STUDIES ON STERILITY IN THE FOWL

by

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INTRODUCTION

The problem of infertility in the domestic fowl has long been one of much concern to the poultryman. In a well-managed flock, the percentage of fertile eggs averages about 88 per cent. This is a direct loss to the hatcheryman as well as the poultry raiser. Every year in the United States losses totaling millions of dollars can be attributed to infertile eggs. A number of possible causes of infertility have been advanced by research workers and practical poultrymen. One of the most common causes is probably the failure of the male to mate or ineffective matings. The production of non-functional sperm by the male or of non-functional eggs by the female could also be contributing factors. Another possible cause may be the rendering of the sperm impotent by the fluids of the oviduct.

Recent work with mammals on sperm morphology and fertility indicates that a direct relationship exists between the two. It has been reported by Moench and Holt (1933) that men of good fertility do not have more than 20 per cent abnormal sperm heads, and sterility is evident in men of over 25 per cent abnormalities. According to Williams and Savage (1927), abnormalities in the semen of normal bulls do not exceed 17. McKenzie and Phillips (1934) found

normal rams not to exhibit over 15 per cent abnormal spermatozoa. The same workers with studies of boar semen demonstrated that the abnormalities of fertile boars ranged from 62 to 104 per 1000 spermatozoa, while boars producing 146 to 501 abnormalities per 1000 were found to be siring small litters containing mummies and weak pigs. The work of Phillips (1935) with boar semen indicated that high incidence of abnormalities in spermatozoa was associated with low fertility.

A technique developed by Burrows and Quinn (1935) makes it possible to easily obtain seminal fluid from the male fowl. The results presented in the study have been made possible largely through this new technique. The problem relative to infertility was approached largely from the standpoint of sperm morphology and concentration.

TECHNIQUE AND METHODS

Method of Obtaining Semen

The method of securing semen recently has been described by Burrows and Quinn (1935). The first technique used, which later proved unsatisfactory, consisted of massaging the soft part of the abdomen a few times until an ejaculatory reflex is set up and the tail is brought down

quickly as in the case of natural copulation. In the last event, semen that is available will be deposited in a receptacle. This method was discarded, since the birds required previous training and handling before satisfactory results could be obtained, and semen was usually accompanied with fecal matter.

The method used is a slight modification of one secured by personal correspondence with Burrows. It consists of one operator holding the bird with the right hand along the right side of the body. The other operator then, with the left hand and the fingers cup-shaped, strokes the back of the bird a few times, the strokes being continued out to the tail region. This motion usually causes a slight eversion of the vent region. At the end of the third or fourth stroke, the left hand is lifted over the tail and, with the index finger and thumb, inward pressure is exerted on each side of the vent, while at the same time considerable pressure is exerted just below the vent by the index finger of the right hand. The stimulations produce an eversion of the rudimentary copulatory organ, and all available semen is deposited. A few repetitions of relaxation and application of the pressure with the fingers in place will sometimes increase the amount of seminal fluid secured. A common tea-

spoon was found to be a very satisfactory receptacle for catching the semen and this is held below the vent in the left hand of the one holding the bird. Instead of lifting the hand over the tail after stimulation, if the thumb and forefinger encircles the base of the tail, pressure may be applied such as to produce the desired effect. Long fingers are an aid in this method. Semen was obtained from some birds without the preliminary stimulation, by only applying pressure as has been previously described. Too much pressure should not be exerted in eversion of the vent region, since one may rupture blood vessels and cause bleeding. As was heretofore intimated, no previous training of the bird is necessary for procuring semen by the above described method. During a visit to a local packing plant, three operators secured samples of semen from 60 males in two hours' time. Not a single male failed to respond to the stimulations.

The expulsion of semen is more or less pulsating in nature; that is, the flow is not regular. Sometimes the seminal fluid is expelled from the copulatory organ in a small stream about the size of the lead in a common pencil, with such force that the stream would be continued for a few inches from the origin. The available seminal fluid at

a single stimulation is probably that carried by the vas deferens and epididymis. The vas deferens each end in the small papillae which are located in the upper wall of the cloaca. The papillae form what is known as the copulatory organ of the fowl.

No breed differences were observed with regard to the ease of obtaining or the quantity of available semen. However, individual differences were evident. Semen could usually be obtained from mature and healthy males. A male which had been kept with females usually would not respond with much if any semen until he had been segregated at least 24 hours.

Obtaining semen from 2 to 4 times at different intervals daily for a period of 6 weeks appeared to have no detrimental effects on the birds.

Method of Artificial Insemination

The method of artificial insemination is that described by Quinn and Burrows (1935). This method consists of exposing the vaginal orifice into the cloaca and the direct injection of seminal fluid into this portion of the oviduct. In exposing the oviduct of the hen, the bird is held against the right side of the operator's body with the fingers of

the left hand under the breast and between the legs. The left thumb is then pressed against the surface of the abdomen below and to the left of the vent. The thumb and index finger of the right hand are slipped over each side of the anal opening and with a slight force and pressing tothether as the downward movement ensues. Considerable pressure is exerted by the thumb of the left hand. These simultaneous motions enable the orifice of the oviduct to be exposed. The orifice appears on the left side of the vent. With practice, the oviduct alone will become everted, leaving the anal opening unexposed in the cloaca. If the vaginal opening is not exposed at the first attempt, it usually could not be accomplished, even by an experienced operator. Prolonged handling of the bird seemed to set up a condition inhibiting eversion. Hens were observed to vary as to ease of everting the cloaca. Only one hen was found in which the orifice of the oviduct could not be exposed. No breed differences were observed to exist. The position of the egg in the oviduct seemed to have no influence on the technique and non-laying hens failed to respond.

In the actual insemination of a hen, a pipette with a slightly curved distal and was found to be the most satisfactory type for introducing the semen into the oviduct. After eversion, the vaginal orifice is kept exposed by the

continued pressure of the hands until the pipette is introduced, after which the pressure is released and retraction of the cloaca permitted.

The pipette is inserted from one-half inch to one inch in the vaginal opening and, if the seminal fluid is discharged rather slowly, there may be no visible evidence of semen at the vaginal opening after the pipette is withdrawn. Frequently, some may be forced out into the cloaca.

Method of Sperm Concentration Studies

A technique relative to this procedure is at its best subject to considerable error. An attempt was made to use a haemocytometer. This device was unsatisfactory because of the inaccuracy of the count resulting from the small size of the spermatozoa. All of the spermatozoa could not be seen in a single focus and refocusing was a source of error. Hutt (1929) used the haemocytometer without mentioning this difficulty. An attempt was made to compare the sperm concentration in a considerable number of males, and the samples of semen were so taken that some measurement could be made of the sperm concentration in a unit of seminal fluid. Two samples from each male were taken at two-day intervals. The semen was well mixed before sampling by considerable agitation. The pipette used was a .2 cc. one callibrated in

millimeters. Twenty cubic millimeters of semen were transferred to a small glass vial. To this was added 200 cu. mm. of a 5 per cent solution of rose bengal. The rose bengal stain was prepared by the addition of 95 per cent Ringers (for birds) solution. It was found that a high concentration of stain prevents an even distribution of spermatozoa on the slides. The stain and semen were thoroughly mixed by forcing air into the solution through the pipette. Ten cubic millimeters of the diluted semen were transferred in the form of a drop and placed near the end of a slide. The end of another slide was placed on this drop and pushed across the length of the first slide. Capillary attraction caused the fluid to distribute itself evenly between the two slides. The operation was repeated, beginning at the end opposite that upon which the drop was placed. The same operation was repeated in an effort to get an even distribution of the semen. The slides were then passed over a gas flame a few times. The taking of two different samples from the same male, plus a thorough mixing of the stain and seminal fluid, together with an attempt to secure an even distribution of the fluid over four slides were believed to give a fairly dependable basis for comparison of sperm concentration in the seminal fluid of different males.

In order to provide a standard for counting sperm over

a uniform area of the slide, a slotted cardboard disc was used in the eye piece to reduce the field to a width of 0.18 millimeter. A mechanical stage was used to draw an area in the middle of the slide across the field of vision. The spermatozoan heads visible in the 0.18 millimeter strip extending across the slide were counted for determining the relative sperm concentration in the semen. The ocular used was a 12.5x and the high, dry objective was .66. In order to prevent a personal bias in the counting, slides were taken at random from the slide box.

In most cases, the spermatozoa were not badly clumped, although this was probably one of the greatest sources of error in sperm counts. The method of fixation did not result in a good preservation of the form of spermatozoa, but this was not necessary, since accurate identification only was desired.

Method of Sperm Morphology Studies

In order to make studies of the morphology of the spermatozoa in relation to fertility, it was necessary to utilize a technique whereby a dependable and rapid fixation was secured. The following method was adopted after trying out a number of different ones:

A small quantity of semen varying from .1 to .22 cc.

was placed on the slide. This was diluted with Ringers solution (for birds) to the extent of at least 3 to 1. The slide was placed in osmic acid vapors for about one and one-half hours. This method of fixation seemed to be the most satisfactory; however, occasional overfixation resulted in rupturing the sperm heads. After fixation, the following procedure by McKenzie (personal correspondence) was used in staining:

1. Clear with 1 per cent chlorazene for 10 minutes.
2. Wash in distilled water.
3. Stain with carbol fuchsin (9 parts of 5 per cent phenal and 1 part fuchsin).
4. Wash in tap water.
5. Dry and cover.

All morphology studies were made using the oil immersion lens.

DESCRIPTION OF NORMAL SPERMATOZOA

The normal spermatozoan is characterized by a typically long cylindrical, moderately pointed head, and a long lash-like tail. As was found by Adamstone and Card (1934) the posterior end of the head merges almost imperceptibly into a long tapering tail. There is seemingly no line or point of demarcation between the head and tail. In studies

by Adamstone and Card, the head was described as possessing a somewhat recurved acrosome. From staining tests with a dilution of rose bengal stain, as was previously described, the head appeared to be composed of three distinct regions, the acrosome taking a dark stain, as well as about an equal length joining the tail, while the major part of the head was only slightly stained. Guyer (1909) mentions that the head proper contains a series of very minute, highly refractile bodies arranged like a string of beads along its entire length. The recent work of Adamstone and Card (1934) substantiates this observation.

According to Payne (1914) the tail measures from 12 to 15 times the length of the body. The figures of Warren and Kilpatrick (1929) also show about the same proportionate length. For a large number of measurements, the length of the head of the spermatozoa ranged from .0175 to .0210 millimeters, which is approximately one-fifth the length of the tail. The entire length of the spermatozoa was found to vary from .0875 to .0963 millimeters. The photomicrograph, Figure 1, shows this relationship to exist.

The movement of spermatozoa is facilitated through the coordination of its parts. In the morphological studies, a number of spermatozoa appeared to have been fixed in motion. That is, the tail was bent in a series of wave-like motions.

Figures 1 and 2.

Explanation of Figure 1

Spermatozoa from a male giving normal fertility.

Explanation of Figure 2

Spermatozoa from a sterile male.

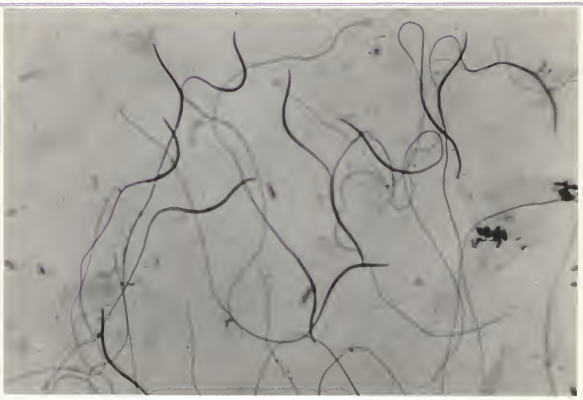

Figure 1

Figure 2

In a few instances, the head was fixed in somewhat the same manner, but to a lesser degree. The latter condition perhaps would indicate that the head also possesses the power of wave-like movements to aid in the migration through the oviduct.

The ejaculate from a normal male is characteristically chalky white in color. The dried fluid on a glass exhibits a crystalline appearance. The ejaculate (Ishikawa, 1930) is odorless and alkaline to phenolphthalein and litmus.

DENSITY OF SPERM SUSPENSION

In previous work, the semen for concentration study has been secured either by interception at copulation or by a recovery of the seminal fluid from the cloacal region. The latter method, as has been used by Payne (1914), and Craft, McElroy and Penquite (1926), is subject to some inaccuracy due to the dilution of semen by fluid in the cloaca. Payne found the density of sperm suspension in the semen of 5 different cocks to range from 1,920,000 to 5,470,000 per cubic millimeter with an average of 2,228,000. Craft, McElroy and Penquite noted a count range from 2,000 to 4,000,000 per cubic millimeter. Butt (1929), in a concentration study of semen from 10 birds, observed an average count from 825,000 to 7,328,500 with an average of 3,998,642

per cubic millimeter. Ishikawa (1930) observed a count range of from 115,000 to 437,500 per cubic millimeter.

In this study, 4 counts were made from each of 68 males. The males had been isolated from females for at least 2 days and in most cases for a considerably longer period. The males were kept in individual batteries while being studied. The counts were considered as the number of sperm seen through the rectangular disc as the slide was passed under the lens. To convert the count in sperm per cubic millimeter, the factor 930 is multiplied by the average count. The derivation of this factor consisted in dividing the length of the slide in millimeters, which is 76.2, by .18 millimeters, the width of the strip covered in counting. This result, which is 423, is divided by the amount of semen spread on one slide, or .45455 cu. mm., and the quotient represents the fractional part (1/930) of a cubic millimeter of semen utilized in making the count. The amount of semen used for dilution with 200 cu. mm. of Ringer's solution was 20 cu. mm. Since 10 cu. mm. of the solution of suspended sperm, or .9090 cu. mm. of pure semen, was spread evenly on the two slides, then on one slide there would be half that amount, or .4545 cu. mm.

Table 1, giving the average counts from samples of semen, shows a considerable variation in concentration for

Table 1. Average counts for 69 males of the number of sperm in an area .18 mm. wide extending across a slide

Male No.	Breed	Count A	Count B	Male No.	Breed	Count A	Count B
396	**1	1018	572	1350M	1	1250	2555
*567	1	612	341	1331M	1	12	141
945	3	121	139	1326M	1	712	717
1160M	3	685	409	1271M	1	758	442
1198M	1	313	543	1245M	3	160	205
1213	3	365	255	1227M	1	526	189
1222	1	50	193	856	1	657	680
*1240	1	1274	520	953	3	304	330
1264	1	55	534	*803	1	533	623
1272M	3	98	420	682	3	570	415
1275M	1	538	677	627	1	305	523
*1299M	1	360	86	550	1	624	801
1321M	1	525	1169	6773	3	541	1220
1355M	1	385	843	5514	1	520	805
*1382M	3	401	130	5513	1	198	1010
*1391M	3	673	1595	5400	1	825	514
1762	3	337	980	5288	2	191	548
1722	3	149	1322	4369	1	962	854
1781	3	757	533	3825	1	923	1670
*2155	1	302	794	*1376M	1	721	543
*2660	1	382	275	1381M	1	67	160
*3058	1	278	437	1393M	2	103	428
3818	1	261	1930	*1402M	1	215	110
3511	3	349	372	2613	1	452	119
*1374M	4	162	876	2714	3	103	219
*1364M	4	408	804	3545	3	1132	95
*1363M	4	404	555	4051	7	1835	788
1352M	1	148	104	4507	1	312	531
*4971	1	1353	385	4971M	1	638	2162
5249	1	852	52	1362	1	272	384
1377M	1	383	722	*1369M	1	302	424
67878	3	76	13	*32549M	2	139	536
*3550	3	159	338	1756	1	351	686
1231M	1	85	186	*1386M	3	536	355
*1405M	2	654	535				

*Males that were tested for fertility
**1 - Crossbred; 2 - White Leghorn; 3 - Rhode Island Red; 4 - White Wyandotte

different males as well as variation in the different counts from the same male. This variation may be attributed to the amount of actual seminal fluid secreted with the spermatozoa, the inaccuracy of the technique, or even the actual variation of the male. It appears that within rather wide limits fertility is not associated with density of sperm suspension. Those numbers preceded by an asterisk were tested and found to give good fertility. Hutt (1929), in a careful study of this factor, found that sperm suspension ranging from 825,960 per cubic millimeter to nine times that figure has no relation to fertility. Walton (1927) found that, with rabbits, a sperm suspension of less than 1,000,000 in 3 cc. resulted in reduced fertility and, that below 10,000 in 3 cc., fertility did not occur.

The mean of the average counts of 69 males was 541. The results were analyzed on the basis of the month of taking the sample. The mean of 30 counts taken from 15 males in February was 406; 82 counts taken from 41 males in March was 557; 20 counts taken from 10 males in April was 729; 6 counts taken from 3 males in May was 380. These results suggest that March and April are the months of greater concentration of sperm in the seminal fluid. However, the amount of available semen was not recorded.

SPERM MORPHOLOGY IN RELATION TO STERILITY IN MALES

In this phase of the problem, an attempt was made to determine the influence of sperm abnormalities on fertility. It has been demonstrated that sperm abnormalities and low fertility have been associated in the ram, bull, boar and man. Semen samples from 86 males were studied. The examination period extended from January to May. Two slides were made from the semen of each male. A number of different areas over the slides were examined. No seasonal influences were noted, and no consistent differences in morphology of spermatozoa of young and older males were found. In the cases studied, normal fertility was secured in the males which averaged from 4 to 8 abnormal spermatozoa per 100. Perhaps the question may be raised as to what percentage of the observed abnormalities, if any, is due to the fixation procedure. However, due to the types of abnormalities observed, it seemed safe to assume that these conditions were not caused by fixation technique. Various samples of semen taken from the same male on different days were very uniform as to the percentages of abnormalities. The main types of abnormalities observed were those found in the sperm head. It thus appears, and is quite reasonable to assume, that the normality of the sperm head will indicate the degree of

potential fertility. It also was demonstrated in man by Moench and Holt (1933), in the bull by Williams and Savage (1927), and in the ram by McKenzie (1934) that the condition of the sperm head is the most important criterion in judging the reproductive potentialities of semen. In the semen of normal fertility males, the chief types of abnormalities appeared to be tailless heads, coiled tails, and blunt heads.

The most striking case of infertility was found in a crossbred male which, in natural matings, had given only one fertile egg in 30, from 5 females and, with the artificial insemination method, no fertile eggs out of 26, from 3 different hens. The artificial inseminations were made every other day. Other males were tested at the same time and fertility ranging from 70 to 90 per cent was secured. A morphological study of this male's spermatozoa revealed characteristic abnormalities. The bird was in good condition at all times. His ejaculate was invariably of a semi-chalky color and watery consistency. The abnormalities of the spermatozoa are listed as follows in the order of decreasing incidence:

1. Head bent in form of loop.
2. Extreme clumping.
3. Blunt heads.
4. Short and broken heads.

5. Coiled tails.
6. Swelling at base of head.
7. Sharp constriction between head and tail.
8. Head bent in a "C" shape.
9. Small immature sperms.
10. Broken tails.

In the photomicrograph, which shows a field of the sperm complex of this male, one can distinctly see the extreme clumping of the spermatozoa and several types of abnormalities. Eighteen seminal observations of this male were made over a period of 4 months and each examination revealed the consistent abnormalities. Twelve sperm concentration counts were made of this male. The average of 12 counts was 113 per strip or 105,090 per cubic millimeter. This is a comparatively low average and, since these sperm abnormalities are estimated to range from 20 to 25 per cent of the total, there would exist considerably fewer functioning spermatozoa as compared to a normal male of the same concentration. From these observations, it would appear that in this case of sterility, low concentration is accompanied by a high percentage of sperm abnormalities.

Since the concentration from a non-chalky ejaculate was found invariably to run quite low, it is suggested that an association also exists between non-chalky ejaculates and a

relatively high percentage of sperm abnormalities. However, enough cases were not found to thoroughly substantiate this observation.

OTHER CAUSES OF STERILITY IN MALES

It is recognized that there are external factors which contribute to low fertility. Extremely cold weather for a period of a few days in the late winter or early spring is considered by hatcherymen as a cause of low fertility through lack of mating. It is also thought that fertility decreases during the late summer or early fall. This likewise probably can be attributed to the lack of mating instinct.

Dove (1928) noted that three out of four males, that possessed a diminutive copulatory organ, proved sterile or sexually abnormal. He also found no correlation between size of the copulatory organ and fertility.

Quinn and Burrows (1938) pointed out that when the body weight of the larger mate becomes much greater than twice that of the smaller mate, a considerable decrease in fertility was noted. These workers also found that no fertility was obtained in natural matings when the body weight of the larger mate was about four times as great as that of the smaller.

Hutt (1929) showed, with eleven cocks, that within quite wide limits, fertility of the male is not correlated upon size of testes. Hutt also cited the instance of low fertility as caused by a mild cloacitis of the male.

Wyandottes have long been regarded by poultrymen as a low fertility breed. From a series of observations of Wyandotte matings, it appeared that approximately 20 per cent were ineffective. Natural matings produced, in this pen, a fertility percentage from 30 to 70 per cent. These males, when artificially tested, produced a fertility percentage ranging from 74 to 100. The insemination test from one of the males produced 14 fertile out of 15 eggs, another 8 out of 11 and the third male only two eggs were produced, but both were fertile. Both the breeding pen matings and the artificial insemination tests were studied during rather severe weather in January and February. Sperm concentration counts of the Wyandotte males averaged 518, which is slightly above the mean of all males studied. Examination of the spermatozoa from Wyandotte males did not reveal any abnormal conditions. The cause of low fertility in Wyandottes may be the result of excessive fluff development on the abdomen. At least the studies on sperm morphology, concentration and artificial insemination indicated that the semen was entirely normal.

Another case of infertility was found in a pen mating of White Leghorns. The male gave a 50 per cent fertility with artificial insemination tests and, at a later date, produced good fertility with natural matings. This male was only in fair condition during the first mating and insemination tests. This might indicate the cause of the low sterility in the natural mating as due to a failure to mate. Another case of periodical sterility was that observed in a crossbred mating. The concentration of spermatozoa as well as morphology was normal. This male, when placed back in the pen about two weeks later, produced good fertility.

It appears that sterility in the male is sometimes periodical. Other possible causes of sterility might be described as too frequent matings, pathological condition of the reproductive organs, and lack of mating instinct.

STERILITY IN FEMALES

It is a possibility that the female is responsible, in some cases, for infertility of eggs. As was heretofore mentioned, the lack of mating instinct on the part of the female, the possession of external characteristics such as excessive fluff, the repulsion of certain males, and the rendering of the spermatozoa non-functional through secretions in the genital tract might be contributing factors of

low fertility. Lamson and Card (1920) point out the individuality of hens as being the prime factor in determining the fertility of eggs from a flock.

Hinchaugh (1932), in an examination of 582 individual matings of White Wyandottes, found that if the females were grouped according to their percentage fertility, 54 per cent show fertilities over 85 per cent, while 10 per cent are under 5 per cent and the remainder (35 per cent) vary from 5 per cent to 85 per cent. This might be interpreted, at least in these cases, that the general lower fertility of Wyandottes is caused by very low fertility of a few females.

In the spring of 1934, on the Kansas State College Poultry Farm, 21 females were found that, from natural matings, had given low or no fertility. These females had been kept in matings where other females had given good fertility, indicating that the male was not at fault. Table 2 gives the detail results from these hens in natural matings and artificial insemination tests. As can be seen from the table, when artificially inseminated, 15 out of the 21 gave a high percentage of fertility. They were inseminated in most cases with semen from the same male to which they previously had been mated, or at least with one of proved fertility. Four of the hens gave fertility percentages from 5 to 30, which was about the same as secured in natural

Table 2. Insemination with sterile and low fertility females

Hen number	Natural mating			Artificial insemination		
	Fertile	Infertile	Dead embryos	Fertile	Infertile	Dead embryos
White Leghorn						
3761	5	25	0	14	24	0
3521	1	14	0	8	2	0
3706	6	34	2	12	3	0
3360	1	23	1	11	1	0
3723	1	21	1	1	19	0
3799	0	28	0	7	3	0
3749	3	24	0	2	8	0
3762	4	17	0	1	0	0
3679	2	29	0	0	6	0
3760	0	27	0	5	14	0
3595	6	23	2	6	5	0
3434	8	16	0	3	1	0
1250	1	19	0	10	1	0
1306	8	35	1	10	2	0
2068	6	34	1	11	2	0
2089	1	26	3	0	10	0
Rhode Island Red						
1597	0	25	0	12	1	0
1525	1	21	0	4	2	0
Crossbred						
336	0	43	0	3	8	0
44	2	36	0	14	2	0
216	2	32	1	13	4	0

matings. The remaining two females, which were both White Leghorns, gave in natural matings fertility percentages of 6 and 13 per cent, and when artificial insemination was used, produced no fertility. It appeared that these females produced eggs which could not be fertilized. These hens went out of production after laying 6 and 11 eggs, respectively.

During the last half of the artificial insemination tests of hens 3761, 3723 and 3760, a Rhode Island Red male was substituted for the White Leghorn. There was no change of fertility in hens 3760 and 3723, but in the case of 3761 the fertility rose from 21 per cent to 90 per cent. The results from hen 3761 suggest that one male's sperm may function where another apparently normal male's sperm have failed to do so.

Another very interesting instance was that observed in a Rhode Island Red hen which, in natural mating, gave no fertile eggs out of 25 and with artificial insemination produced a fertility percentage of 86. Since this hen possessed a drooped tail, it is assumed that the deformity interfered with effective copulation.

In a number of instances, natural matings gave either no or extremely low fertility and with artificial insemination the fertility ranged from 75 to 91 percent. This

would indicate either a lack of mating instinct or a failure to mate successfully, as the causes of infertility.

It is also possible that cases of supposedly infertile egg production were caused by very early embryonic mortality which could not be detected without microscopic aid. The chance of a lethal character appearing in the formation of the gametes cannot be ignored.

COMPETITIVE ACTION OF SPERMATOZOA

It has been suggested that selective fertilization occurs, the advantage being in the case of sperm meeting eggs from mates of his own breed. Crew (1926), in a study of this problem using a Leghorn male and a somewhat inactive Redcap male, observed that the majority of the chicks were sired by the Leghorn male. The 2 males were used on 6 Leghorn females during alternate half days. This work, however, is not conclusive, due to the small number of females, the extreme differences in vigor of the males, and the unreliability of natural copulation. Curtis and Lambert (1928) noted with comparatively meager data from Rhode Island Red and Plymouth Rock birds a slight indication of selective fertilization. All tests heretofore reported were made through the use of natural matings. The chief difficulty arises from preferential mating, which cannot be

controlled in the use of this method. The development of the method of artificial insemination provided a more exact technique for the study of competitive sperm action.

In this study, 10 Rhode Island Red and 10 White Leghorn females were used. The two males, which were slightly over one year of age, were both normal and vigorous representatives of the breed. The sperm concentration count resulting from samples taken from the White Leghorn male averaged 811 while the Rhode Island Red male averaged 508. All birds were kept in individual laying batteries throughout the experiment. The females were inseminated at intervals varying from daily, every other day, and every two or three days. The purpose of varying the interval between insemination was to determine approximately the length of interval that would give the highest fertility. The hens were arbitrarily divided into three series, each series containing three hens of each breed. The inseminations were somewhat irregular the first half of the study, while during the last half, two series or 12 birds were inseminated daily.

The average ejaculate from each male at one time was approximately .6 cc. A .2 cc. pipette was used in measuring and transferring semen from the spoon to a small glass vial. The seminal fluid from the two males was transferred alternately as long as equal amounts remained. The semen of the

Leghorn and Rhode Island Red male was thoroughly mixed by forcing air through the pipette. Each female thus received approximately .2 cc. of the mixture. A microscopic examination of the mixed seminal fluids did not reveal any abnormalities that might be incurred in mixing.

The eggs were hatched in individual pedigree bags. The sire identification of the chicks was determined by the factor interaction of red and dominant white and early and late feathering of the chicks. The results summarized in Table 3 clearly indicate the preponderance of chicks sired by the White Leghorn male. As was indicated heretofore, the density of the spermatozoa from the White Leghorn male averaged 811, while the Rhode Island Red male averaged 503. If the chances of fertilization were corrected, taking into consideration the concentration differences, there would still remain an unaccounted for preponderance of Leghorn male offspring. Since over 85 per cent of the chicks were sired by the White Leghorn, it is suggestive of a higher activity of spermatozoa of this male.

The fact that 88 per cent of the chicks from the Rhode Island Red females and 83 per cent of the chicks from White Leghorn females were sired by the Leghorn male would indicate that selective fertilization did not occur.

Table 3. Results of competitive sperm studies

Hen No.	Chicks sired by W. Leghorn male	Chicks sired by R. I. Red male	Dead embryos	Infertiles
R. I. Red				
1515	15	2	2	2
1475	0	2	0	0
1351	5	0	3	2
1512	13	0	4	2
1442	11	2	3	2
1482	7	2	1	2
1462	13	2	8	3
1464	12	1	0	2
1454	1	0	0	3
1541	3	0	0	0
Total	80	11	21	18
W. Leghorn				
3328	3	3	2	1
3029	4	0	17	0
2068	6	3	4	0
3347	9	0	4	1
3336	7	2	11	2
3344	7	1	6	1
3356	5	0	0	5
3386	2	1	13	3
3303	0	0	1	17
3354	5	0	0	0
Total	48	10	58	30
GRAND TOTAL	128	21	79	48

AGE OF MALE IN RELATION TO FERTILITY

It has usually been recommended that growing cockerels be separated from the pullets quite early, as well as to be isolated from the laying flock. The purpose of this recommendation, among other things, is to insure infertile eggs. An examination of the literature reveals no critical studies on the approximate age at which cockerels are capable of fertilizing eggs. Phillips (1935), in studies with young Hereford bulls, found that the testes of this animal produced spermatozoa between 26 and 32 weeks of age.

This problem was approached by attempting to obtain seminal fluid from the Leghorn cockerels at as early an age as possible. From only one cockerel, out of approximately 50 cockerels examined, could seminal fluid be obtained at 8 weeks of age. The ejaculate of this cockerel was of about one-half normal as judged by color and viscosity. A microscopical examination revealed a very low concentration as well as a high percentage of abnormal spermatozoa. This cockerel showed somewhat precocious sexual development as evidenced by the comb and plumage.

Insemination tests with this cockerel on White Leghorn hens beginning at the 8 weeks' age did not result in any fertility until 9.5 weeks of age. Three other White Leghorn

cockerels were tested, but no fertility was obtained from the ejaculate up to 11 weeks of age. Successive semen samples from this cockerel approached normality and, at the eleventh week of age, semen obtained was normal in all visible respects.

Semen was secured from other White Leghorn cockerels, beginning usually at about 9.5 weeks of age. In most cases, normality was reached by at least 12 to 14 weeks of age. The concentration count of semen from 12 weeks old cockerels showed a normal density of sperm suspension as compared to mature cocks. Two eleven-weeks old cockerels were placed in a pen with 4 hens to observe mating. It appeared that all early attempts of the cockerels to mate were futile. However, 3 out of 6 eggs laid during the thirteenth week were fertile. This would indicate that Leghorn cockerels begin to reach normal reproductive ability at 12 weeks of age.

It is usually found by poultrymen and breeders that fertility of the male decreases with age, especially after the second year. Practical experience has often demonstrated that the use of old males results in low fertility. Jull (1935) demonstrated that White Leghorns and Rhode Island Red cockerels were higher in fertility in every case than cocks. However, the results were not significant as a whole in White Leghorn comparisons, but were in the case of

the Rhode Island Red. In the study of density of sperm suspension, cock birds, two years of age or older, compared very favorably with younger cock birds. No noticeable differences were found in a comparison of the sperm morphology as well as the amount, viscosity, and color of the ejaculate of one and two year old cocks. If it is true that old cocks give significantly lower fertility than young cocks, then perhaps it would be justifiable to consider the difference due either to the physiological inefficiency of the spermatozoa or to the failure to mate, or ineffective matings.

EFFECT OF REPEATED STIMULATIONS

It has been shown by Hutt (1929) that spermatozoa counts following sexual activity were reduced. Lewis (1911), in studies with the stallion, observed also a progressive decrease in the amount of semen and number of spermatozoa following successive services. Penquite, Craft and Thompson (1930) showed that there was a tendency for the concentration of sperm to be reduced following successive matings.

In this study, two Rhode Island Red and two White Leghorn cocks were used. All the birds were slightly over one year old and in good condition. The birds were stimulated at hourly intervals, beginning at 8 o'clock in the morning and continuing until 6 o'clock in the evening. All the

semen available was taken from the bird at one time. Concentration counts were made every two hours. As is noted in Table 4, the amount of ejaculate from Leghorn male 933 is comparatively small. The only explanation offered for variation is that this male had not been used for insemination work previously, while the other three males had been used as such up to two weeks before the test. The first two ejaculates from all the males were normal except the second ejaculate from No. 933 male, which was considerably less viscous. All samples of semen as indicated above were estimated to be from 60 to 80 per cent normal in viscosity. The same method of sperm counting was used as in the previous concentration studies. These results seem to indicate that from hourly stimualtions there is a progressive decrease in amount of ejaculate, but not such a consistent or comparable decrease in the amount of sperm in suspension.

In work on artificial insemination with males No. 1035 and No. 1404 prior to this study, it was found that three semen samples of normal viscosity ranging in volume from .4 to .6 cc. each could be secured at morning, noon, and evening.

DURATION OF FERTILITY

This problem has been investigated by a large number of workers in the case of natural matings. Curtis and Lambert

Table 4. Results of successive stimulations on the amount of ejaculate and concentration of spermatozoa

TIME	R.I.Red male 1404		R.I.Red male 3550		W.Leghorn male 1035		W.Leghorn male 933	
	Amount of ejaculate per cc.	Average sperm count	Amount of ejaculate per cc.	Average sperm count	Amount of ejaculate per cc.	Average sperm count	Amount of ejaculate per cc.	Average sperm count
8 a.m.	.2	770	.2	460	.4	439	.15	1733
9 a.m.	.3		.35		.35		.05	
10 a.m.	.2	203	.4	288	.35	226	.05	62
11 a.m.	.2		.35		.2		.07	
12 a.m.	.2	213	.2	490	.25	198	.03	106
1 p.m.	.2		.35		.38		.03	
2 p.m.	.18	120	.16	104	.23	120	.00	
3 p.m.	.18		.23		.2		.00	
4 p.m.	.23	102	.15	169	.2	121	.00	
5 p.m.	.1		.05		.13		.00	
6 p.m.	.1	203	.18	310	.15	124	.03	122

(1928) observed that the mean duration of fertility was about 11 days with 21 days as the longest duration. Other workers report periods varying from 5 to 19 days. Dunn (1927) obtained one fertile egg 30 days after the removal of the male. Jull (1930) concluded that reasonably good fertility may be expected for about 2 weeks after the removal of the male from the flock.

The results of using the artificial insemination method showed a duration of fertility ranging from 5 to 18 days. This was based upon the time of the last insemination. The amount of ejaculate varies from .2 cc. to .6 cc. with each insemination. Inseminations were practiced about every other day.

SUMMARY

A recently developed method of obtaining seminal fluid from the male bird and artificial insemination of the female gave very satisfactory results. Artificial insemination of females every other day with .2 cc. of semen will produce good fertility.

The normal spermatozoan averages approximately .09 mm. in length. The long narrow tail is from 4 to 5 times the length of the head.

The density of sperm suspension was found to be quite

variable among males and samples of the same male. No association was found to exist between density of sperm suspension and fertility.

Morphological abnormalities in spermatozoa of normal fertility males range from nil to approximately 8 per cent. Low concentration plus abnormalities above 20 per cent resulted in sterility in one male.

Extreme clumping of the spermatozoa, hooked, broken and blunt heads were the most common types of abnormalities of the spermatozoa of the sterile male.

Other cases of sterility in males seemed to be due to lack of mating instinct and ineffective matings. Wyandotte males giving poor fertility were found to be normal in density of sperm suspension and morphology of the spermatozoa.

Observed cases of sterility or low fertility in the female were usually the result of failure to mate successfully, since artificial insemination usually produced good fertility. Some females produce eggs which could not be fertilized.

Selective fertilization did not occur in recripocal crosses of Rhode Island Reds and White Leghorns.

Fertile eggs were produced by artificial insemination from a White Leghorn cockerel of 9.5 weeks age, and from natural matings between 12 and 13 weeks of age.

Successive hourly stimulations of the male resulted in a decreasing amount of ejaculate, but not a corresponding decrease in the density of sperm suspension.

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LITERATURE CITED

Adamstone, F. B., and Card, L. E.
A study of the spermatozoan of the fowl with particular reference to osmiophilic bodies in the sperm head. Jour. Morph. 56: 325-337. 1934.

Burrows, W. H., and Quinn, J. P.
A method of obtaining spermatozoa from the domestic fowl. Poultry Sci. 14: 251-253. 1935.

Craft, W. A., McElroy, C. H., and Penquite, Robert.
The influence of certain feeds upon the production of spermatozoa by the domestic chicken. Poultry Sci. 5: 187-196. 1926.

Crew, F. A.
On fertility in the domestic fowl. Roy. Soc. Edinb. Proc. 46: 230-238. 1926.

Curtis, V., and Lambert, W. V.
A study of fertility in poultry. Poultry Sci. 8: 142-150. 1928.

Dove, W. F.
Sex sterility and the diminutive copulatory organ in the domestic fowl. Science 68: 327. 1928.

Dunn, L. C.
Selective fertilization in fowls. Poultry Sci. 6: 201-214. 1927.

Guyer, M. F.
The spermatogenesis of the domestic chicken. Anat. Anz. 34: 573-580. 1909.

Hindhaugh, S. L. S.
Some observations on fertility in White Wyandottes. Internatl. Rev. Poultry Sci. 6: 37. 1932.

Hutt, F. B.
On the relation of fertility in fowls to the amount of testicular material and density of sperm suspension. Roy. Soc. Edinb. Proc. 49: 102-117. 1929.

Ishikawa, Ryozaburo.
The life duration of cock spermatozoa outside the body. Proc. of the 4th World's Poultry Congress. p. 90-94. 1930.

Jull, Morley A.
Poultry husbandry. New York. McGraw-Hill. 639p. 1930. (Ref. p. 150).

_____ Studies in fertility in the domestic fowl. Poultry Sci. 14: 37-41. 1935.

Lamson, G. H. Jr., and Card, L. E.
Factors in incubation. Storrs Agr. Expt. Sta. Bul. 105, p. 4-35. 1920.

Lewis, L. L.
The vitality of reproductive cells. Okla. Agr. Expt. Sta. Bul. 96, 48p. 1911.

McKenzie, F. F., and Phillips, R. W.
Measuring fertility in the ram. Jour. Amer. Vet. Med. Assoc. 84: 189-202. 1934.

Moench, G. L., and Holt, Helen.
Sperm morphology in relation to fertility. Biol. Abs. 7: 869. 1933.

Payne, L. F.
Vitality and activity of sperm cells and artificial insemination of the chicken. Okla. Expt. Sta. Circ. 30. 8p. 1914.

Penquite, R., Craft, W. A., and Thompson, R. B.
Variation in activity and production of spermatozoa by White Leghorn males. Poultry Sci. 9: 247-256. 1930.

Phillips, R. W.
The physiology of spermatozoa. Proc. Amer. Soc. Anim. Prod. p. 222-235. 1935.

Quinn, J. P., and Burrows, W. H.
Artificial insemination in fowls. Jour. Heredity. 27: 31-37. 1936.

Walton, A.
The relation between density of sperm suspension and fertility as determined by artificial insemination of rabbits. Roy. Soc. Edinb. Proc. 101: 303-315. 1927.

Warren, D. C., and Kilpatrick, L.
Fertilization in the domestic fowl. Poultry Sci. 8: 237-256. 1929.

Williams, W. W., and Savage, A.
Methods of determining the reproductive health and fertility of bulls. Cornell Vet. 17: 374-385. 1927.