions. The spermatozoa showed a morphology similar to fowl sperm, but on visual comparison it appeared that pigeon spermatozoa heads were longer and thinner than fowl spermatozoa. The tails showed thin ends.

The morphological abnormalities which were only observed in semen of one bird, number P. 53, were the curled up heads and irregular staining (which latter may have been due to technical defects).

The semen of fowls being better known and larger in amount could be studied in greater detail. During all seasons over a period of roughly two years 279 samples, collected by the author's method, were examined and the semen was in many cases subjected to specific tests as listed in Table 45. Some of the findings are suitable for comparison with previously published reports in the literature and some may form a basis for future work on the semen of fowls in South Africa.

The following results were obtained:—

(i) The percentage of samples soiled and partly or wholly unsuitable for examination or insemination varied with different males and averaged about one quarter, when all the untrained males were included.

(ii) The volume obtained per collection varied with different cocks at different times, but in not one case did the average exceed 0·75 c.c., which is less than the amount obtained by Burrows and Quinn (1935, 1937, 1939). The total average volume was 0·45 c.c., per collection which compares favourably with the average recorded by Wheeler and Andrews (viz.: 0·41 c.c.) (1943).

(iii) The viscosity of semen also varied in different males, and in the same male at different times. The range of variation was from a watery appearance to a thick oily consistency, estimated to run about a quarter as fast as water through a glass tube of 0·2 cm. bore at room-temperature and atmospheric pressure.

(iv) The colour of pure fowl semen was ivory-white except when very watery, when a slight yellow tinge was observed.

(v) Almost all sperms in the fresh samples examined were found vigorously motile, but a large percentage usually lost all motility in the first day of storage *in vitro*. Only one case of some progressive motility after 96 hour storage at room-temperature was noticed but a weak oscillatory motion of some cells was often noticed even after 10 days' storage under liquid paraffin at room-temperature.

(vi) The density estimated from wet preparations, from dry films and from opacity was lower than the haemocytometer counts and varied between 1·0 and 5·5 millions sperm per c.mm. Two samples counted by milk " Breed-clump-count " method showed a figure of 8·5 millions. The haemocytometer counts were between 1·5 and 7·9 millions of sperms per c.mm. All these values fall within the range reported by Wheeler and Andrews (1943) and Parker, McKenzie and Kempster (1942).

(vii) The pH measurements of semen by means of the " B.D.H. capillator " gave readings of 7·1 to 7·8. Accurate determinations with " Beckman's pH meter " gave readings of 7·0 to 7·74.

(viii) Morphological abnormalities were very rarely observed and only occurred in large numbers in the semen from one cockerel No. 23 and from a relatively infertile but not sterile cock No. 44 The curled up head, the protoplasmic drop and irregular staining all occurred in the case of the latter. The curled up head which was found in the semen of pigeon P. 53 and in semen of cockerel No. 23 (i.e. in young birds) was a very common feature of smears from semen stored *in vitro* which suggests that it may be an abnormality associated with fairly aged cells occurring in the semen.

(ix) The fertility of semen samples inseminated by the new intraperitoneal method was lower in the case of cock No. 6 (Tables 49 and 50) than from samples inseminated *per vaginam*, but the duration and percentage hatchability of the fertile eggs was better. Two out of eight intraperitoneal inseminations with semen from cock No. 34 and five out of 28 with semen from cock No. 38 were successful in producing fertility, whereas three inseminations *per vaginam* from each of these two birds failed to produce any conclusive results. The variation between the results from different cocks was greater than between the results from different methods of insemination in the first series of inseminations.

The dose of semen used in the intraperitoneal operations carried out during the *first* and the *third* series of inseminations was shown to have an effect on the results, as the higher percentage of fertile eggs was obtained from larger doses of semen injected. This finding was particularly accentuated with the mixed samples of semen used (Table 49). Hatchability was apparently not markedly affected (Table 50).

The storage and dilution of fowl semen was in general a failure.

Diluted semen gave only two cases of fertility out of 24 inseminations. One was the 1:1 dilution in fresh egg albumen, giving the results reported in Table 11 (and Table 51 line a) and the other a case in which only a single fertile egg was obtained after dilution with an egg-yolk diluent, (line g, Table 51). Before storage and dilution of fowl semen can be practised, better methods will have to be found, particularly in respect of preparation of diluents for fowl semen.

Stored samples kept by the " Cambridge " method (Walton, 1933) showed a drop in fertilizing power after 60 minutes at room-temperature, i.e. when in fact the cooling had not proceeded sufficiently even to reach room-temperature. This is in agreement with the results reported by Warren and Gish (1943), who found a drop of fertility to 66 per cent. after 5 hours' storage (see Table 52).

(10) *Biological relations between fowl spermatozoa of different origin.*

Crew (1926) noticed that fertility in the fowl hen resulting from a single mating could be reduced both in the length of the fertile period and in the number of eggs fertilized by the effect of a subsequent coitus, i.e., the fresh spermatozoa replaced the older sperm-cells in the fertilization of the ova as they became available. Warren and Kilpatrick (1929) found that sperm of a new cock introduced into a flock of hens after removing the previous male replaced the sperm present in the hens within about 48 hours to a few days i.e. the fresh sperm " superseded " the older in the organs of the hen. Warren and Gish (1943) presented statistical data on the subject.

This phenomenon was observed with intraperitoneal insemination when the recessive coloured hen No. 9 was inseminated by this method with semen from a dominant coloured cock 72 hours after the last previous insemination with semen of a cock of different colour (inseminations Nos. 18 and 19, series 1).

This information is set out in table 53.

TABLE 53.

Spermatozoa aged in the Hen Superseded by Fresh Sperm from a Different Male.

Hours after First Insemination.	Description of Semen Injected.		Number of Eggs laid and Set.	Incubation Results.	Colour of Chick.
	Number of Cock.	Breed of Cock.			
0.....	6	W.L.	—	—	—
43.....	—	—	1	Hatched.....	White.
72.....	18	B.P.R.	—	—	—
90.....	—	—	1	Dead embryo	—
118.....	—	—	1	Hatched.....	Black.
160.....	—	—	1	Hatched.....	Black.
190.....	—	—	1	Hatched.....	Black.

Although several workers had used mixed semen samples in order to offset the effect of low quality fertilizing power of sperm from one male by the higher qualities of other, few tests have been made of the effect of competition between sperm of equal age from different cocks. (Parker, McKenzie and Kempster 1942 b.)

To obtain information on this point the following 57 inseminations in the first series were executed with more than one type of semen, with the results indicated as follows:—

(*i*) Mixed samples of semen from different fowl cocks inseminated into hens by:—

(i) *Intraperitoneal injection*: 31 *cases.*

Eight cases in this group were followed by fertile eggs and in three of these the donor of the semen responsible for fertility could be determined. In the one case 0·25 c.c. from cock No. 22 with a recessive buff colour was mixed fresh with an equal amount from White Leghorn cock No. 6, and injected into the ovarian region of the recessive coloured Rhode Island Red hen No. 16. Eggs laid on the second and the eleventh day after the operation proved fertile and from the latter a white chick was hatched. In the other two cases fresh mixed semen from cocks Nos. 6 and 38 and from Nos. 44, 6 and 38 were used and the spermatozoa from the latter (cock No. 38) fertilized the single egg that hatched in each case.

(ii) *Insemination* per vaginam: *Two cases.*

One of these cases showed fertility but the five fertile eggs failed to develop far enough for determination of paternity.

(*ii*) *Separate samples* of semen from different cocks inseminated into fowl hens by separate operations or matings:—

(i) *Intraperitoneal injections*: *Seven cases.*

All the seven cases were followed by eggs from which chicks were hatched but three results were doubtful. The four cases where paternity could be determined are reported in detail, being of particular interest because conditions were not equal:—

Insemination number 106.—After two intraperitoneal injections of semen within a few minutes of each other into hen No. 9 (Buff Plymouth Rock) she was kept under observation for 24 "hen-days" during which time fourteen eggs were laid. All eggs were set and three proved fertile of which one hatched and two contained dead embryos. The chick was from the egg laid on the eighth day.

The results are given in Table 54.

Insemination No. 107: Hen No. 16 (R. Island Red) was under observation 24 days after two intraperitoneal inseminations at the same time. 16 Eggs were collected, of which 14 could be set and four proved fertile, developing into three chicks and one dead embryo. Table 55 contains the results.

Insemination No. 109: Recessive coloured hen No. 20 (Rhode Island Red) was kept under observation for 32 days after two simultaneous intraperitoneal inseminations. 24 Eggs were set, fifteen proved fertile, three hatched, one chick died in the shell and eleven showed dead embryos. The last fertile egg was laid on the 21st day after the operation and this egg hatched. Table 57 was compiled to show the results.

TABLE 54.

The Results of two Separate Simultaneous Inseminations with Different Semen Samples by the Intraperitoneal Method.

Item.	Description.	
Cocks used	White Leghorn No. 6	Barred Plymouth Rock No. 44.
Amount of semen	0·1 c.c.	0·3 c.c.
Needles used	Wax-coated, coarse, short, sharp, single aperture [Type (xi)]	Wax-coated, fine, long blunt, triple aperture [Type (ix)].
Depth of penetration into abdomen	2·0 cm.	6·0 cm.
Paternity of chicks	Nil chicks.	One chick.

TABLE 55.

The Results of the Separate Intraperitoneal Inseminations with Semen from Different Cocks in one Hen at the Same Time.

Item.	Description.	
Cocks used	White Leghorn No. 6,	Barred Plymouth Rock No. 44.
Amount of semen	0·1 c.c.	0·3 c.c.
Needles used	Wax-coated, coarse, long, sharp, single aperture [Type (vii)]	Wax-coated, coarse, short, sharp, single aperture [Type (xi)].
Depth of penetration into abdomen	6·0 cm.	2·0 cm.
Paternity of chicks	Three chicks.	Nil chicks.

TABLE 56.

The Results of two Separate Intraperitoneal Inseminations at the Same Time with Semen from Different Cocks into one Hen.

Item.	Description.	
Cocks used	White Leghorn No. 6.	Rhode Island Red No. 38.
Amount of semen	0·1 c.c.	0·2 c.c.
Needles used	Wax-coated, fine, long, blunt and triple aperture [Type (ix)]	Wax-coated, coarse, long, sharp, single aperture [Type (vii)].
Depth of penetration into abdomen	6·0 cm.	6·0 cm.
Paternity of chicks	Nil chicks.	Nine chicks.

TABLE 57.

The Results of two Separate Simultaneous Intraperitoneal Inseminations with Semen from Different Cocks into the Same Hen.

Item.	Description.	
Samples	Pure	Mixed.
Cocks used	White Leghorn No. 6.	Rhode Island Red No. 38. Barred Plymouth Rock No. 44. Wyandotte No. 47.
Amount of semen	0·4 c.c.	0·75 c.c. in equal amounts (0·25 c.c.) from each.
Needles used	Wax-coated, fine, long, blunt, triple aperture [Type (ix)]	Wax-coated, coarse, long, sharp, single aperture [Type (vii)].
Depth of penetration into abdomen	6·0 cm.	6·0 cm.
Paternity of chicks	Three chicks.	Nil chicks.

(ii) *Inseminations by different methods*: 17 *Cases.*

Chicks were obtained in twelve cases, but paternity was only established in 11 cases. The results are shortly summarized in Table 58. For details see Appendix B, Table 58.

The results reported here show that in the *first* series of inseminations 18 cases were recorded in which the paternity of chicks was determined, when the eggs were fertilized at a time when the sperms of different cocks were present in the hen. All the eggs of each hen were fertilized by sperms from one cock only, as far as determined. The results of Parker, McKenzie and Kempster, (1942) who concluded, that the fertilization of the ovum by different spermatozoa present in the hen at the same time, was largely a matter of chance, stimulated further work in this direction. In the *third* series, inseminations with large doses of mixed semen were carred out. The results again showed that after both intraperitoneal and *per vaginam* insemination, the chicks produced in each case, were sired by a single male until cock No. 61 was introduced. This bird, (Indian Game) had recessive colour and dominant comb shape (pea comb). When his semen was inseminated mixed with other semen, he supplied in two cases sperms that fertilized some eggs laid after other eggs fertilized by sperms from other cocks, had been produced. (See Table 59.) In one of these two cases, a South African Australorp hen, No. 59, was dosed with 1·2 c.c. of mixed semen from cocks Nos. 6 (S. Combed W. Leghorn), 47 (Wyandotte) and 61 (Indian Game) *per intraperitoneal* injection. The eggs laid on the 3rd and 5th day after insemination were fertilized by sperms from cock No. 6 (white down single comb) and the egg laid on the sixth day was fertilized

by sperm from cock No. 61 (black down, peacomb [line i, column (7), Table 59, Appendix B]. On post-mortem examination, when this hen was sacrificed for location of spermatozoa in the genital organs six days after insemination, she was found to have a double oviduct both sides being fully developed for egg-production. In the second case mixed semen from cocks Nos. 6, 55 and 61 (0·5 c.c. from each) was introduced *per vaginam* into the S.A.A. hen number 60. One egg was laid on the first day and one on the second day after insemination. The former contained an embryo which developed white down and a single comb, the latter black down and a pea-comb. When killed on the sixth day this hen had a normal single oviduct. [Line j, column (7), Table 59, Appendix B.]

One other case is of interest in this connection. In this, mixed semen from cocks Nos. 47 (Wyandotte), 53 (B.P.R.) and 55 (R.I.R.) was given per intraperitoneal injection into hen No. 46 (Buff P.R.) immediately after she was allowed a single natural copulation with cock No. 36 (W.L.). The first day afterwards she laid an egg which proved to have been fertilized by cock number 47 (white down, rose comb) but from the eggs laid between the third and the 22nd day after insemination eight chicks of cock No. 36 (white down, single comb) were obtained. This was considered as a case of simple supersession or replacement of the intraperitoneally introduced sperm, by the naturally inseminated sperm on the arrival of the latter at the upper end of the oviduct [Line i, column (6), Table 59, Appendix B].

TABLE 58.—(See Appendix B, Table 58.)

Summary of Analysis of Simultaneous Inseminations by Different Methods During the First Series.

Line	Description of Type of Test.	Number of Tests.	Result.
	Column (1)	Col. (2)	Column (3)
a	Simultaneous *per vaginam* and intraperitoneal insemination with equal amounts of pure semen from cocks of good and indifferent fertility	5	All fertile eggs consistently sired by male of good fertility, whether inseminated *per vaginam* or by intraperitoneal injection.
b	Simultaneous *per vaginam* and intraperitoneal insemination with larger amounts of semen from cocks of indifferent fertility than from cocks of good fertility	1	All fertile eggs sired by male of good fertility, notwithstanding smaller dose.
c	Naturally mated hens artificially inseminated with semen from cocks of good and indifferent fertility	3	Sperm from cocks of good fertility immediately superseded all other sperm present, but naturally introduced sperm maintained superiority over inseminated sperm from cocks of indifferent fertility.
d	Naturally mated hens inseminated also artificially with mixed semen from males of good and poor fertility	2	Sperm from mixed semen of good and bad quality ineffective in competition with sperm present in the hen after natural mating.

TABLE 59.—(See Appendix B, Table 59.)

Summary of the Results of all Inseminations Performed in the First and Third Series (Doses of Semen delivered unmixed, Separate or Simultaneous in Different Amounts and by Different Methods.)

Line	Class of Dosage.	Number of Cases of Insemination with the Fertile Eggs following all fertilized by identified Sperm.	Percentage of Cases in which Eggs Fertilized by Sperm from one Male only.
	Column (1)	Column (2)	Column (3)
a	Pure semen alone.....	41	Obviously 100%
b	Mixed semen from different males.....	10	80%
c	Separate doses of different samples of semen from different males.....	21	95%

Discussion.—The results of insemination with mixed samples of semen were inferior to those with pure semen (Tables 57 and 58) and provided evidence to suggest that a mixed semen sample tends to be reduced to the quality of the poorest of its components, rather than that the poor quality of a given sample of semen can be offset by admixture with semen of better quality. This fact requires fuller investigation as several workers have made use of mixed semen in artificial insemination work on fowls. (Parker, McKenzie and Kempster, 1940, 1942.)

Except in two instances one of which was a case with a double oviduct and the other a vaginal insemination, all the chicks hatched from each insemination of a mixed sample of semen were the progeny of only one of the males which contributed semen to the sample.

With mixed semen inseminated *per vaginam* into 16 hens Parker *e.a.* (1942) obtained chicks from different sires in every case, but only about half the number of progeny from White Leghorn males as compared with New Hampshire and Barred Rock males when New Hampshire females were used.

Insemination of separate samples of semen by the intraperitoneal method into a hen at the same time showed the following: (*a*) Discharge of the semen in the region of the ovary was more successful in producing fertility than depositing the dose in the peritoneal cavity near the point of entry, even if the latter was larger. (Tables 54 and 55.) (*b*) The coarse needle was more favourable to successful intraperitoneal insemination than the fine needle (Table 56.) (*c*) Pure semen produced fertility although injected through a fine needle, whereas mixed semen injected through a coarse needle into the same bird at the same time, failed (Table 57), even though the mixed semen contained sperms (cock No. 38) which on two occasions had proved superior to the sperms (cock No. 6) in the pure semen sample if these two were inseminated in a state of admixture.

Insemination with separate samples of semen by the different methods into a hen at the same time showed that—

(*a*) the fertilizing quality of semen was more important than the route of introduction in determining which of two kinds of sperm would gain the advantage in the competition for fertilization of ova when present in the hen at the same time (Table 58);

(*b*) the fertilizing quality was also more important than the dose; provided the minimum of 0·1 c.c. was maintained [Tables 57 and 58, (lines f and i)];

(*c*) the superior fertilizing quality of a given semen was lost on admixture with a semen sample not shown to possess such quality (Table 58, lines j and k and Table 57).

(11) *Determination of the site of storage* in vivo *for spermatozoa maintained in the body of the fowl hen during the fertile period after insemination.*

At the time of the first successful intraperitoneal insemination [experiment (4) part (2)] it was decided to plan investigations for determining the tissues and organs in which the spermatozoa were located during the fertile period in the fowl hen. The lack of information and the contradictory views held on the subject have been mentioned in the review of the literature (second chapter) and in preliminary articles (v. Drimmelen; 1945 b and 1946 a).

On the basis of the most recent work, the first experiment was made on a few hens slaughtered about 24 hours after mating or insemination *per vaginam*.

EXPERIMENT (5).

Object.—To determine the sites where spermatozoa may be found in the fowl hen 24 hours after artificial insemination *per vaginam*, after intraperitoneal injection, and after natural mating.

Material.—Four birds were obtained from various sources and killed after an isolation period of one day following different methods of insemination.

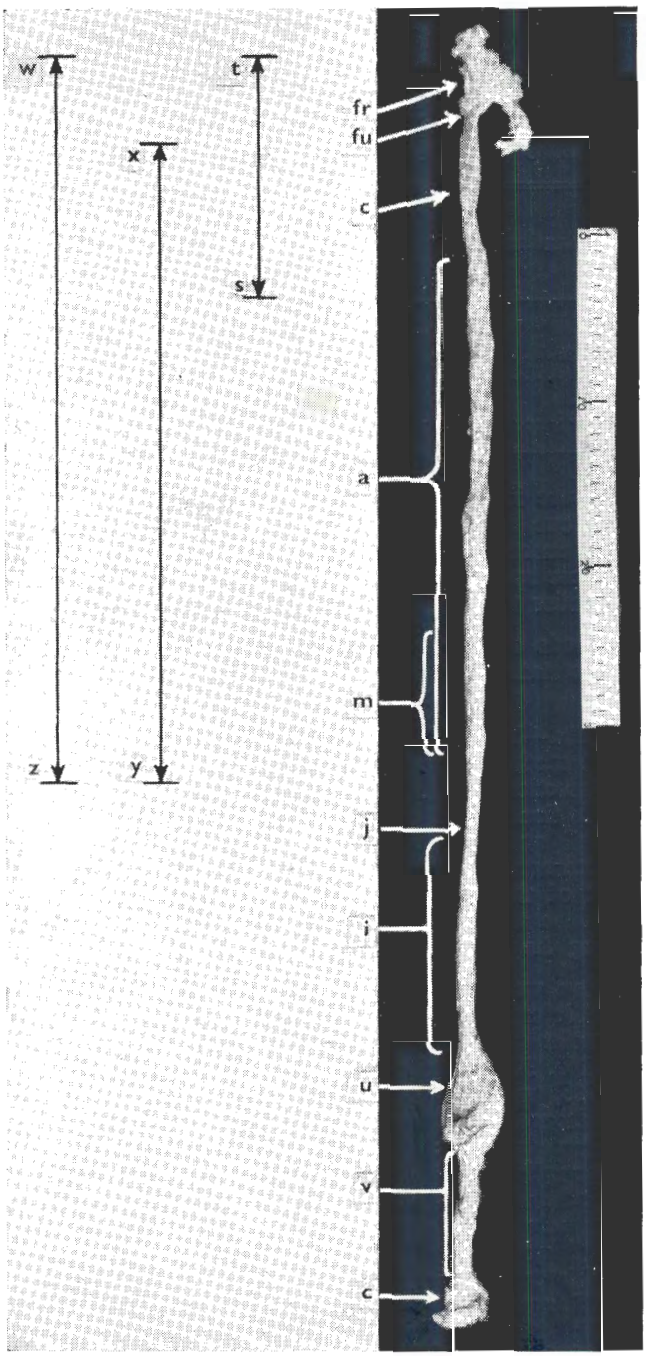
Method.—The fowls were killed by thrusting a small scalpel into the base of the brain through the cleft in the palate, followed by immediate bleeding through the severing of the right jugular vein and the jugular anastomosis, by means of a scalpel introduced via the pharynx and oesophagus. During the killing and bleeding the bird was fastened on her back on a post-mortem table, the head and neck hanging over the edge in a small basin fitted to the side of the table. For examination the skin was removed from the abdomen and pectoral region. The sternum, lower ribs and muscles of the ventral body wall were removed first, followed by the heart, liver and intestines.

For microscopical examination of the tissues in the vicinity of the ovary, and of the mucosa of the oviduct, scrapings were made with the edge of a glass slide and spread on another glass slide in the form of a very thin film, which was dried immediately and later stained. Giemsa's azur-eosin was used for staining and they were stained and examined within a few weeks after the post-mortem preparations were collected.

Results.—The results of the examination of specimens are summarized in Table 60.

The results of experiment (5) were irregular and inconclusive, which was discouraging in view of the expense involved in killing laying hens. Nevertheless experiment (6) was planned to obtain more information on the site of storage of spermatozoa in fertile hens.

FIGURE (xii).—The Genital Tract of the Fowl Hen.



Photograph of a complete genital tract of the fowl : Taken from a bird in active laying condition (No. 939) and stripped of all attachments. Total length: 78 cm. ; (fr) : fimbriae ; (fu) : funnel ; (c) : chalaziferous region ; (a) albumen region ; (m) : mucous region ; (j) : translucent region and junction of albumen secreting part with isthmus ; (i) : isthmus ; (u) : uterus ; (v) vagina ; (c) : cloaca ; (x-y) : " magnum " (Warren and Scott, 1935) ; (w-z) Infundibulum (Ellenberger and Baum, 1939) : (t-s) : Infundibulum (Surface, 1912 ; Bradley, 1928 ; Richardson, 1935).

(Photo by Mr. Th. Meyer, for the Director of Veterinary Services, Onderstepoort.)

EXPERIMENT (6).

Object.—To determine the site of sperm storage in the organs of fowl hens, slaughtered at various times after insemination.

TABLE 60.—(See Appendix C, Table 60.)

The Results of Microscopical Examination of Scrapings from the Genital Organs of Four Hens, 24 hours after Insemination by Different Methods.

Line	Method of Insemination.	Number of Hens.	Localities in which indications of Live Sperm were found.
a	Intraperitoneal insemination.....	1	Infundibulum.
b	Insemination *per vaginam*.....	1	Isthmus, Vagina, Mucous part of Albumen region.
c	Natural mating.....	2	Infundibulum, Vagina, Cloaca.

Material.—Nine birds were included in this investigation, five of which were killed for other purposes, but as the genital organs were not required, the opportunity was grasped to inseminate the hens and to examine the organs at post-mortem. Four birds were specially inseminated and their fertility established before they were killed in order to locate the spermatozoa.

Method.—Various methods of artificial insemination were employed in this series of nine cases, and the hens were not disturbed during the interval before examination.

Post-mortem Examination.—In the case of the first four examinations (hens Nos. 30, 24, 51 and 20) the method of preparation was similar to the procedure for routine post-mortem dissection [Experiment (5)] viz.: the bird was fastened on the table in a supine position, killed by destruction of the brain through the palate and base of the skull and bled by section of the jugulars through the mouth. After death the sternum with pectoral muscles and ventral ribs was removed. The caudal part of the bird was then raised to prevent blood from cut vessels to interfere with the examination of abdominal organs and these were exposed in turn by separating the viscera from the carcase.

Wet and dry preparations were examined in each case and, as reported in a preliminary publication (v. Drimmelen 1945 b) a special technique for the collection of material from moist organ surfaces was evolved. In the first P.M. of this series (23.10.44 hen No. 30) a sterile platinum loop was used applied after the method of Mimura (1939) with a drop of Ringer's solution to each of the surfaces searched for spermatozoa. The material collected was thus a dilution of the natural fluid. A thin Pasteur, bacterial pipette was used in the second P.M. (23.10.44 hen No. 24) also applied with a drop of Ringer's solution. The third and fourth P.M.'s. (19.1.45. hen No. 51 and 26.2.45. hen No. 20) were carried out using pipettes drawn out in a flame to very thin capillary glass tubes but still with the aid of a small quantity of Ringer's solution. From now on the pipettes used were made with capillary points so fine that it was found possible to obtain material of liquid nature in very minute quantities from almost all surfaces and these could be examined in the natural state under a coverslip, and were of course very much better for the preparation of dry films.