

Experimental Studies on the Artificial Insemination of Domestic Ducks with Special Reference to the Production of Mule-Ducks

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I. INTRODUCTION

Duck raising in Japan is a relatively small industry. Its relative smallness, however, does not mean that its production is valueless. In fact, Kyoto and Osaka of Middle Japan are great consuming cities of duck meat. Also in many countries of Asia, the duck is mostly produced by a family industry, but its eggs and meat are very valuable resources of animal protein foods. The indispensability of duck meat accompanied with pork in Chinese dishes is wellknown, not to mention, that the world-famous Pekin duck was bred some centuries ago for this purpose. Beside the great domestic consumption, the duck egg processed in various ways as salted, powdered etc. is an important export of China. For the Filipino, the boiled duck-egg, called balut, is a delicacy; balut is an egg in which the embryo has been allowed to develop usually up to the thirteenth or nineteenth day of incubation (BURGOS, 1934).

Duck raising, particularly in South-East Asia, is very closely connected with the rice cultivation. The majority of the inhabitants of this area are good duck-raisers keeping ducks around their living houses. Most of them purchase ducklings from hatcheries and rear them by providing only kitchen or garden refuse, sometimes with a small amount of paddy or rice-bran, while the greater part of feeds is sought by ducklings themselves in the nearby waters as ditches, brooks, pools, paddy-fields, etc. The total amount of eggs and meat thus produced is enormous; although the most part of it is consumed at home, hence statistics are not available.

In addition, it is a common custom among the inhabitants of South-East Asia to raise the hybrid bird between the Common duck (*Anas platyrhyncha* var. *domestica*) and the Muscovy drake (*Cairina moschata*). This hybrid bird is commonly known as mule-duck.¹⁾ The mule-duck, although completely sterile in both sexes, has much merit as a meat producer, exhibiting a rapid growth, a strong constitution and good foraging qualities due to heterosis. In Taiwan, for example, the Common duck of penguin type is bred for egg production and called *tsai-ah* (菜鴨), and the Muscovy duck, named *fan-ah* (蕃鴨) is kept for the production of the mule-duck, which is known as *too-fan-ah* (土蕃鴨); the mule-duck is raised exclusively for meat purpose. The same method of duck keeping for the production of eggs and meat seems to be prevalent all over the areas of South-East Asia, not only among the Oversea-Chinese but also natives.

The domestic duck, i.e. the Common duck, is a polygamous bird, but its polygamous habit is not so extensive as the fowl. As a matter of fact, in China the

1) According to KURODA (1958) the mule-duck is known in binominal terms as *Anas hybrida*, *Anas maxima* and *Anas sterilis*.

breeder of Pekin-duck mates a drake with four ducks at most in order to obtain 80 percent of the resultant fertility of eggs (KUMASAKA, 1942). Also, the low fertility of intergeneric eggs in the mule-duck production is often reported. In view of such a low fertility of the Common duck as well as the Muscovy, it is considered that the application of artificial insemination to the duck breeding should be of great significance for the production of the largest possible number of progeny.

The artificial insemination, however, has not so far been applied to the duck breeding industry, perhaps for two reasons: Little understanding of the nature of this industry and the technical incompleteness of insemination.

From this standpoint, the present experiments were performed from 1953 to 1960 at the Faculty of Fisheries and Animal Husbandry, Hiroshima University, Fukuyama, Hiroshima Prefecture. The research program was concerned primarily with improving the technique of artificial insemination for applying to the Common duck and secondly with promoting the fertility rate of hybrid eggs between the Muscovy drake and the Common duck by using the improved technique.

The author wishes to express his sincere thanks to Prof. emer. Dr. J. YAMANE, President of Hiroshima Agricultural College, formerly Dean of Faculty of Fisheries and Animal Husbandry, Hiroshima University, for suggesting this study as well as for constant guidance and encouragement during the course of the work.

II. COPULATORY ORGANS AND COPULATORY BEHAVIOR OF COMMON DUCKS

Since the structure of copulatory organs and the copulatory behavior of the Common duck seem to be fundamental knowledge for the application of artificial insemination to this bird, some observations should be first taken in consideration.

1. Copulatory Organs

The drake, unlike the cock, has a long penis which is developed from the phallus in embryonal stages. According to HASHIMOTO (1939), the phallus begins to develop into an elongated penis when the duckling is 5 months old. In the grown bird its length attains 8-10 cm. Normally the penis is found involuted in a membranous fold in the ventral wall of the cloaca. On sexual excitement the penis evaginates itself spiralling two and a half or three times outside of the cloaca. Along the spiral of the penis a groove, *sulcus ejaculatorius*, passes from the urodeum of the cloaca to the apex of the penis. On erection of the penis, both ridges of the sulcus come in contact so closely with each other that it forms a canal-like passage, through which semen flows out at ejaculation. Since the ureters open into the urodeum, the urinary and reproductive ducts in ducks, unlike mammals, are completely separated, although the penis is developed. In the center of the penis a mucous canal runs longitudinally, leading from its root to its apex. While the canal ends in blindness at the root of the penis, it opens at the apex of the penis and secretes mucus which lubricates the evagination of the penis and facilitates the copulatory act. Most peculiar fact is that the wall of this mucous canal serves as a sheath for the invagination of the penis upon diminishing of its erection. HASHIMOTO

(1939) found a great number of Herbst's corpuscle, an end organ of nerves peculiar to the bird, in the submucosa at the root of the penis, and suggested that this part should be most sensitive to sexual excitement of the drake.

In the female duck, the organ homologous to the penis of drake remains merely as rudimentary phallus, representing a slightly flattened protuberance throughout the life. Whereas the rectal opening is seen on the median wall of the cloaca, the vagina leads to the dorso-sinistral wall of the cloaca because of the unilateral development of the Müllerian ducts. The vagina measuring about 2 cm. in length leads from the uterus with a slight bend at its root.

Comparing the full length of the penis at erection with the depth of the cloaca (about 1.0 cm.) and the length of the vagina (about 2.0 cm.), it seems to be logical to deduce that at copulation the penis is inserted through the vaginal orifice as deep as the uterus. On this account, the spiralling of the penis appears to be very fitted for its penetration into the deviated vaginal orifice, leading through the utero-vaginal bend.

2. Copulatory Behavior

In natural breeding the Common duck (*Anas platyrhynchos* var. *domestica*) has a water-copulatory habit but it can mate on land also if confined in a pen or left out of doors devoid of water. Present observations were made in a yard without any other pool or puddle than a basin for drinking water.

Prior to copulation, the drake approaches a duck bending his neck wave-like near the ground, and then pecks repeatedly at the nape of duck with his bill until the duck carries her body downward. The duck's carriage being low down enough, the drake seizes firmly the duck's nape with his bill and then mounts on the duck. Upon riding on her back, the drake exerts massage-like motions with his toes upon the duck's ilio-femoral regions and excites her vent with the erected penis for 10 to 20 seconds. When the excited duck keeps her vent upward enough, the penis is inserted momentarily through the vaginal orifice to reject the semen. The drake retreats from the duck's back after ejaculation, flaps strongly, runs to a basin and cleans his feathers all over the body. The time required for a copulatory act from seizing the duck to the ejaculation usually does not exceed 20-30 seconds.

The duck receiving the drake's mounting bends her tail upward and rhythmically opens and shuts the vent, sometimes secreting a mucus. At the penetration of the penis the duck quacks in a under-tone and after being freed from the drake, the duck also runs to a basin in order to clean her body thoroughly as the drake does.

III. TECHNIQUES OF SEMEN COLLECTION

Present experiments were undertaken with the two aims: firstly to find out if there is any room for improvement in both massage and electrical stimulation methods, and secondly to determine the relative advantages and disadvantages of the two.

Ducks used here were of a Japanese White Breed, so-called "Osaka-duck" which

had been bred by crossing the native duck with the Pekin duck. The name of "Osaka-duck" is originated from the city of Osaka in Middle Japan where a great amount of duck meat is yearly consumed. Unlike the penguin form of native ducks of South-East Asia, the "Osaka" shows a canoe-type in body form; it is an outstanding meat-producer with rapid growth. The average weights are: adult drake, 2.3 kg and adult duck, 1.8 kg.

During the course of the experiments, the drakes were strictly isolated in individual coops day and night; the ducks were ranged ashore at day time but each confined into its own trap-nest from evening on. They were supplied with an ordinary forage of breeder ration.

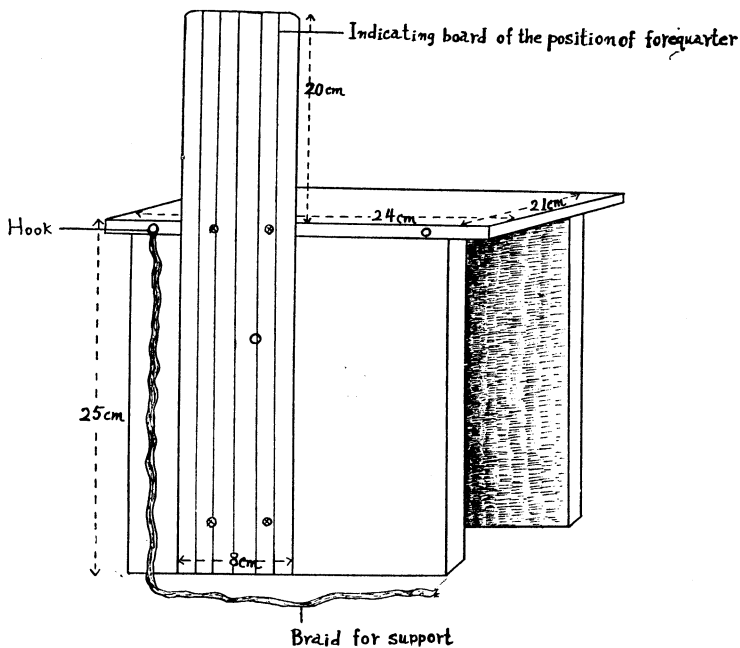
1. Application of Massage Method

The first method applied to the cock by BURROWS and QUINN (1935) was to massage the keel and the soft part of the abdomen above gizzard and below the pelvic bones of the bird which was held by an assistant standing opposite with its head downward. In applying similar massage method to the drake, ŌNISHI *et al.* (1950, 1955) kept the bird in sitting position on the ground by holding it firmly by the roots of the both wings with the left hand. With the right hand an irritable massage was repeatedly exerted upon the both thighs of the drake with the thumb on one side and the fore- and middle-fingers on the other.

On re-examining ŌNISHI's method, it was found that for drakes also the new method of BURROWS and QUINN (1937) using a bird holder was more convenient and successful. For this purpose WATANABE and SUGIMORI (1957) have newly developed the application of a drake holder. This holder consists of a small wooden table with a height of 25 cm., a width of 21 cm. and a length of 24 cm. and a side-plate of 45 cm. in height and 8 cm. in width perpendicularly attached to it (Text-fig. 1). At manipulation the table is hung either on a wall or on a post. A drake is fixed on the table with its both wings and legs bound with a braid, the left side of the forequarter of the bird being situated along the side plate (Pl. II, Fig. 8, 9, 10). Thus, the position of the penis for operation can be ensured to come outside of the hind margin of the table (Pl. I, Fig. 1 & 2).

After the skin around the vent has been cleanly wiped with the sterilized physiological saline solution, the drake is stimulated on its both thighs (ilio-femoral regions) by rapidly massaging with the operator's right hand. This massage upon both thighs, however, is not massage in a real sense but quickly repeated flippings with the thumb on one side and the fore- and middle-finger on the other in ventro-dorsal direction from the acetabulum.

The first response to this massage is a stiffness of the penis in its original position and this is followed by a spasm of the whole body of the drake, specially of his neck and tail. At this moment, the stiff penis is to be pressed down for its complete evagination with the operator's right hand, then the ejaculate is caught into the receptacle which is beforehand kept beneath the cloaca with the operator's left hand (Pl. I, Fig. 3 & 4). The semen receptacle here employed is a glass cup of 5 cm. in diameter and 3 cm. in depth, its bottom being elongated into a graduated conical



Text-fig. 1. Drake holder for collecting semen.

tube of 12 cm. long (about 20 ml. content) (Pl. III, Fig. 13). The receptacle has to be sterilized before use.

Although an ejaculatory response seems to occur prior to this milking action, an ejaculate can be collected without loss into the receptacle because of long spiral passage of the ejaculatory groove of the penis.

The ilio-femoral massage thus applied to the drake does not vary in principle from the new method of BURROWS and QUINN (1937) applied to the cock, but because of morphological difference in the copulatory organs of the two birds, semen of the drake thus collected is less contaminated than that of the cock.

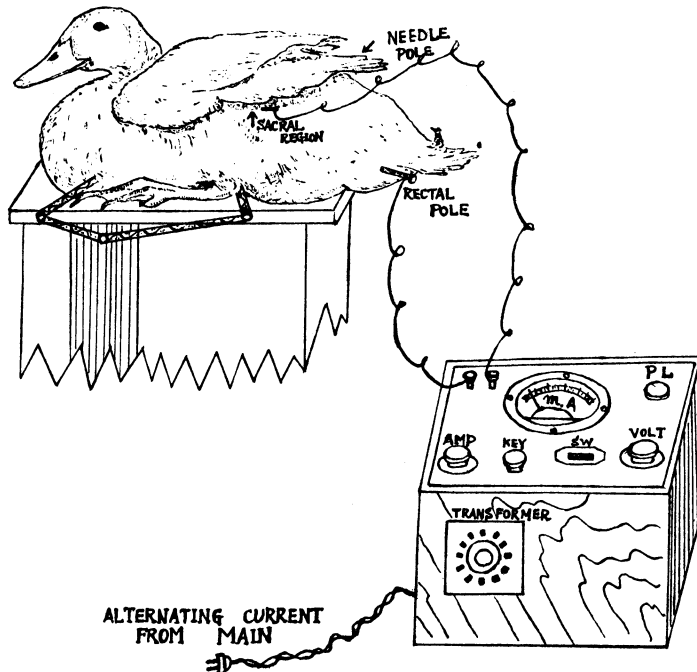
The time required for inducing an ejaculation by the massage is usually 30 seconds in the drakes well trained but cases were not seldom where an ejaculation occurred within 5-10 seconds. It is interesting to note that some trained drakes acquired a habit of erecting the penis or even ejaculating the semen before massage due to the conditioned reflex. Training of drakes, however, is fairly elaborate work as it takes usually a period of from 10 to 15 days until the bird shows an ejaculatory response to the massage. On the other hand, the newly trained drake requires much longer time, from 8 to 15 minutes, though this can be gradually shortened. As regards the individual difference in the ejaculatory response, of 47 drakes tested about 10 reacted to the massage without training, 24 needed training more or less and the remaining 13 did not react upon massage at all and the training had to be given up because of skin injuries effected by this repeated massage.

Semen always has to be collected early in the morning before supplying forages: 6 to 7 o'clock in summer and 7 to 8 o'clock in winter in order to avoid spoil-

ing of the semen with excrements. Further, just prior to the operation, the cloaca is pressed so that feces accumulated at the end of the rectum is expelled. Notwithstanding these precautions, it happened often that the semen thus procured had to be discarded because of soiling. It is also to be noticed that the semen volume here presented is not a volume in "physiological" sense, as it is hardly possible to measure the volume accurately because of its extremely small quantities.

2. Application of Electrical Stimulation

In the experiments of using the electrical method on drakes, an electro-ejaculator was employed. The apparatus was properly developed by SHIBATA *et al.* (1938) for applying to the rabbit, later commercially constructed by the FHK Co. Ltd., Tokyo, for applying a 60 cycles alternating current to the goat and sheep. The apparatus consists of a transformer, a voltmeter of 0–30 volts and a milliammeter of 0–100 milliamps and a switch of push button (Pl. II, Fig. 5). In operation, an electric pole, a sharp needle which is led from the apparatus, was stuck into the hypodermis of the ilial region; and the opposite pole, a blunt rod, was inserted about 4 cm. deep into the rectum through the cloaca in the same manner as GUNN (1936) originally devised for the ram (Pl. II, Fig. 6; Text-fig. 2). At first the alternating current of 30 volts and 60–80 milliamps was turned on for 3 seconds and repeated by using a push button from three to five times with an interval of 5 seconds. Since the experiments with Muscovy drakes, as it will be later described, showed the cur-



Text-fig. 2. Diagram illustrating the method of electrical stimulation for collecting the semen of drake.

rent of 20 volts and 40–50 milliamps to be strong enough for inducing ejaculatory response, the same current was also applied subsequently to the Common duck with the same success. The operation occurred in the following way: A drake was first fixed on the bird-holder in the same manner as described in the massage method, then the current was turned on. At every turning of the current the drake showed a momentary rigidity of its body (Pl. II, Fig. 7 & 8). Upon sufficient stimulation, the bird flapped reflexively its tail revealing emission of semen from the vas deferens presumably into the urodeum (Pl. II, Fig. 9 & 10). It is peculiar to the electrical stimulation that the penis, contrary to the massage method, always remained in its original position without evaginating, though it showed some stiffness. For receiving the semen into a receptacle, therefore, further manipulation or “milking” was necessary in order to expel the semen from the urodeum and ejaculatory groove. This was simply done by pressing with fingers the penis somewhat stiffened in its original position. The time required for milking to induce the semen dropping into a receptacle was usually one minute. From the standpoint of avoiding contamination, it was more advantageous to recover the semen from the penis in its invaginated state than in its evaginated state.

It should not be left here unnoted that there is no evidence of injuries on the drakes treated with electricity of such a weak current as used here: Neither loss of appetite, nor decline of live weight, nor paralysis of body parts were observed. Ten birds subjected to the regular electrical stimulation during the past two years are producing normal semen still now.

SUMMARY

1. The value of practical use of both the massage and electrical stimulation in collecting the semen from Common ducks was comparatively studied.

2. By using a drake-holder, the massage applied upon both the thighs of a drake induces easily an ejaculatory response. It is required, however, that the drake should be trained beforehand for about 10–15 days.

3. The repetition of massage for a long period of time gives sometimes injuries to the skin of the thighs operated upon.

4. As the reaction is partly of psychological nature, the length of time which is required from manipulation to ejaculation is fairly manifold: 50 to 5 seconds according to the training received. There are, however, some birds that do not react at all.

5. Application of an alternating current with 20 volts and 40–50 milliamps is strong enough to induce an ejaculatory response within one minute.

6. In the electrical stimulation, reaction being completely of reflexive nature, the collection of semen can be done with regular success. No deleterious effects has been found in birds thus treated.

7. The advantages and disadvantages of both techniques of semen collection shall be later discussed in association with other factors concerned.

IV. CHARACTERISTICS OF SEMEN OF COMMON DUCKS

The semen which was collected by both the massage and electrical method was examined every time just after collection with respect to volume, sperm concentration, deformity percentage, pH-value and motility of sperms. Determination of sperm concentration was performed with a Thoma-Zeiss haemocytometer as usual. For determining the pH-values of semen the brom-thymol-blue test paper was used. The term deformity used here means a chain of the secondary changes sperm cells: looping of tail, bending or breaking at the middle piece, isolation of the head, etc.

1. Characteristics of Semen Collected by Massage Method

Massage method was applied to two drakes in the same season: April 25 to July 22, 1957. Microscopical examinations of semen carried out just after collection revealed that in the majority of cases, about 80–90 percent of sperms, were most active producing strong currents in semen drops, if the semen was collected in a clean state.

The results obtained from 6 samples of semen are summarized in Table I, in comparison with those secured by ŌNISHI *et al.* (1955), who have determined the per-ejaculate volume on 150 samples, and the per-unit-volume sperm concentration on 199 samples.

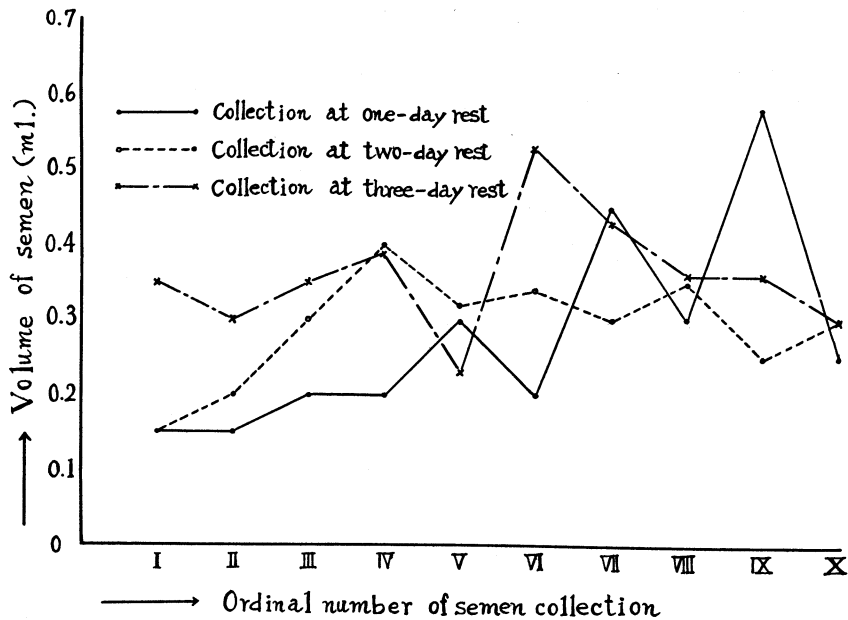
From the table it is seen that the semen collected by massage has an average volume of 0.233 ml. per ejaculate, an average number of 883,000 sperms per cubic millimeter, totalling 213.7 millions sperms per ejaculate. The average of pH-value of the semen is 6.73 and the average percentage of sperm deformity is 20.87. The first four figures of the average in the table show only slight differences from those determined by ŌNISHI *et al.* (1955), though the ranges are not always coincident.

In mammals, it has been known that semen volume and sperm concentration vary exceedingly at different intervals of collection, especially when this occurs repeatedly within a short time as in the twice-a-day collection. Since the duck receiving massage with such a frequency is very likely to sustain skin injuries, it is

Table I. Average volume, sperm concentration, pH-value and deformity percentage of the semen collected by massage per bird per collection.

	WATANABE (1957 b)			ŌNISHI <i>et al.</i> (1955)	
	Mean	Range	Confidence limits(95%)	Mean	Range
Volume per ejaculate (ml.)	0.233	0.05–0.45	±0.14	0.32	0.1–1.0
Sperm concentration per cu. mm. (millions)	0.883	0.340–1.360	±0.45	0.905*	0.07–3.587*
Total number of sperms per ejaculate (millions)	213.7	68.0–562.5	±2.09	263	1–1,550
pH-value	6.73	6.6–6.8	±0.10	7.31	6.6–7.8
Sperm deformity (%)	20.87	19.32–23.94	±1.66	—	—

* Converted from the number of sperms per ml. ejaculate.



Text-fig. 3. Relationship between the intervals of collection and the volume of semen.

desirable to determine the optimum interval of semen collections. In this connection, the figure cited from the previous paper (WATANABE & SUGIMORI, 1957a) may give some suggestions for it, if not conclusive. Each graph denotes an average of semen volume per bird per collection obtained from two drakes at a one-day rest, a two-day rest and a three-day rest (Text-fig. 3). In the trials where semen collections were made after a two-day or a three day-rest, the semen volume was greater than in the trial of a one-day rest, within a limited period of time, but when the collection occurred more continuously, the results showed a reverse tendency; it seemed to show that the bird can gradually adapt to massage and produce more semen after a one-day rest.

2. Characteristics of Semen Collected by Electrical Stimulation

The collection of semen by electrical stimulation was first performed with eight 2-year-old drakes during the season from April 25 to July 22, 1957, taking the above described two drakes as control to which massage was applied (WATANABE, 1957b). The results obtained are shown in Table II.

In comparing Tables II with Table I, most conspicuous fact is that both in semen volume and in sperm concentration the samples collected by electrical stimulation are always greater than those collected by massage. On account of technical difficulties of determining the exact volume below 1 ml., it is hardly possible to show any statistical significance for the semen volume, in consequence, also for the total number of sperm per ejaculate. Nevertheless, a significant difference in the sperm concentration per cubic millimeter is clearly found,

$$\text{revealing } t_0 = 2.807 < t = 6.446$$

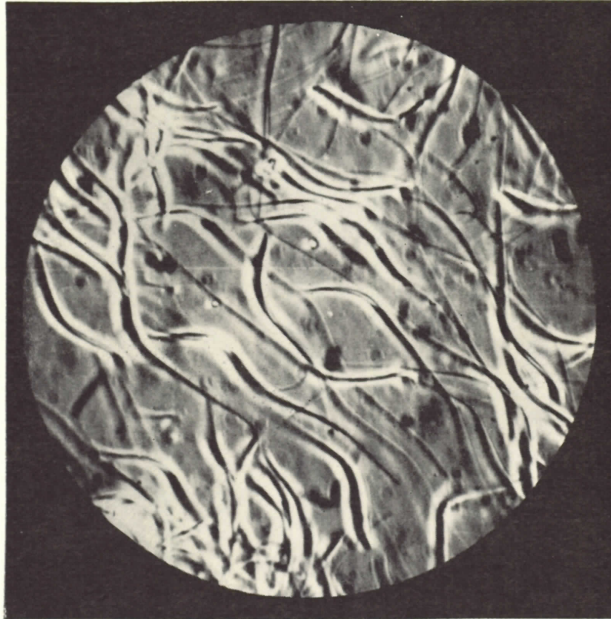
Table II. Characteristics of semen collected by electrical stimulation per bird per collection.

	Mean	Range	Confidence limits (95%)
Volume per ejaculate (ml.)	0.326	0.10–0.75	±0.08
Sperm concentration per cu. mm. (millions)	4.624	2.210–8.660	±0.89
Total number of sperms per ejaculate (millions)	1,608.2	442.0–5,196.0	±6.12
pH-value	6.78	6.4–7.0	±0.09
Sperm deformity (%)	20.13	16.06–23.44	±0.98

In fact, the semen obtained by electrical method appears unexceptionally thick and viscous in consistence with creamy color, whereas that obtained by massage is thin and less viscous and milky white in color.

In morphological character, the sperm cell of the duck seems apparently not to differ much from that of the fowl as shown in Text-fig. 4; the mean of the length of the heads and tails of 100 sperm cells measured were $15.02 \pm 0.469 \mu$ and $56.41 \pm 0.552 \mu$ respectively.

As regards the initial motility of sperms in the semen collected by electrical stimulation, it may be said that the motility did not vary from the sample collected by massage.



Text-fig. 4. Sperm cells of Common duck.
Phase-contrast microscopy.
Magnification about 850.

Further experiments in semen collection by electrical stimulation were carried out on four 2-year-old drakes at every seven days for the period from April 1957 to March 1958 with the aim of its application to routine artificial insemination practice on one hand and, on the other hand, of examining the seasonal variation of semen characteristics.

The quantitative characters of semen thus obtained are shown in Table III in

Table III. Seasonal variation of characteristics of semen obtained by electric stimulation from Common duck (during the period from April 4, 1957 to March 27, 1958). Each number is an average of 4 ducks except those which are marked: One asterisk shows the case where only one drake responded to the stimulation; two, where two responded, and three, where three responded.

Week No.	Volume per ejaculate (ml.)	Sperm concentration per cu. mm.	Total number of sperms per ejaculate	Monthly average
Jan.	1.	0.10	2,640,000	246,200,000
	2.	0.10	3,210,000	
	3.	0.10	1,240,000	
	4.	0.10	2,760,000	
Feb.	1.	0.20	4,080,000	641,500,000
	2.	0.20	3,120,000	
	3.	0.20	2,490,000	
	4.	0.20	3,140,000	
Mar.	1.	0.20	1,348,000	1,607,550,000
	2.	0.30	8,362,000	
	3.	0.20	16,710,000	
	4.	0.20	1,550,000	
Apr.	1.	0.60	6,460,000	3,079,000,000
	2.	0.40	7,900,000	
	3.	0.40	4,620,000	
	4.	0.60	5,720,000	
May	1.	0.40	14,100,000	3,570,500,000
	2.	0.30	6,740,000	
	3.	0.50	7,960,000	
	4.	0.40	6,600,000	
Jun.	1.	0.20	2,520,000	1,731,250,000
	2.	0.30	5,140,000	
	3.	0.50	4,270,000	
	4.	0.80	3,430,000	
Jul.	1.	0.40	5,580,000	1,126,750,000
	2.	0.20	4,850,000	
	3.	0.10	7,560,000	
	4.***	0.10	5,490,000	
Aug.	1.*	0.20	2,620,000	431,500,000
	2.*	0.10	3,390,000	
	3.	0	0	
	4.	0	0	
Sep.	1.**	0.10	1,240,000	159,400,000
	2.**	0.10	2,577,000	
	3.***	0.10	1,310,000	
	4.	0.10	1,250,000	
Oct.	1.	0.10	2,810,000	319,750,000
	2.	0.10	1,570,000	
	3.	0.20	2,890,000	
	4.	0.10	2,630,000	
Nov.	1.	0.20	2,800,000	524,000,000
	2.	0.20	3,180,000	
	3.	0.10	3,380,000	
	4.	0.20	2,810,000	
Dec.	1.	0.10	3,080,000	330,750,000
	2.	0.10	2,840,000	
	3.	0.10	4,170,000	
	4.	0.10	3,410,000	

average values per bird per collection.

The table shows that the total number of sperms per ejaculate is at its highest during the period from the middle of March to the beginning of July, rising to a peak in May. A very conspicuous fact is that none of the drakes responded to the electrical stimulation in the last half of August although they were under the normal raising condition. Occasionally no response was also observed in another drakes over a period from the fourth week of July to the third week of September. Hence, this period appears to be the worst breeding season for the Common duck.

SUMMARY

1. The results of examining 6 samples from 2 drakes showed that the average volume of semen collected by massage was 0.233 ml. per ejaculate, containing 883,000 sperms per cubic millimeter, thus totalling 213.7 millions sperms per ejaculate (Table I).

2. The average of 19 semen samples electrically collected from 8 drakes revealed 0.326 ml. in volume per ejaculate and 4,624,000 sperms per cubic millimeter, totalling 1,608.2 millions sperms per ejaculate (Table II).

3. Hence, the semen electrically collected is larger in its volume and about 5 times higher in sperm concentration than the semen collected by massage.

4. As a consequence, the total per ejaculate number of sperms is about 8 times bigger in the electro-ejaculated semen than in the semen procured by massage.

5. There were, however, no substantial difference in pH-value and deformity percentage of sperms between the semen collected by both the methods when examined immediately after collection (Table I and II).

6. Aside from the methods of collecting semen, a close relationship between the volume and frequency of collection of semen was found. A greater volume of semen was usually obtained at an interval of 2- or 3-day rest than at an interval of 1-day rest within a limited period of time. But when the collection occurred continuously for a longer period of time, the results appeared reversely.

7. A remarkable seasonal variation in sperm production was observed in a full-year record, showing a remnant of breeding season in wild life (Table III). A high testicular activity lasted from the middle of March to the beginning of July, rising to a peak in May. It declined, however, from the end of July to the third week of September, dropping off completely in the last half of August: the worst breeding season for the Common duck.

V. TECHNIQUES OF SEMEN INTRODUCTION

Throughout present experiments, the insemination of ducks was carried out generally between 9 and 10 o'clock a. m. and at 11 o'clock a. m. at the latest. This relates, on one hand, to the time of semen collection before foraging the drakes, and on the other hand, to avoiding the new egg appearing in the uterus.

Introduction of semen into the oviduct of ducks was comparably tested by the

two methods: firstly by palpating the orifice and secondly by employing a speculum.

1. Techniques of Semen Introduction by Palpation

a. Experiments with semen collected by massage

Semen introduction by palpating method was applied to fifteen ducks in full lay which had been isolated from drakes for one month. Semen for the experiments was collected simultaneously from three drakes by massage. Every three samples thus collected were mixed immediately and each of the composite semen was diluted from 5 to 20 times according to the lot of experiments. To each duck, 0.3 ml. of the diluted composite semen was introduced into the uterus with the following procedure: After evacuating the bowel, a duck is held by an assistant sitting on a stool, the body of the bird being firmly held between both thighs of the assistant in order that the abdomen of the bird may be directed outwards and its vent upwards. An operator, after cleaning the vent with absorbent cotton, inserts his left index finger into it to search the vaginal orifice which can be palpated slightly shifting to the left at the upper part of the cloaca. As above mentioned, in the duck, the anterior part of the vagina leads to the uterus with a bend which can be felt by a weak pressure on the finger. The finger should be carefully inserted along its inner wall until it reaches the uterus.

Leaving the left finger at this position, a fine rubber tube with the length of about 10 cm. is introduced along it into the uterus by right hand. When the tip of the rubber tube reaches the uterus, the left finger is withdrawn and a common 1 ml. tuberculin-type syringe containing the semen is attached to the free end of the rubber tube and the content is slowly introduced. The rubber tube for the insemination of the rabbit used by YAMANE (1925) was found to be most satisfactory in this case, too.

The first experiment was performed on September 2, 1953 and the second on September 29, 1953. In both experiments the same birds were used, the insemination being done once per bird. The fertility of the semen thus introduced, was tested with eggs laid after the insemination, which were put into an incubator at 8.30 p. m. every evening and candled first on the 5th day of incubation. For the sake of confirmation, incubation was continued further for a week after the first candling. Tables IV and V show the laying records of the ducks in the two series of experiments, introducing the semen by palpation, and both these tables are combined into the Table VI by expressing in figures.

At a glance of these three tables it will be noticed that all the eggs produced on the first day following insemination are infertile and the first fertile eggs appear at the earliest on the second day after insemination.

In the duck, the time of onset of fertilization is not exactly known but the above data in association with the facts that the inseminations in the present study were always performed between 9 and 10 o'clock, and the eggs laid were collected between 6 and 7 o'clock every morning, it may be inferred that the eggs laid on the first day following insemination have no concern with the sperms introduced, but

Table IV. Laying record of 15 ducks inseminated with semen collected by massage and introduced by palpation (First experiment: September 2 to 16, 1953).

No. day following insemination	No. duck inseminated														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2	-	-	-	+	+	+	-	+	+	+	0	-	+	+	+
3	-	-	-	+	+	+	-	+	+	+	+	-	+	+	+
4	-	-	-	+	+	+	-	+	+	+	-	-	+	+	+
5	-	-	-	+	+	+	-	+	+	+	+	-	+	+	+
6	-	-	-	+	+	-	-	+	-	+	+	-	+	0	+
7	-	-	-	+	+	-	-	+	-	-	-	-	+	+	+
8	-	-	-	0	+	-	-	-	-	-	+	-	0	-	+
9	-	-	0	0	+	-	-	+	-	+	-	-	-	-	-
10	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
11	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
12	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
13	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
14	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Remarks: +=Fertile; -=Infertile; 0=No egg laid.

Table V. Laying record of the same 15 ducks as in Table IV, inseminated with semen collected by massage and introduced by palpation (Second experiment: September 29 to October 13, 1953).

No. day following insemination	No. duck inseminated														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1	0	-	0	-	-	-	-	-	-	0	-	-	-	0	-
2	-	+	0	+	+	+	-	0	+	+	+	+	0	+	+
3	-	+	0	+	+	+	+	+	+	+	+	+	0	+	+
4	0	+	0	0	0	-	+	0	+	+	+	+	0	+	+
5	0	+	0	+	0	+	-	+	0	-	-	-	+	+	+
6	0	+	0	+	0	-	-	+	+	-	-	-	-	0	+
7	+	+	0	+	+	-	-	-	+	-	-	+	0	-	-
8	+	+	-	+	+	-	-	0	-	-	-	0	-	-	+
9	-	+	0	+	+	+	-	+	+	-	-	-	-	+	+
10	+	+	0	+	-	-	-	-	-	-	-	-	-	-	-
11	-	-	0	+	-	-	-	-	-	-	-	-	-	-	-
12	0	-	0	+	+	-	-	-	-	-	-	-	-	-	-
13	-	-	0	-	-	-	-	-	-	-	-	-	-	-	-
14	-	-	0	-	-	-	-	-	-	-	-	-	-	-	-

Remarks: +=Fertile; -=Infertile; 0=No egg laid.

the fertilization should occur just on that day. It follows, therefore, that the eggs produced on the first day after insemination should not be considered in calculating the fertility rate. On the same account, the duration of the fertilizing capacity of

Table VI. Fertility test of semen collected by massage and injected to 15 ducks by palpation (Compiled from Table IV and V).

No. day following insemination	Number of egg laid		Number of fertile eggs		*Daily fertility (%)		
	Exp. I	Exp. II	Exp. I	Exp. II	Exp. I	Exp. II	Average
1	15	11	0	0	0	0	0
2	14	12	9	10	64.3	83.3	73.8
3	15	13	10	12	66.7	92.3	84.5
4	15	9	9	8	60.0	88.9	75.0
5	15	11	10	7	66.7	63.6	65.1
6	14	11	7	5	50.0	45.5	47.8
7	15	13	6	6	40.0	46.2	43.1
8	13	13	3	5	23.1	38.5	30.8
9	13	14	3	8	23.1	57.1	40.1
10	14	14	0	3	0	21.4	10.7
11	15	14	0	1	0	7.1	3.6
12	15	13	0	2	0	15.4	7.7
13	15	14	0	0	0	0	0
14	15	14	0	0	0	0	0

* In testing the fertilizing capacity of the semen of poultry, the fertility rate may be calculated from two aspects as reported in earlier papers (WATANABE and SUGIMORI, 1957 a; WATANABE, 1957 b): the rate of the number of birds inseminated and produced fertile eggs to the number of all birds inseminated, viz. absolute fertility, and the rate of the number of fertile eggs to the total number of eggs produced per bird per insemination, viz. relative fertility. Since it has been subsequently found that the use of the fertility rate per bird or per flock per day or per week or per some days is more rational for judging the fertility of semen samples inseminated, this term will be used hereafter.

sperms may be indicated by the day before the last fertile egg has been laid. Considering the approximate time of onset of fertilization as above mentioned, further analysis of the data presented in the Table VI reveals that the fertility rate of eggs laid during the period from the second day following insemination to the day producing the last fertile egg is 50.0 percent (57:114) in the first experiment, and 48.9 percent (67:137) in the second experiment; thus the results of both experiments was almost identical.

It is peculiar, however, to find the less fertilizing capacity of semen in the first experiment as compared to that in the second experiment, manifesting clearly in the daily fertility rates and the durations of retaining fertility potential. In this connection, it must be remembered that the first experiment was performed during the first half of September when the semen qualities were inferior as has been indicated in the foregoing chapter. On the contrary, the second experiment was conducted during the first half of October when the semen was already improve quantitatively as well as qualitatively.

b. Experiments with the semen collected by electrical stimulation

As it has been shown, the semen collected by electrical method is strikingly

thick compared with that collected by massage, and the introduction of the semen electrically obtained should be also tested. All the treatments of the semen did not differ from the preceding experiment, except that it was diluted only 10 times in spite of its greater density.

Ten ducks were used for this experiment; the results obtained are shown in Table VII.

Table VII. Fertility test with semen electrically collected and introduced to 10 ducks by palpation (May 19 to June 3, 1957).

No. day following insemination	Number of eggs laid	Number of fertile eggs	Daily fertility (%)
1	8	0	0
2	8	4	50.0
3	8	5	62.5
4	7	2	28.6
5	7	3	42.9
6	6	2	33.3
7	4	1	25.0
8	5	2	40.0
9	1	0	0
10	3	0	0
11	2	0	0
Total number of eggs from 2 to 8 day after insemination	45	19	7-day fertility (%) 42.2 (19:45)

The first fertile eggs appeared on the second day after insemination and the daily maximum fertility rate was gained on the third day as shown in the foregoing experiment.

It is worthy of note that the electro-ejaculated semen exhibited a lower fertility than that collected by massage. This, however, is not likely to be attributed to the introducing technique, but rather to the characteristics of the semen collected by electrical stimulation. The matter will be discussed below.

2. Techniques of Semen Introduction by Employing a Speculum

In the artificial insemination of mammals, a vaginal speculum is of common use, but no attempt has been made of its use for birds; this may be due to the smallness and peculiar form of the cloaca. In the present experiments, however, it was shown that the speculum is easily applicable to the duck and very useful. A metallic speculum was devised, approximating the anatomical structure of copulatory organs of the duck. The part to be inserted of the speculum is 7 cm. in length, 1.3 cm. in depth and 1.5 cm. in width and its handle bends at 145°, and measures 14 cm. in direct length; the bend of the handle firmly lets the speculum fix on the operator's index finger (Pl. III, Fig. 11 & 12). For semen introduction, in this case,

a glass pipette of 10 cm. in length and 0.3 ml. content which was connected to a rubber bulb at its hind end was used. The bird was held in a similar manner by an assistant and cleaned at its vent as described before.

On inserting the speculum through the vent, the firmly closed anal opening can be found at the center of anterior wall of the cloaca. Slightly shifting from the anal opening to the upper left lies the vaginal orifice loosely closing. Further manipulation with the speculum easily enables the insertion of it about 7 cm. deep through the vaginal orifice. Hereupon, the insemination pipette sterilized beforehand is introduced along the speculum until its tip reaches the fore-end of the speculum, and the content is injected by pressing the rubber bulb attached (Pl. III, Fig. 13-16).

The whole procedure from insertion of the speculum to the semen introduction was only one minute's work; insemination could be performed quickly without worry of bringing injury or infection to the female birds.

A further fertility test was made as before in a flock of another 10 ducks from February 4 to 21, 1957. The result shown in Table VIII indicates slightly higher fertility rates than those found in the case of semen introduction by palpation method (Table VII & VIII). Hence, there is no reason to believe that at semen introduction the palpation method is more preferable to the use of speculum.

Table VIII. Fertility test with semen electrically collected and introduced by applying speculum in a flock of 10 ducks (February 4 to 21, 1957).

No. day following insemination	Number of eggs laid	Number of fertile eggs	Daily fertility (%)
1	9	0	0
2	10	8	80.0
3	10	5	50.0
4	9	5	55.6
5	9	2	22.2
6	9	1	11.1
7	10	1	10.0
8	9	0	0
9	9	0	0
10	9	0	0
Total number of eggs laid from 2 to 8 day after insemination	57	22	6-day fertility (%) 38.6 (22:57)

SUMMARY

1. In all cases of the present experiments, semen introduction was carried out between 9 to 10, rarely 11 o'clock a. m., considering the time of semen collection as well as the time of forming the egg-shell within the oviduct.
2. For introducing semen to the oviduct the two techniques, viz., palpation

method and method employing a speculum, were compared, both the semen collected electrically and by massage being used.

3. Semen diluted with the physiological saline was introduced into the uterus by palpating the vaginal orifice with the index finger on one hand, and by inserting a speculum through the vaginal orifice, on the other.

4. The palpation method with semen obtained by massage (Exp. a), brought a result that the maximum fertility was obtained on the third day after insemination, showing the fertility of 66.7 and 92.3 percent with the average of 84.5 percent. The maximum period during which the sperm maintained its fertility was 11 days.

5. By the palpation method with the semen obtained by electrical stimulation, the daily fertility rate dropped to 62.5 percent at maximum and the duration of maintaining fertility was shortened to 7 days (Exp. b). This is, however, unlikely to be attributed to the introducing technique itself as discussed below.

6. The use of a speculum specially devised for the purpose of semen introduction was found to be decidedly advantageous. It could be performed quickly without giving any damage to ducks.

7. The fact that the fertility rates of semen electrically obtained for both experiments by palpation method and employing a speculum was lower than that of semen secured by massage, seem to indicate the proper characteristics of the semen collected by electrical stimulation. The matter is considered below.

VI. EFFECTS OF DILUTORS AND STORAGE ON FERTILITY OF DUCK SEMEN

1. Effects of Dilution Rates

In the fowl, the relationship between the dilution rate and the fertility of semen have been reported by a number of workers but there is very little information available on the duck.

In the present experiments, the semen collected by massage from 4 drakes was added with 0.85 percent physiological saline solution, each sample being subjected to the dilution rates of 1:0, 1:5, 1:10 and 1:20. Each semen thus diluted was fractionally inseminated to five ducks, with a dose of 0.3 ml. per bird. All eggs laid after the insemination were incubated for fertility test. Table IX indicates clearly the beneficial effect of dilution but within a limited extent.

While the undiluted semen showed 100 percent fertility until the third day after the insemination, the semen diluted with a rate of 5, 10 and 20 times resulted 100 percent fertility until the seventh, third and fourth day, respectively, and most satisfactory fertility level was maintained in the 5-time dilution. As these resultant fertility rates have been obtained by a single insemination per bird, it should be inferred that the most satisfactory fertility level will be continuously retained, if the insemination is periodically performed with a cycle of every five days by using a fresh semen.

Table IX. Relationships between the dilution rate and the fertility of semen obtained by massage and diluted with physiological saline solution (September 2 to October 13, 1953). Sperm suspension of each rate was allotted to five ducks in a volume of 0.3 ml. per bird.

No. day following insemination	Number of eggs laid				Number of fertile eggs				Daily fertility (%)			
	Dilution rate				Dilution rate				Dilution rate			
	0	5	10	20	0	5	10	20	0	5	10	20
1	5	3	4	4	0	0	0	0	0	0	0	0
2	4	4	4	4	4	3	3	4	100	75	75	100
3	4	4	5	4	4	3	5	4	100	75	100	100
4	5	1	4	3	3	1	3	3	60	100	75	100
5	5	2	4	5	4	2	2	3	80	100	50	60
6	5	2	4	4	4	2	2	1	80	100	50	25
7	5	4	5	4	3	4	1	1	60	100	20	25
8	4	5	4	4	3	4	0	1	75	80	0	25
9	5	4	5	5	2	3	3	2	40	75	60	40
10	4	4	5	5	1	3	0	0	25	75	0	0
11	3	4	5	5	1	1	0	0	33.3	25	0	0
12	4	3	5	5	0	2	0	0	0	66.7	0	0
13	5	4	5	5	1	0	0	0	20	0	0	0
14	4	4	4	5	0	0	0	0	0	0	0	0
15	4	4	5	5	0	0	0	0	0	0	0	0

2. Effects of Egg-yolk Citrate Buffers

In all the experiments above mentioned, 0.85 percent saline solution was used as dilutor of semen, because no adequate dilutor so far had been known for duck semen. Nevertheless, the physiological saline solution is not always suitable dilutor in keeping the fertility of sperm for a long period of time *in vitro*. In order to promote fertility of the sperms, regardless of immediate use after collection or long-term storage *in vitro*, the preparation of an expedient dilutor is indispensable.

In the present experiments three kinds of egg-yolk buffers were provisionally used:

1. Fowl egg-yolk citrate solution consisting of 3g $\text{Na}_3\text{C}_6\text{H}_5\text{O}_7 \cdot 2\text{H}_2\text{O}$ dissolved in 100 ml. distilled water and 100 g fresh fowl egg-yolk,
2. Duck egg-yolk citrate solution prepared in the same ratio as above, and
3. "Seminan", a commercial fowl egg-yolk citrate buffer devised by NISHIKAWA (1954) for diluting the bull semen.

Semen samples for the test were simultaneously collected by electrical method from three 8-month-old drakes and the three semen samples were mixed and put into 5 vials in an equal proportion; each content of the vials was diluted five times with the dilutors above mentioned. After dilution, all the samples were gradually cooled to 5°C within half an hour, then stored at this temperature in a refrigerator. Every 24 hours, sperm motility was examined under the microscope equipped with a stage thermostat. During the first 24 hours after collection each semen sample showed very active motility of sperm, regardless of dilution or non-dilution or kinds

of dilutors; the difference in the viability, expressed by the maximum duration of motility, occurred from the second day on. The results of the experiments with 8 lots are summarized in Table X.

It is seen that, compared with the undiluted semen, all dilutors here tested were more or less effective for the viability of sperm, but among them the fowl egg-yolk citrate was decidedly effective. It was noteworthy that the duck egg-yolk citrate buffer and "Seminan" were less effective than the simple saline medium.

Table X. Average viability of duck sperms in various dilutors.

	Mean (hrs.)	Range (hrs.)	Confidence limits (95%)
Undiluted	36	24—72	± 12. 63
Physiological saline solution (0.85%)	78	48—96	± 20. 72
Fowl egg-yolk citrate	126	48—192	± 41. 11
Duck egg-yolk citrate	63	24—96	± 21. 22
"Seminan"	60	24—96	± 21. 41

3. Storage and Fertility of Semen in Egg-Yolk Buffer

Based on the foregoing result that the fowl egg-yolk citrate solution was most effective for maintaining the motility of semen, further experiments on the insemination with the semen stored in this solution were made to a greater extent during the period from November 23, 1959 to March 4, 1960.

Semen was collected electrically; a composite sample from five 8-month-old drakes was diluted 5 times with the fowl egg-yolk citrate buffer, gradually cooled to 5°C within half an hour and stored in a refrigerator at this temperature as before described. Thirty ducks of 8-months of age were divided into 5 flocks, each consisting of 6 ducks (Flock I to V). Each buffered semen sample was injected simultaneously to each flock with a dose of 0.3 ml. per bird. Fertility test was made with the semen stored for 0, 24, 48, 72 and 96 hours but the results of the last two tests were quite negative, no fertile egg being produced; thence the results obtained in Flock IV and V were discarded here (Table XI).

In the Flock I where the buffered semen was injected immediately after collection, the most satisfactory fertility rate was gained until the 5th day following insemination and the maximum duration of fertility was 13 days. Considering that this was the result of a single insemination will be repeated every five days, a full fertility can be gained in practice. In the case of the Flocks II and III tested with semen stored for 24 and 48 hours respectively, the duration of fertility was remarkably short. These cases also show that, a repeated insemination with a cycle of every three days would result in 80 percent fertility or more. A longer storage than 48 hours has little likelihood of retaining fertility potential under the present condition, although about two-thirds of sperms in the microscopical field exhibited

Table XI. Fertility of the semen electrically collected and stored in a fowl egg-yolk citrate buffer at dilution rate of 1:5. Insemination took place once for each flock, consisting of 6 ducks.

No. day following insemination	Flock I. 0-hour storage			Flock II. 24-hour storage			Flock III. 48-hour storage		
	Number of eggs laid	Number of fertile eggs	Daily fertility (%)	Number of eggs laid	Number of fertile eggs	Daily fertility (%)	Number of eggs laid	Number of fertile eggs	Daily fertility (%)
1	3	0	0	5	0	0	4	0	0
2	6	4	66.7	6	3	50.0	5	4	80.0
3	6	6	100.0	6	5	83.3	5	4	80.0
4	4	3	75.0	6	2	33.3	3	1	33.3
5	4	4	100.0	5	2	40.0	5	3	60.0
6	4	2	50.0	6	2	33.3	5	1	20.0
7	6	2	33.3	6	2	33.3	6	0	0
8	6	2	33.3	5	2	40.0	6	0	0
9	5	1	20.0	6	2	33.3	6	0	0
10	3	1	33.3	6	1	16.7	4	0	0
11	4	2	50.0	6	0	0	6	0	0
12	6	1	16.7	6	0	0	6	0	0
13	6	1	16.7	6	0	0	6	0	0
14	4	0	0	5	0	0	6	0	0

very active motion at the time of insemination. The fertilizing capacity of the stored semen can be measured neither by motility nor viability as PARKER, MCKENZIE and KEMPSTER (1942) and WILCOX and SHAFFNER (1957) have already pointed out.

SUMMARY

1. The beneficial effects of dilution were clearly shown. In the 5-time dilution of semen a full fertility appeared until the 7th day after insemination, whereas this lasted only for 3 days in the undiluted semen.

2. Further dilution than 5 times lowers the fertility rate; this may be due to the excessive reduction of sperm number.

3. Three kinds of egg-yolk citrate buffer were compared with the physiological saline in respect to the fertilizing capacity of stored semen.

4. In 5-time dilution the best result was obtained from the semen buffered with fowl egg-yolk citrate.

5. In this buffer the semen injected immediately after collection showed 100 percent daily fertility with a slight intermediate dropping from the third to fifth day following insemination. The fertility potential was maintained until the twelfth day after insemination (Table XI, Flock I).

6. The semen of 24-hour storage showed 83.3 percent fertility on the third day following insemination; the fertility potential was maintained until the ninth day (Table XI, Flock II).

7. The daily fertility of the semen stored for 48 hours was almost the same

as that of the semen stored for 24 hours. However, the duration of the fertility was quite different; it was maintained only for 5 days in the case of 48-hour storage (Table XI, Flock III).

8. Considering that only little is known about the successful storage of avian semen, the above findings about 24- and 48-hour storage times should be considered very promising for practical purpose.

9. The problem of maintaining a full fertility level will be easily solved by a periodical insemination with a cycle of five days in the case of immediate use of semen after collecting and with a cycle of three days in the case of 24- or 48-hour storage.

10. Further extension of the cycle of periodical insemination will be possible by developing a more suitable buffer.

VII. APPLICATION OF ARTIFICIAL INSEMINATION FOR MULE-DUCK PRODUCTION

The mule-duck is a hybrid bird produced by mating of the Common duck with the Muscovy drake. In spite of its excellent meat qualities, its production is not a little impeded because of the low fertility of intergeneric hybrid eggs. According to a report of the Taichung Animal Industry Association, Taiwan (1943), of 4,952 hybrid eggs incubated only 2,094 eggs were fertile, showing a fertility of 42.3 percent. This partial infertility has been believed by some people of Taiwan to be caused by a difference in mating behavior of the parent species: the Common duck has a water-copulatory habit and the Muscovy, a land-copulatory habit.

On account of the importance of mule-duck raising from the practical point of view, the experiments of artificial insemination for mule-duck production were undertaken with the aim of promoting the fertility of intergeneric hybrid eggs.

The birds used for the present experiments consisted of an adult Colored Muscovy drake and five 2-year-old Common ducks. While this Muscovy drake was that raised in Okayama Prefecture, the Common ducks were of the same strain of Osaka breed as used in the foregoing experiments. Besides the said drake, 5 males and 3 females of Muscovy ducks, raised in the author's laboratory pens, were subjected to subsequent observations. They were secured by incubating either the eggs transported by air from Taipei, Taiwan, or the eggs produced in this laboratory. The initial eggs were kindly presented by Dr. E. RYU, Professor of The College of Agriculture, National Taiwan University, Taipei, Taiwan, to whom the author wishes to express his heart-felt thanks on this occasion.¹⁾

1. Copulatory Behavior of Muscovy Drake in Mating with Common Duck

The average live weight of the Common duck used in the present experiments was 2.3 kg in the male and 1.8 kg in the female. On the other hand, the Muscovy

1) Of 22 eggs shipped from Taiwan 5 samples were damaged by transportation. No data on the time of laying were accessible but 17 eggs incubated resulted 35.2 percent fertility (6: 17), 66.7 percent hatchability (4: 6) and 33.3 percent embryonic mortality (2: 6).

drake here used for mating showed the live weight of 4.2 kg which was much heavier than the average value of $3,752.50 \pm 146.86$ g given by SOKOLOVSKAJA (1935) (Pl. IV, Fig. 17 and 18).

The Muscovy drake exhibited much stronger sexual activity against the Common duck than the Common drake but a great difference both in body size and weight is likely to cause a copulatory hindrance. The Muscovy drake mounting on the back of the Common duck has to stabilize his body with great effort though the duck showed her receptivity. This would make the burden too heavy for the duck sitting on ground instead of floating on water. As a matter of fact, the Muscovy drake, upon ejaculating, falls down aside the duck without withdrawing the penis from the duck's body, until the latter escapes from the former, stands up and cleans her body with her bill. Unlike the Common duck, the Muscovy drake standing up later runs to a basin to clean his body. In all cases, so far no copulation was observed ending without ejaculatory response.

2. Collection of Semen from Muscovy Drake

Unlikely the Common drake, the Muscovy drake hardly shows an ejaculatory response to massage. This is partly due to the lack of docility of the Muscovy drake and also to too much breadth between both the thighs of this bird to apply the massage. Therefore, in the present experiment, the electrical method had to be employed for the collection of semen.

The same procedures as those described for the Common drake were taken except that a weaker current was applied: 20 volts and 40–50 milliamps in place of 30 volts and 60–80 milliamps. In this case also, the electrical stimulation could be applied without any deleterious effects to the bird: the drake has been used for the semen collection for these three years and still it is being used.

Semen was regularly collected once a week from December 5, 1957 to November 30, 1958 in order to find out if there is any seasonal variation as well as to use for occasional insemination.

3. Characteristics of Muscovy Semen

Every ejaculate was examined immediately after collecting in respect to its characteristics. Generally speaking, the semen in spite of electrical collection, was very thin and appeared milky white in color, similarly to the semen of the Common duck collected by massage. At examination just after collection, about 85 percent of sperms always exhibited very active motility.

Table XII gives a record of the characteristics of Muscovy semen examined throughout the year.

A certain seasonal variation of the semen characteristics of the Common duck has been shown above (Table III). In the case of the Muscovy also, the similar variation was found. The total number of sperm per ejaculate is greatest in June, and smallest in November and December, although the curve, if drawn, will never be smooth. In comparing the Table XII with the Table III obtained from the Common duck, the most conspicuous fact is that, of the Muscovy semen, the sperm

Table XII. Seasonal variation of characteristics of Muscovy semen obtained electrically
(from December 5, 1957 to November 30, 1958).

Week No.	Volume per ejaculate (ml.)	Sperm concentration per cu. mm.	Total number of sperms per ejaculate	Monthly average
Jan.	1. 0.18 2. 0.35 3. 0.32 4. 0.35	599,000 305,000 220,000 70,000	107,820,000 106,750,000 70,400,000 24,500,000	77,368,000
Feb.	1. 0.35 2. 0.50 3. 0.20 4. 0.30	430,000 190,000 90,000 450,000	150,500,000 95,000,000 18,000,000 135,000,000	99,625,000
Mar.	1. 0.50 2. 0.30 3. 0.20 4. 0.32	240,000 300,000 380,000 366,000	120,000,000 90,000,000 76,000,000 117,120,000	100,780,000
Apr.	1. 0.35 2. 0.30 3. 0.40 4. 0.40	32,000 22,000 45,000 50,000	11,200,000 6,600,000 18,000,000 20,000,000	13,950,000
May	1. 0.20 2. 0.60 3. 0.50 4. 0.60	61,000 114,000 64,000 158,000	12,200,000 68,400,000 32,000,000 94,800,000	51,850,000
Jun.	1. 0.60 2. 0.70 3. 0.30 4. 0.60	330,000 298,000 134,000 284,000	198,000,000 208,600,000 40,200,000 170,400,000	154,300,000
Jul.	1. 0.20 2. 0.40 3. 0.50 4. 0.40	208,000 96,000 80,000 108,000	41,600,000 38,400,000 40,000,000 43,200,000	40,800,000
Aug.	1. 0.30 2. 0.50 3. 0.30 4. 0.40	236,000 294,000 142,000 166,000	70,800,000 147,000,000 42,000,000 66,400,000	81,550,000
Sep.	1. 0.40 2. 0.50 3. 0.50 4. 0.30	158,000 102,000 218,000 460,000	63,200,000 51,000,000 109,000,000 138,000,000	90,300,000
Oct.	1. 0.30 2. 0.20 3. 0.30 4. 0.10	80,000 4,000 96,000 14,000	24,000,000 800,000 28,800,000 1,400,000	13,750,000
Nov.	1. 0.10 2. 0.30 3. 0.10 4. 0.20	20,000 3,000 10,000 1,000	2,000,000 900,000 1,000,000 200,000	1,025,000
Dec.	1. 0.20 2. 0.10 3. 0.05 4. 0.05	35,000 83,000 128,000 212,000	7,000,000 8,300,000 6,400,000 10,600,000	8,075,000

number both per cubic millimeter and per ejaculate are strikingly smaller than those of the Common duck, although the semen volume in the former is apparently larger than in the latter; in fact, Muscovy semen shows macroscopically much thinner consistency. In maximum sperm concentration, the Muscovy semen is about 28 times smaller than that of the Common duck (Jan. 1st wk. in Table XII and March 3rd wk. in Table III), and the Muscovy is about 23 times inferior to the Common duck in its sperm productivity at their respective peaks on monthly average (June-average in Table XII and May-average in Table III).

4. Insemination of Common Ducks with Muscovy Semen

Inseminating experiments with the aim of hybridizing Common ducks with a Muscovy drake were conducted on September 23, 1958, though it was an unfavorable breeding season for ducks as seen above in the sperm productivity. The results obtained from the inseminations in a worse breeding season, however, may give expectations of gaining better fertility rates, if these would be done in a better breeding season.

An ejaculate recovered from a Muscovy drake was allotted for insemination to five Common ducks which were beforehand proved to be good layers and strictly isolated from drakes. The semen secured was immediately diluted by 10 times with 0.85 percent physiological saline solution and each 0.3 ml. of this mixture was simultaneously injected into the five ducks. Since the total volume of the ejaculate was 0.5 ml. containing 102,000 sperms per cubic millimeter, the total number of sperms each duck received was 3,060,000, each sample containing 10,200 sperms per cubic millimeter. At the microscopical examination of semen immediately after collection, about 85 percent of sperms exhibited very active motility. The introduction of the semen was made by employing a speculum.

All the eggs laid during two weeks following the insemination were tested by putting into an incubator at 10.00 a. m. every day and the eggs verified to be fertile by candlings were further incubated to hatching.

The total number of hybrid eggs produced by the five ducks during a week following the insemination were 28 consisting of 20 infertile and 8 fertile eggs. Since the onset of fertilization takes place on the second day following insemination, 3 eggs laid on the first day following the insemination should be eliminated from accounting the fertility of introduced semen. If so be, the 6-day fertility rate at a single insemination for this flock will be 32.0 percent (8:25). Table XIII gives the results of insemination with the heterologous semen accompanying the fertility percentage per bird per day.

The Muscovy semen released in the oviduct of the Common duck kept fertility for 6 days at most, which did not differ from the insemination with the homologous semen (cf. Table VIII). However, as far as the daily fertility rate was concerned, it appeared that the semen of these two duck species were not always identical in physiological efficiency. On the second day following the insemination when the maximum fertility rate was expected, the heteroinsemination showed 75 percent fertility whereas it was 80 percent in the homoinsemination. On the fourth day it

Table XIII. Results of simultaneous inseminations of 5 Common ducks with fractions of a Muscovy ejaculate electrically obtained. The semen was diluted 10 times with physiological saline solution (September 23, 1958).

No. day following insemination	No. ducks inseminated					Number of eggs laid	Number of fertile eggs	Daily fertility (%)
	1	2	3	4	5			
1	0	—	0	—	—	3	0	0
2	0	+	+	—	+	4	3	75.0
3	+	—	+	—	0	4	2	50.0
4	+	—	+	0	—	4	2	50.0
5	—	—	—	—	—	5	0	0
6	—	0	0	—	—	3	0	0
7	—	—	+	—	—	5	1	20.0
8	0	0	0	—	0	1	0	0
9	0	0	—	0	—	2	0	0
10	0	0	—	—	—	3	0	0
Total number of eggs laid from 2 to 7 day following insemination						25	8	6-day fertility rate 32.0 (8:25)

decreased to 50 percent in both cases. The ratio of the number of fertile eggs to the total number of eggs produced during the period from the second to the seventh day following a single insemination of Common ducks with Muscovy semen was 32.0 percent (8:25), comparing with 38.6 percent (22:57) fertility of the homologous semen. Although the number of birds tested here is small, it is clear from this experiment that the repetition of every-three-day insemination will be very effective for retaining a satisfactory level of fertility higher than that obtainable in natural matings.

5. Hatchability, Pre- and Post-natal Development of Mule-Ducks

As for the hatching, 6 hybrid ducklings were secured from 8 fertile eggs showing 75 percent (6:8) hatchability and 25 percent (2:8) embryonic mortality. Although the two unhatched eggs had exhibited a vascular development at the first candling carried out on the fifth day of incubation, they showed no trace of embryonal tissues excepting the remains of the vascular system when they were examined by breaking their shells a week later. This suggests that the embryos had died in a very early stage of incubation, probably at the time of the first candling or thereabout. The secondary sex ratio revealed 200 (♂♂ 4:♀♀ 2), the male being exceedingly predominating though the number of hatched eggs is not enough to give a decisive conclusion.

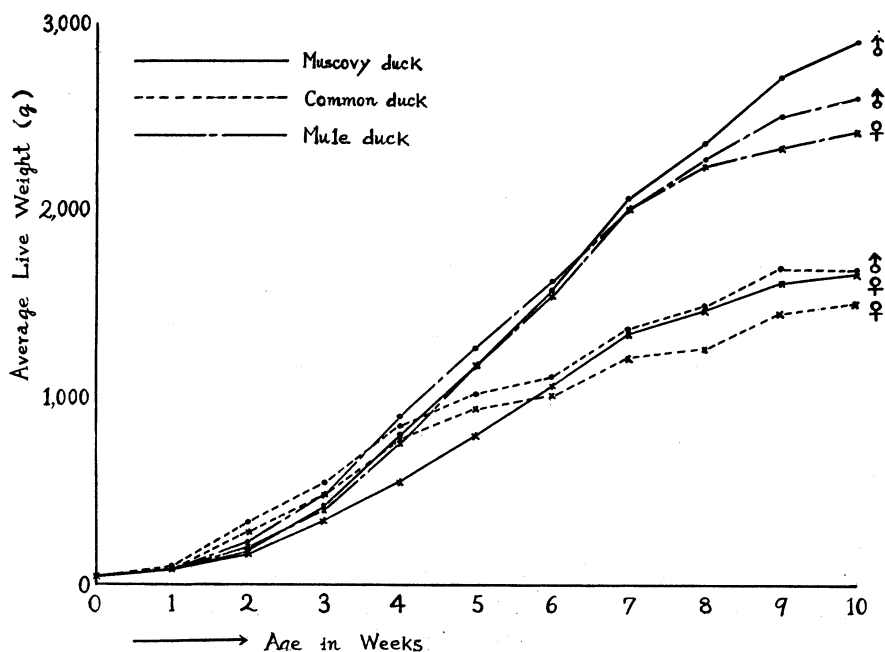
In the Common duck here observed, the length of incubation varied from 27 to 29 days with an average of 28 days, while that of the Muscovy varied from 35 to 37 days with an average of 36 days. Hybrid eggs occupied an intermediate position between those of the two parental species, ranging from 29 to 32 days with an average of 31 days in consistence with the finding of FABER (1957).

In live weight from hatching to the third week of age there were very little differences among the three breeds of ducks but with age they exhibited their own growth rates (Table XIV; Text-fig. 5).

Among them the Common duck grew most slowly, both the male and female showing almost the same growth rates. In 10 weeks after hatching, the male Com-

Table XIV. The average rates of growth of mule-ducks produced by insemination in comparing with those of their parent ducks. The figure in a parenthesis indicates the number of birds determined with regards their live weight (g).

Age of ducklings	Common duck		Muscovy duck		Mule-duck	
	♂ (3)	♀ (14)	♂ (3)	♀ (1)	♂ (3)	♀ (1)
At hatching	46	43	50	47	43	42
1 week after hatching	99	84	81	78	85	82
2 weeks " "	331	280	182	164	232	202
3 " " "	542	480	421	342	483	400
4 " " "	847	782	803	550	905	761
5 " " "	1,020	949	1,177	800	1,267	1,181
6 " " "	1,121	1,023	1,579	1,065	1,628	1,551
7 " " "	1,384	1,225	2,076	1,350	2,013	2,020
8 " " "	1,505	1,277	2,372	1,480	2,293	2,250
9 " " "	1,716	1,468	2,728	1,625	2,520	2,350
10 " " "	1,703	1,526	2,920	1,680	2,618	2,440



Text-fig. 5. Graphs showing the growth rates of Common ducks, Muscovy and Mule-ducks.

mon duck weighed 1,703 g and the female, 1,526 g. On the contrary, the Muscovy duck exhibited a conspicuous sexual dimorphism in live weight; the male Muscovy showed the most rapid growth, whereas the growth rate of the female Muscovy did not vary greatly from that of the male Common duck. The live weight of the 10-week old male was 2,920 g and that of the female of the same age was 1,680 g. In this respect, the findings on the mule-duck were most remarkable; the male mule-duck had very high growth rate similar to that of the male Muscovy, attaining 2,618 g live weight at the age of 10 weeks and the female mule-duck of the same age also attained almost the same live weight as its male, that is, 2,440 g. Thus the mule-duck produced by artificial insemination shows the prominent character as a meat bird so well as that produced by natural mating.

SUMMARY

1. An attempt was made to increase the fertility rate of hybrid eggs in mule-duck raising, applying the same technique of artificial insemination as used for the Common duck.

2. The semen of the Muscovy drake could be obtained by electrical stimulation with regular success, whereas the massage method was hardly applicable for this bird.

3. It was found that there is a vital difference in testicular activity between both the duck species, Muscovy and Common duck, the semen of the former showing fewer sperms both per cubic millimeter of semen and per ejaculate than that of the latter. The Muscovy semen, compared with that of the Common duck, is about 28 times smaller in the maximum sperm concentration and 23 times smaller in the maximum number of sperms of the monthly average (Tables III and XII).

4. Beside the difference in quantitative characters of the semen between the Muscovy and the Common duck, there exists a qualitative difference indicating lower physiological efficiency of sperms of the Muscovy.

5. In spite of lower fertility in the Muscovy, a single insemination with its semen allotted to five Common ducks showed 75 percent daily fertility per flock on the second day and 50 percent on the third and fourth day after the insemination. This suggests a high possibility of utilizing artificial insemination for mule-duck production, when it is performed every two or three days per bird.

6. Since the Muscovy semen in the present study was used for insemination without buffering but at relatively high dilution with the physiological saline solution, more satisfactory fertility level may be obtained, if the two points, a suitable buffer solution and a less dilution rate, will be further considered.

7. There was a tendency for prenatal disturbances which seemed to be caused by an intergeneric hybridization: A high embryonic mortality (25 percent) and a predomination of the male in the secondary sex ratio (=200) of the hybrid bird.

VIII. DISCUSSION

The present paper dealing with the artificial insemination of ducks in connec-

tion with their reproductive physiology involves many problems hitherto obscure. Among them are some main problems taken in consideration for discussing in order.

1. Techniques of Semen Collection

Since the publication of classical work of IVANOV (1913), a number of methods of semen collection from the male bird have been reported. Aside from the post mortem collection by IVANOV, the techniques which are combined with the natural mating have many disadvantages in training the birds and in receiving semen without contamination or loss. In this impasse BURROWS and QUINN (1935, 1937) made the way of collecting semen, either applying abdominal massage or milking the copulatory organ of the male bird. In this respect KAMMAR (1958) has given a very useful review categorizing the techniques of collecting cock semen in a chronological order.

For the water-fowl, however, semen collection has been rarely reported and still now remains with a limited scope of experiments.

KUZIMINA (1933) was the first to report on the semen collection with success from drakes by applying a pessary to the cloaca of ducks. YAMASHINA (1950), ŌNISHI, FUTAMURA, KATO and AKAMATSU (1950) and ŌNISHI, KATO and FUTAMURA (1955) successfully applied massage method to domestic ducks, and likewise ROSE (1953), JOHNSON (1954) and KINNY and BURGER (1960), to geese. Another technique of semen collection an ejaculatory response by electrically stimulating the male bird. SEREBROVSKII and SOKOLOVSKAJA (1934) seemed to be the first authors who reported about the electrical stimulation applied to birds: 1 cock, 1 goose, 2 drakes, several pheasants and 13 guinea fowls. As the original paper of the authors was not accessible, a reference may be made here from an abstract in the Animal Breeding Abstract 3:73-74 (1935):

"Ejaculation could be induced by an electric current, the positive pole being applied to the skin of the bird in the sacral region, the negative pole placed in a basin of water, into which the beak was immersed. As a rule a weak shock of 45 volts was given first, which caused spasms and sometimes ejaculation, but usually ejaculation was only produced by four stronger shocks (80 volts), each of 3-4 seconds' duration, with 1-2 seconds' intervals. Most of the experiments were successful, and normal sperm was regularly collected, though in small quantities. In several instances (e. g. some of the pheasants) failure was found to be due to sterility. No injurious influence was noticed and experimental casualties were few."

NISHIKAWA (1951) describes briefly his observations on the electrical stimulation of the fowl with alternating current at 40 volts applied per rectum and hypodermis of the sacral region in accordance with the method of GUNN (1936) devised for the ram. The author, however, is of opinion that the cock semen thus procured is too small in quantity to be used for practical purpose.

In this uncertain state of affairs, the present experiments were undertaken in using the two methods of semen collection: massage and electrical stimulation, with the aim of some technical improvements. Both the methods showed more or less advantages in practical use.

The massage method relying on the psychic response of the bird needs generally a long period of time for training the bird, commonly 10 to 15 days, which is

undoubtedly a laborious work for duck breeders. Besides, there are some birds which never respond to this operation. On the contrary, the electrical stimulation induces an ejaculation of semen quite reflexively regardless of preceding training or individuality of birds. No evil effect upon the operated bird is found and some skin injuries sporadically seen at massage can be avoided by this method. For semen qualities, too, the electrical method has a certain advantage as the semen is obtainable in less contaminated state. Hence, from the standpoint of operation, the electrical method is decidedly preferable to the massage. Two questions must, however, be raised before conclusion. One relates to the fertility and the second to the hereditary effect of the semen thus obtained.

Since the electro-ejaculation is an entire reflex without accompanying orgasm, the ejaculate thus secured is deficient in accessory sex secretions commonly known as seminal plasma. The sex secretions cannot be considered as ineffective for the viability and fertility of sperms. In the Common duck, the sperm concentration was found about 5 times higher and the total per-ejaculate sperm number 8 times greater in the case of electrical stimulation than in the case of massage. On the other hand, in the fertility tests with the semen likewise diluted ten times, the sperms from the electro-ejaculation showed clearly less daily fertility rates and a shorter duration of retaining fertility potential than those from the massage-ejaculation (Table VI, VII & VIII). In that condition, therefore, the semen collected by electrical stimulation is unlikely to be suitable for practical use.

There has been a generalized concept for mammals that the male accessory sex secretions cause a decreased viability and fertility of sperms (GUNN, 1936). The above data on the duck semen electrically collected are certainly in contradiction to this postulation. As a consequence, it seems to be more reasonable to attribute the low resultant fertility of the electro-ejaculated semen to the direct influence of electric current applied, possibly not only upon sperm cells but also upon their precursors during the spermatogenesis. In the present experiments, however, the electro-ejaculated semen, when it is diluted ten times with fowl egg-yolk citrate buffer, exhibited an average viability of 126 ± 41.11 hours with a range of 48–192 hours (Table IX) and a possibility of retaining 100 percent daily fertility for 4 days in association with 12 days of the maximum duration of fertility (Table X, Flock I). Hence, there is little reason to believe any possible effect of electric current applied and the electro-ejaculated semen can be used safely for practical purpose, if it is diluted with a suitable buffer.

More important is the problem of the effect of continuous electrical stimulation of drakes upon the offspring produced by insemination with their semen. As above mentioned, there has been no deleterious effect upon the parent birds operated but this does not always mean the absence of the indirect effect of electric current upon their offspring. With regard to the effect of electric stimulation on the chromosomes of germ cells the present knowledge is almost nil. Unless a comprehensive progeny test is made, no reliable conclusion will be gained, but it is outside the scope of the present experiments performed on a laboratory scale. At any

rate, there is nothing to worry about this, as far as it concerns the production of meat animals or animals of F_1 generation as mule-duck.

2. Techniques of Semen Introduction

Although application of a vaginal speculum for inducing the semen pipette is a usual technique for the insemination of mammals, no speculum has yet been used for birds. In the above experiments the convenient use of the speculum newly devised accommodating to duck's genital organs was demonstrated with regular success by a lot of fertility tests; it could be used with striking readiness neither giving any injury to birds nor reducing the fertility rate of eggs produced. It has so far been fully established that the use of speculum has the advantage over the palpation technique in the semen introduction to the oviduct.

As for the time of semen injection, there have been somewhat contradictory opinions for the fowl. It has been a generally accepted opinion that the presence of a hard-shelled egg in the uterus affects the fertility of forthcoming eggs, thence the insemination should be performed in the afternoon when the uterus is empty. The other opinion represented by SCHINDLER *et al.* (1957) is that no difference is found between the hens inseminated while containing a shell-egg in their uterus and those not containing such an egg with regard both to the fertility of hens and eggs laid.

In the duck there is as yet no criterion concerning the relation of the presence of a shell-egg to the fertility of forthcoming eggs. However, because of the observations that the semen was usefully obtained without being soiled early in the morning before supplying forages, and that the insemination could be performed more successfully with semen as fresh as possible, the forenoon insemination is more preferable for the duck. If the presence of shell-egg plays an inhibitory role in the fertilization of subsequently released eggs, the insemination in the forenoon should be decidedly preferable because ducks lay their eggs very early in the morning and the forenoon must be just the time when their uterus is empty.

3. Characteristics of Semen of Common Ducks

As regards the initial motility of sperms, the fresh, unsoiled ejaculate apparently shows the most satisfactory quality, regardless of methods of collection, about 80–90 percent sperms exhibiting very active movement producing strong currents in semen drops. The quantitative characteristics of semen, however, are remarkably different according to the techniques of collection. The sperm concentration of the semen electrically collected is 5 times higher and the semen volume is markedly smaller than the semen collected by massage; consequently the total per ejaculate number of sperms of the former is about 8 times as great as that of the latter. Besides, the interval of times of collecting semen, that is, the frequency of collection even by the same method, plays a great role in the variation of the numbers of sperms per cubic millimeter as well as per ejaculate. Generally speaking, a greater volume of semen is obtained after two- or three-day rest than after one-day rest within a limited period of time. As a rule, this is consistent with the findings of MUNRO (1938)

and BURROWS and TITUS (1939) in fowls, though the relationship between the frequency of collection and the volume of semen is no absolute one in the case of ducks; it was peculiar to observe that the drake could gradually adapt to the massage producing a greater volume of semen at an one-day rest than the volume of semen collected at a two- or three-day rest.

As one of the effective factors causing fluctuating in semen characteristics, not to be overlooked is the season, as has been reported on the fowl semen by several investigators: PARKER, MCKENZIE and KEMPSTER (1942), PARKER and McSPADDEN (1943), WHEELER and ANDREWS (1943). In the duck, the total number of sperms per ejaculate is at its highest during the period from the middle of March to the beginning of July, rising to a peak in May, dropping to a bottom in the last half of August and recovering gradually from the end of September. Since the total number of sperms per ejaculate is regarded as a reflexion of degree of testicular activity, of the Common duck, this being consistent with the season of the highest production of eggs. On the contrary, the period from the fourth week of July to the third week of September appears to be the most unfavorable season for the breeding of Common ducks (Table III). This is also supported by the two experiments of insemination of 15 ducks at two different periods: the first half of September and of October; greater fertility rates and longer duration of retaining the fertilizing capacity of sperms were found in the experiment in October compared with the results in September.

Thus, the problem of semen characteristics seems to be too complicated to get a generalized concept, because of too many factors involved, not to say the nutrition, leading to fluctuation both in quality and quantity of the semen.

4. Effects of Dilution Rates of Semen of Common Ducks upon Fertility

The practical importance of artificial insemination in poultry is to make the best use of the sire of top performance for breeding, by allotting a single ejaculate to as many dams as possible. On account of this, the problem of dilution of semen must be the first to take into consideration.

The effects of dilution of semen upon the viability and fertility of sperms are to be considered from the two aspects, provided that the dilutor itself is not harmful: One from the reduction of the effect of accessory sex secretions and the other from the reduction of sperm concentration.

It has been known in mammals that a ripe sperm stores a limited amount of energy, thence, whatever stimulates activity or the expenditure of that energy shortens its life, and that the violent activity displayed by sperms when dispersed in accessory secretions, is a main factor in shortening their length of life (HARTMAN, 1932; GUNN, 1936). There may be a similar phenomenon in birds, too.

In the present experiments, duck sperms just after collection exhibited always violent activity and retained a full fertility rate until the third day after insemination but a remarkable decrease in fertility was observed from later on. On the contrary, when the semen was diluted 5 times with physiological saline solution, it showed a full fertility rate until 7th day after insemination. This indicates clearly

a beneficial effect of dilution of the accessory secretions to a certain extent for the viability and fertility of sperms (Table IX). Since this was the result of a single insemination per bird, it follows naturally that a full fertility level will be maintained, if the insemination is carried out periodically with a cycle of six or seven days in the case of using the fresh semen (Table IX).

Considering that the physiological saline solution used for dilution contains no sources of energy for sperm activity, its beneficial action should be quite of physical nature and attributable, on one hand, to the reduction of the accessory secretions stimulating sperm activity, on the other, to the reduction of sperm concentration. The latter effect obviously relates to lowering the accumulation of metabolic products released from sperm cells. There is, however, a limit of beneficial effects of dilution because the experimental results showed that the time retaining a full fertility level was remarkably shortened when the semen was diluted 10 and 20 times. This concerns undoubtedly the excessive reduction of sperm concentration which naturally reduce the total number of sperms per fraction of semen to be injected.

According to PARKER, McKENZIE and KEMPATER (1940) the fowl semen obtained by massage contains on the average 2,340,000 sperms per cu. mm. of semen and 868 millions per ejaculate. Using Ringer's solution, BONNIER and TRULSSON (1939) found that inseminations of hens with the semen of 3 times dilution gave better fertility than with pure semen and with the semen of 10-time dilution about the same fertility as with undiluted, but there was a decline in fertility from the semen diluted 50 times. ALLEN and SKALLER (1958) reported also that fowl semen, diluted 10 to 13 times with Tyrode's solution, when inseminated at the rate of 0.2 ml. twice weekly gave fertility results equal to those expected from undiluted semen or natural matings. Despite the fact that the duck semen contains a smaller number of sperms than that of the fowl, and that the dilutor used was deficient of nutritive substances, 10-time dilution of duck semen showed better fertility than in the fowl. This seems to indicate that duck sperms have a greater physiological efficiency than the fowl semen. There are, however, a considerable number of the authors who could not confirm such a beneficial effect of dilution: MUNRO (1938), GORDON and PHILLIPS (1951), SCHINDLER *et al.* (1955), ROWELL and COOPER (1957), and WILCOX (1958) for fowl semen, and GILBREATH and DAVIS (1949), Van TIENHOVEN and STEEL (1957), and SMYTH and KINNEY (1958) for turkey semen. This contradiction in view of the dilution of semen is very likely to be ascribed rather to the chemical properties of the dilutors which associated with the viability of sperms.

5. Effects of Chemical Composition of Dilutors upon Fertility of Semen of Common Ducks.

An analysis of the data (Table XI, Flock I) appears to indicate the full fertility of duck semen lasting to the fourth day after insemination when it was diluted 5 times with a fowl egg-yolk citrate buffer. Although the rates dropped on the second and fourth day, these can be assumed to fall into the period of retaining the full fertility potential. In contrast to this, for the semen diluted likewise 5 times with the physiological saline solution, the full fertility lasted until the sixth day after in-

semination (Table IX), showing two days longer duration of fertility potential. This difference in the effects of both the dilutors, the fowl egg-yolk citrate buffer and physiological saline solution, upon maintaining the fertility potential seems to show that the egg-yolk citrate buffer hitherto applied to the mammalian semen is not always the most suitable one for the duck semen.

Up-to-date, unlike mammals, the successful insemination of birds with stored semen is very little known, though the viability of stored sperms has been manifoldly reported. According to BURROWS and QUINN (1939) the cock semen stored both at 4.4°C and 20°C loses its fertilizing capacity within two and eight hours respectively. JASPER (1950) could obtain only one fertile egg by inseminating with cock semen that had been stored for 20 hours at 4.4°C in human serum, while GARREN and SHAFFNER (1952) secured 38 and 6 percent fertile eggs with undiluted cock semen stored at 5°C for 3 and 4 hours respectively. In the similar experiments of SCHINDLER *et al.* (1955) they found that the fowl semen diluted with whole milk and stored for 24 hours at 4°C gave only 19 percent resultant fertility though undiluted semen and the semen diluted with either Ringer's or Rock's solution or with whole milk, retained a full fertilizing capacity after 4 hour's storage at 10°C. The experiments of LAKE, SCHINDLER and WILCOX (1959) transporting fowl semen by air from U. S. A. to Israel and Scotland gave better results. The semen buffered with a phosphate solution containing antibiotics was cooled in an insulated container. The fertility rate of the semen thus transported to Israel was 38 percent after 38 hours and that arrived in Scotland, 36 percent after 37 hours, computed from eggs laid within the first week following insemination. Quite recently LAKE (1960) reports the most promising results obtaining 64 and 47 percent fertility from the semen stored for 24 and 48 hours respectively at 0° to 2°C in a glutamate-containing saline solution added with fructose.

The present data on duck semen reveal also that, for the storage of semen, the more suitable buffers should be developed if its storage is necessary, although this appears to be of secondary importance in poultry husbandry.

6. Mule-Duck Production by Applying Artificial Insemination

In the present study, a striking performance of the mule-duck as a meat bird has been definitely shown (Table XIV and Text-fig. 5).

The male mule-duck gains almost the same live weight in 10 weeks as the male Muscovy. There is but little sexual dimorphism of live weight in the mule-duck, while this is much pronounced in the Muscovy. The Common duck shows only a little sexual difference in live weight but both the live weight and growth rate of this bird are small compared with those of the mule-duck. Sexual similarity in the live weight of the mule-duck is probably inherited from the mother bird, Common duck, and the big final body weight and the rapid growth rate may be due partly to the paternal inheritance and the heterosis.

The bottleneck in the mule-duck raising is, however, that the fertility of hybrid eggs is exceedingly low as has been above referred. This partial infertility of hybrid eggs has been ascribed by some duck-breeders in Taiwan to a difference in mating

behaviors between the parental species, the Muscovy being a land-copulatory bird and the Common duck, a water-copulatory bird. In the present study, observations of natural matings between the Muscovy drake and the Common duck, has revealed that the copulation is actually impeded by the difference in body weight between both the duck mates, the adult Muscovy drake being 2.3 times as heavy as that of the adult Common ducks. In spite of the vigorous sexual activity of the Muscovy drake and the receptivity of the Common duck, the latter can hardly bear the weight of the former riding on her back. It appeared that the conditions should be different if the copulation happened floating on the water, because this is usual habit of the Common duck. Nevertheless, no case was met with where the copulation was interrupted without showing ejaculatory reflex. It is therefore not likely that the partial infertility of hybrid eggs is caused by the physical hindrance in natural matings of the two duck species. The main reason should be rather sought in the characteristics of Muscovy semen (WATANABE, 1959).

The Muscovy semen, compared with that of the Common duck, is about 28 times smaller in the maximum sperm concentration and 23 times smaller in the peak number of sperms of the monthly average (Table III and XII). Therefore, it may well be doubted that the inseminations of Common ducks with the Muscovy semen diluted 10 times will result in lower fertility rate than in the case of inseminations with the Common duck semen of the same dilution rate. As a matter of fact, it appeared a marked difference in the weekly fertility, revealing 32.0 percent (8:25) of the Muscovy semen and 42.2 percent (19:45) of the Common duck semen, notwithstanding that the semen of both ducks were collected in the same manner by electrical stimulation (Tables VII and XIII). A further analysis of the daily fertility rates resulting from the inseminations of Common ducks with the homologous and heterologous semen, seems to indicate less physiological efficiency of the sperms of the Muscovy duck compared with the semen of the Common duck. There is a tendency of losing the fertilizing capacity earlier of the Muscovy sperms than those of the Common duck.

In the literature of duck breeding, YAMASHINA (1950) was the first worker who attempted to apply the artificial insemination to the mule-duck production; he secured 6 birds out of 14 intergeneric eggs produced by mating the female Muscovy ducks with a male Common duck, showing a fertility of 42.8 percent. However, a reciprocal cross, namely a cross between the Common ducks with a Muscovy drake brought no satisfactory results in fertility. The cause of this failure was ascribed by the author to the sterility of the male Muscovy employed, because the semen was found to be devoid of sperm cells. Later, ŌNISHI & KATO (1955) succeeded in obtaining fertile eggs by artificial insemination of Common ducks with Muscovy semen. However, no marked increase in the fertility rate of hybrid eggs was attained by inseminating beyond that secured by natural matings, the rate of the former being 34.2 percent (26:76) and that of the latter 32.0 percent (96:300). Thus, the fertility rate of hybrid eggs, whether artificially inseminated or naturally mated, was lower than that of Common duck's eggs procured by the homo-insemination in

another experiment (ÖNISHI, KATO & FUTAMURA, 1955); in the latter experiments the fertility rate was 54.2 percent (130 fertile eggs to 240 produced).

It is known that the Common duck in its domesticated state is polygamous and exhibits a high testicular activity almost all the year round excepting a few months, whereas its wild ancestor, the Mallard, is monogamous and breeds only in a certain season. A conversion of the Mallard from monogamous bird into polygamous bird and an acquisition of a habit of breeding all through the year is regarded as the effect of constant selection with respect to its high fecundity, to which this bird has been subjected from time immemorial. In contrast to this, the Muscovy duck of South American origin, though domesticated prior to importation to Europe, seems to have been very little selected with respect to its fecundity. This is to be seen in the lower yield of sperms throughout the year in the Muscovy. It seems, therefore, to be fair to conclude that less resultant fertility rate of the semen of Muscovy is a reflexion of less selection of this bird in respect to fecundity. As for the lower physiological efficiency of Muscovy sperms manifested in the daily fertility rate, it is not certain whether this is of genetical nature or of environmental influence caused by the genital tracts of Common ducks or of both effects in association.

Although the present data applying the artificial insemination for the production of mule-ducks has shown low fertility rate of hybrid eggs, it is not discouraging, if considered, that despite a single insemination made per bird during an unfavorable breeding season (the 4th week, September), the daily fertility was 75 and 50 percent on the second and third and fourth day after insemination respectively. If the insemination had been done every two or three days per bird, the fairly satisfactory fertility level would have been continuously retained. And there is every reason to believe that the fertility rate can be promoted further by using a more suitable dilutor and diluting to a smaller degree than that used here.

From this point of view, it may fairly be said that the development of the mule-duck industry is now within reach by the use of the techniques of artificial insemination described above.

IX. CONCLUSION

Recent advances in the artificial insemination of farm animals have amazingly contributed to the revolutionary development of animal industry.

In the poultry husbandry, however, the situation has been so far quite otherwise; the application of artificial insemination to the poultry has been quite handicapped until BURROWS and QUINN (1935) reported a technique of collection of cock semen. This seems partly to be due to less understanding of the economic importance of artificial insemination for the poultry industry, associated with the technical difficulties of semen collection in birds.

Especially for the duck, no attempt has so far been made for applying the artificial insemination to the practical breeding to any great extent.

In the *status quo*, the results of the foregoing experiments of artificial insemination with ducks justify the conclusion that the techniques there described in collect-

ing and introducing the semen can be put into practice with great ease. The use of an electro-ejaculator for collecting the semen and the use of a speculum newly devised for inseminating have exceedingly simplified the whole procedure for routine insemination purposes. For the Common duck, it has been shown that a full fertility level can be retained by a periodical insemination with a cycle of five days in the case of immediate use of semen after collecting and of three days in the case of 24- or 48-hour storage of it. As regards the promotion of the fertility rate of hybrid eggs in mule-duck production, the results were not so satisfactory as in the Common duck with homologous semen in spite of the same technique applied. This should be partly attributed to the intergeneric crossing of both the birds remotely related and also to the lower spermatogenic activity of the Muscovy than that of the Common duck. Nevertheless, the application of the above techniques resulted in higher fertility rate of hybrid eggs than by natural matings; it suggests also, that this fertility level will be retained, if the insemination will be done periodically every two or three days per bird.

Technique is still being improved, especially for preparing the dilutor most suitable for the semen of duck species. So one may conclude that there is the commercial possibility of setting up the hatcheries applying the artificial insemination on an extensive scale and producing the largest possible number of ducklings. Thereby, a considerable expansion of duck industry is expected in the rice-cultivating countries of Asia, where meat and eggs of this bird are enormously demanded and the great potentialities of forage supply and pasturing are existing.

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EXPLANATION OF PLATES I-IV

Plate I.

- Fig. 1. The pose of a drake fixed on a holder before being massaged; the side-plate is removed.
- Fig. 2. Receiving semen into a tube.
- Fig. 3. Receiving semen from the spirally evaginated penis following massage. The spiral ejaculatory groove is seen on the penis.
- Fig. 4. The same as Fig. 3.

Plate II.

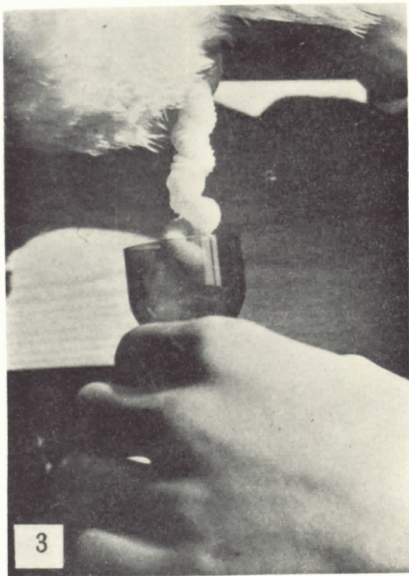
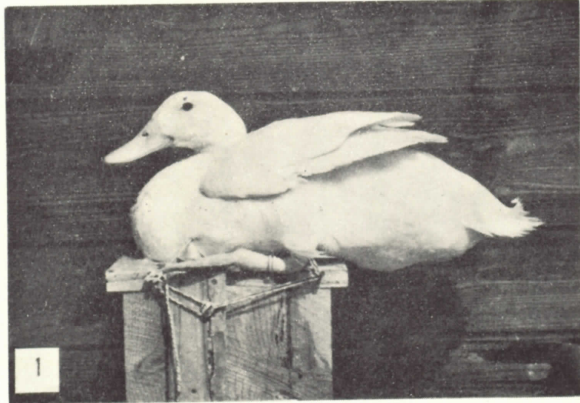
- Fig. 5. An electro-ejaculator constructed by FHK Co. Ltd., Tokyo; the apparatus was applied to drakes in the present experiment.
- Fig. 6. Two electric poles of the electro-ejaculator: the sharp one for sticking to the hypodermis of the ilial region and the blunt rod for inserting into the vent.
- Fig. 7. A drake prepared for turning the current, the two poles being attached.
- Fig. 8. At the moment of turning the current; the bird shows the rigidity of its body.
- Fig. 9. Pressing of the stiff penis with fingers.
- Fig. 10. Collection of ejaculated semen into a receptacle.

Plate III.

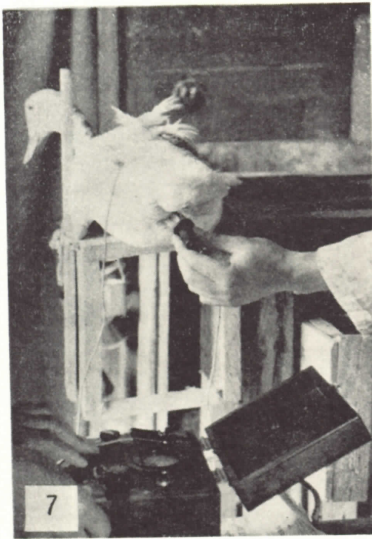
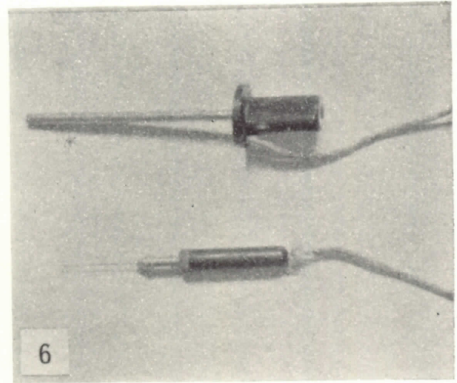
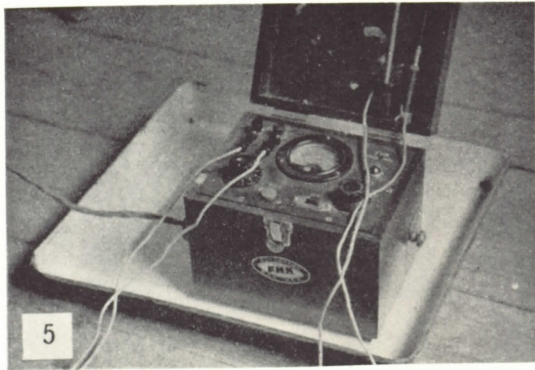
- Fig. 11. A vaginal speculum devised for ducks; a side view.
- Fig. 12. The same as Fig. 11; upper view.
- Fig. 13. Equipments for semen injection: semen vials, speculum and insemination pipette.
- Fig. 14. Orienting the vaginal orifice in the vent.
- Fig. 15. Insertion of the speculum through the vaginal orifice.
- Fig. 16. Insertion of an insemination pipette along the speculum.

Plate IV.

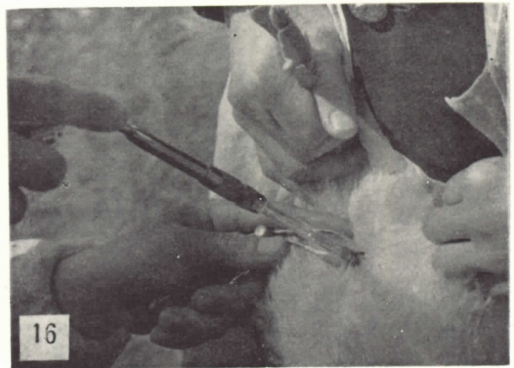
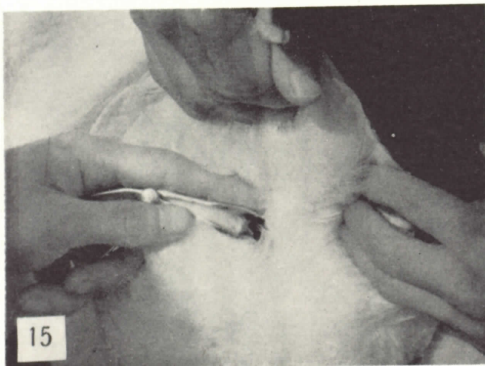
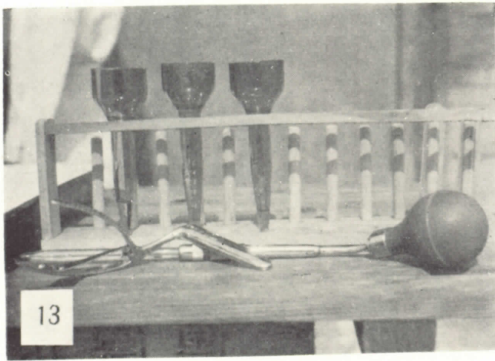
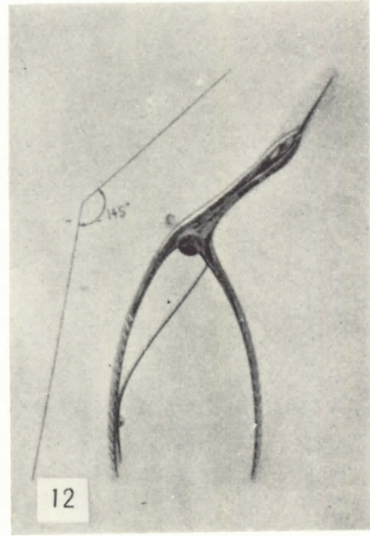
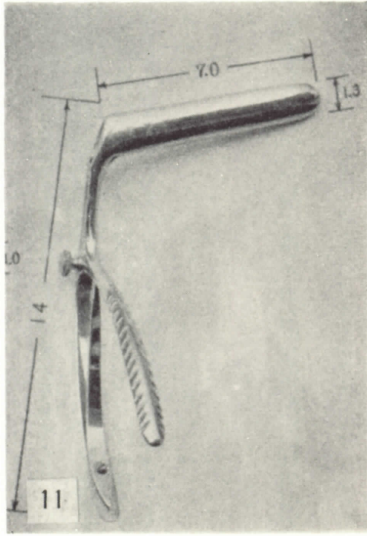
- Fig. 17. A Muscovy drake used for collection of semen; about 2 years old.
- Fig. 18. A Common duck inseminated with the Muscovy semen; about 1 year old.
- Fig. 19. A male mule-duck produced by the insemination; about 14 weeks old.
- Fig. 20. A female mule-duck produced by the insemination; about 14 weeks old.

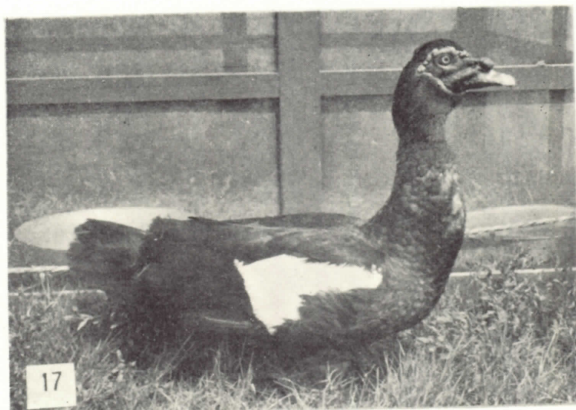


WATANABE : Artificial Insemination of Domestic Ducks



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