On the Ejection of the Accessory Reproductive Fluid of the Cock During Natural Copulation

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Introduction

In a previous paper (Nishiyama, 1955), it was reported that the accessory reproductive organs of the cock consisted of a pair of vascular bodies and a pair of lymph-folds, and the fluid from the accessory reproductive organs of the cock, the so-called transparent fluid, was added to the semen at the moment when it was ejected from the vasa deferentia. The ejaculated semen of the cock was, therefore, a mixture of the semen contained in the vas deferens and the transparent fluid. The origin of the transparent fluid was from blood and therefore was unlike the secretion of the accessory reproductive organs of mammals (Nishiyama, 1955, 1957).

On the contrary, Lake (1956, 1957b) stated that the transparent fluid (Lake termed this fluid blood exudate or blood transudate) was only obtained as a result of forcible compression of the engorged copulatory organ by the massage technique of semen collection, and the fluid was not likely to be a part of the seminal fluid found during natural copulation. He implied (1957b) that the semen obtained by the abdominal massage method (Burrows and Quinn, 1937) was not a normal or true semen of the cock.

On the other hand, the abdominal massage technique has been considered as the most desirable one for the collection of semen from a cock and the technique or its modifications have been widely and successfully used by many workers.

Hence, the problem whether the transparent fluid is a normal constituent of the ejaculate of the cock, is very important, if one is to determine the intrinsic nature of the cock's semen, to study the artificial insemination of the cock and to determine whether the semen which has been studied in the past is normal or abnormal.

The present study was designed to clarify these questions. The study was divided into three phases and each phase was composed of one or more experiments. In the first phase of study, an investigation was made to determine if the fluid from the accessory reproductive organs of the cock was ejected during natural copulation. In the second phase, experiments were performed to ascertain whether the fluid ejected during natural copulation was the same as the transparent fluid which was obtained by the massage method. In the third phase, the detection of aldose in the ejaculated semen was reexamined by the method described by Lake (1957b).

Materials and method

In the first phase of study, three S. C. White Leghorn cocks (Bird No. 361, 398, 399)

and a Barred Plymouth Rock cock (Bird No. 400) were used. Anterior parts of both vasa deferentia of these cocks were ligated with surgical threads, and their openings (Papillae of the vasa deferentia) were also destroyed with an electric cautery, by the method described in the previous paper (Nishiyama, 1954), (these cocks were called vasectomized cocks thereafter). After complete recovery from the operation, i. e., at least 2 weeks later, the vasectomized cocks were released into a pen of laying hens and the ejected fluid was collected with a semen collector which was affixed to the cock.

Released period was 20 minutes, but when the cock mated with a hen, he was returned to his cage. During the period, various mating behaviors, for example, courting, attempted mating and completed mating were described every time.

As soon as the cock mated with a hen, the fluid ejected into the semen collector was removed with a medicine dropper and its volume was determined with a micropipette. The fluid obtained by this means was used in analyses of the 2nd phase and a part of 3rd phase of study.

The semen collector used in this experiment was a small vinyl pouch attached to an oval wire ring, about 5 cm. in diameter. An elastic sling constructed of 7 or 8 common rubber bands connected together, was attached to the sides of the semen collector (Fig. 1). The length of the elastic sling and the size of the collector should be adjusted to the cock in question. To affix the collector, the elastic sling was put around the base of both

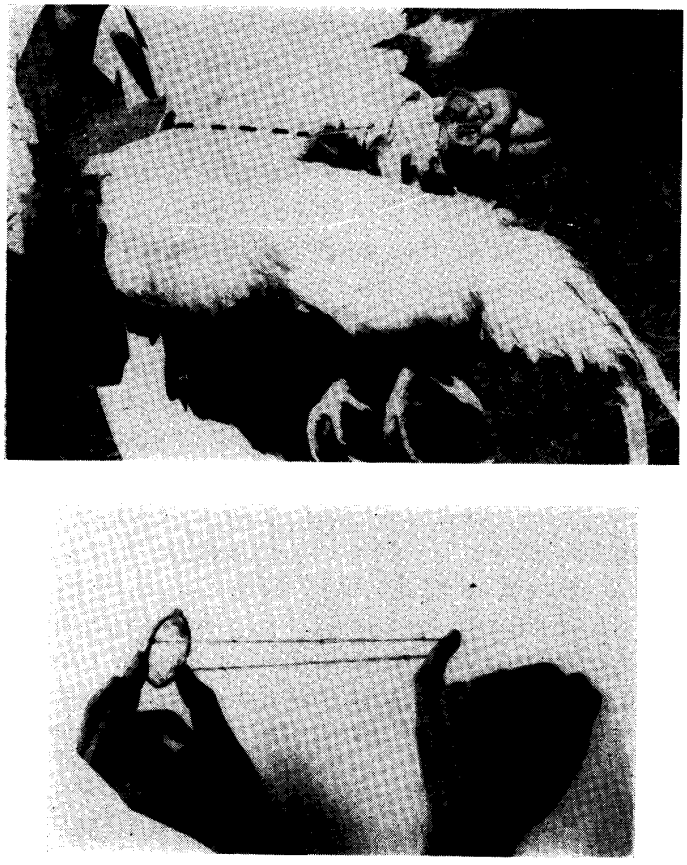


Fig. 1. Semen collector.

Fig. 2. Semen collector affixed to the vent region of the cock.

wings and then the collector was streched rearward and fitted to the vent region (Fig. 2). In this condition, the collector covered the vent and the processes of both pubic bones, and was held fairly well in place by them. If the elastic sling was streched in equal intensities on both sides and was not too loose, the collector scarcely slipped from the vent during the mating action, even when the cock tried vigorously. This semen collector was very light and the semen could be collected in a state of natural copulation.

The second phase of study was divided into two experiments, i. e., one concerning the characteristics of the fluid and the other concerning the origin of the fluid.

i) *Experiment to determine the characteristics of the fluid*: The ejected fluid obtained in the 1st phase of study was used as the sample, and some of the characteristics of the fluid, i. e., pH value, per cent of protein and viscosity were determined and the results of these determinations were compared to those made on the transparent fluid which had been reported in the previous paper (Nishiyama, 1955, 1957).

The pH value of the fluid was determined with the pH test paper (BTB, PR and CR) of Tōyō test paper Co. make. The per cent of protein and viscosity of the fluid was determined with the protein refractometer of Hitachi make, and a Hess-Viscosimeter, respectively.

ii) *Experiment to determine the origin of the fluid*: Three of the 4 cocks which had been used in the 1st phase of study (361, 398 and 400) were used. Four hundred microcuries of Isotop ^{32}P diluted with 0.84 ml. of physiological saline were injected into the left wing vein.

A semen collector was affixed to the injected cock and he was released with laying hens at various times following injection; 2 to 4 minutes (as soon as possible after injection), 10 to 30 min., 1 hr., 3 hrs., 6 hrs. and 12 hrs., and 1 to 6 days after injection. This experiment required 25 collectors since a collector was used only once.

The volume of the fluid obtained was determined accurately by a micropipette and poured into a small dish, 15 mm. in diameter, 6 mm. in depth. Immediately after the fluid sample had been obtained, a blood sample, about 1 ml. in volume was collected from the right wing vein of the cock. The blood was poured into a small citrated test tube (0.2 ml. of 3.8 per cent sodium citrate dried at 80° C), mixed and centrifuged at a speed of 4000 RPM for 20 minutes. A volume of blood plasma equal to that of the corresponding ejected transparent fluid was put into a small dish. The plasma and ejected fluid in the dishes were dried at 80° C., and radioactivities of both the plasma and the fluid samples were determined at one time with a Geiger-Müller counter at the end of the experiment.

In the 3rd phase of study, 20 S. C. White Leghorn cocks were used for collection of ejaculated semen and 6 for vas deferens semen (the semen contained in the vas deferens). The ejaculated semen was collected with a semen collector as described in phase 1, and the vas deferens semen was obtained by squeezing the vas deferens at autopsy. Just after copulation, one drop of the ejaculated semen was spotted on a filter paper and the detection of aldose on the chromatograph was done by the method described by Lake (1957b). The transparent fluid obtained from vasectomized cocks in phase 1, vas deferens semen, and blood serum were also chromatographed for aldose. Degree of reaction, i. e., degree of brown coloration, was scored on a basis of 0 to 3 in which 0 was negative reaction

(no coloration), and 3 had brown coloration as deep as that of blood serum.

Results and discussion

(1) The ejection of the accessory reproductive fluid (transparent fluid) in a state of natural mating.

The mating behaviors of the vasectomized cocks were shown in Table 1. Bird No. 398 and 400 were active in sexual behavior and they usually copulated with a female as soon as they were released with the hens. On the other hand, bird 361 was moderate in sexual behavior and it needed a few minutes to mount or copulate. Bird 399 was sexually active in the early part of the experiment but this activity was lost on and after 16th day, and he was discarded from the data on the 20th day.

Table 1. The mating behavior activities of the vasectomized cocks.

Bird No.	Courts only	Courts and Mounts	Matings	Ejection of transparent fluid (No.)
361	5	6	15	13
398*	6	0	45	41
399**	9	5	6	2
400	3	3	20	17

* Including the data from preliminary experiment.
** The bird 399 was discarded from experiment after 20th day, because he lost sexual libido after the 16th day.

Table 2. Volume of the transparent fluid collected with a semen collector.

Bird No.	No. of collections	Volume of transparent fluid (cub. mm.)		
		Mean	Standard error	Range
361	13	43	8.6	10–100
398	41	77	9.8	10–200
399	2	55	25.0	30, 80
400	17	74	18.5	10–330
average	—	62	16.7	—

When the vasectomized cocks mated with females they ejected a watery transparent fluid in most cases as shown in Table 2. In the vasectomized cocks, the fluid ejected at copulation must be from accessory reproductive organs, since the ejaculation from their vasa deferentia had been blocked. Thus, it may be stated that the cock ejects accessory reproductive fluid during natural copulation.

The ejected fluid was usually collected in a very clean state, but in some cases, a somewhat opaque fluid was ejected and in a few cases the fluid was contaminated with urine. If the ejected fluid was somewhat opaque or tinged with white and very minute globules were found under microscopical examination, it was assumed that the transparent fluid was contaminated with the fluid from the vas deferens. This could be due to incomplete blockage of the openings of vas deferens at cauterization, or their reopening by ejaculatory action. In preliminary experiments, it was observed that the fluid contained in the vas deferens of the vasectomized cock was opaque milk white in color, and contained numerous microscopical globules. Lake (1957b) also reported a slightly opaque fluid from secretions of the seminiferous tubules, epididymal region and vasa deferentia, and a slightly yellow brown pigmented fatty material from the epididymal region. The samples contaminated with the fluid from the vas deferens or urine were discarded from the data.

The volume of the transparent fluid ejected from each bird, was quite variable and ranged from 0.01 to 0.33 ml. and in a few cases none was ejected (Table 2). In general, the volume of the fluid was related to the type of mating action of the vasectomized cock; i. e., when he copulated very rapidly or incompletely, the quantity of the fluid was very small and the more completely he copulated the more the fluid was ejected. When he repeated the copulatory action (dropping his tail feather) 2 to 3 times, the ejected fluid was very large in volume.

As the ejection of the transparent fluid is usually delayed in relation to the ejaculation of the semen from the vasa deferentia, the fluid might not be ejected at all if the copulation was very rapidly completed. Other case where the fluid was not ejected might be ordinal aspermia which occured about 14–17% in normal copulation (Penquite et al., 1929, Parker et al., 1940, Lake 1957b). The great variability in volume of the ejected transparent fluid which was observed in this experiment might be due to varying duration of copulation, degree of sexual excitement, and the number of copulatory actions.

(2) **Identification of accessory reproductive fluid ejected during natural copulation with the transparent fluid obtained by massage.**

From the experiment described above, it is evident that the cock ejects accessory reproductive fluid during natural copulation. To ascertain whether this fluid was the identical one with the transparent fluid described in the previous papers (Nishiyama, 1955, 1957), the following two experiments were conducted:

a) *Some of the characteristics of the fluid collected with a semen collector.*

The appearance of the accessory reproductive fluid collected with the semen collector from the vasectomized cock was quite similar to the "transparent fluid" which had been obtained from vasectomized cocks by means of abdominal massage and described in a previous paper (Nishiyama, 1955). The mean value of the viscosity and per cent of protein of the fluid was 1.16 and 0.41 respectively, and these values were also similar to those of the "transparent fluid" (Table 3 and Table 4). The pH value of the fluid was 8.6 on the average, and this value was rather higher than that of the "transparent semen" which had been reported in a previous paper (Nishiyama, 1955). Part of this difference may be due to technical errors, but part may be the fact that the transparent semen reported in the previous paper (1955) was not the pure transparent fluid. A translucent adhesive di-

Table 3. Some characteristics of the transparent fluid obtained with a semen collector.

Characteristics	No. of birds	No. of samples	Mean	Standard error
Protein (%)	4	19	0.41	0.006
Viscosity	4	16	1.16	0.016
pH	4	14	8.6	0.035

Table 4. Comparison of some characteristics of two transparent fluids obtained by the abdominal massage method and with a semen collector.

	Appearance	Protein (%)	Viscosity	pH	Dilute gelatinous substance	Origin
Massage method	Watery and transparent	0.4(1)	1.1(2)	7.9(3)	Offen(4) developed	Blood(5)
Semen collector	ditto	0.4	1.2	8.6	ditto	ditto

(1) Nishiyama, 1957.
(2) (3) (5) Nishiyama, 1955.
(3): pH of the "transparent semen".
(4) Nishiyama, 1955, 1954.

lute gelatinous substance or substances developed frequently in the accessory reproductive fluid in this experiment, within 10 to 20 minutes after collection, and the same phenomenon also had been observed in the transparent fluid which was collected by abdominal massage (Nishiyama 1955)*.

From these comparisons, it may be considered that the accessory reproductive fluid obtained by natural copulation was the same as the transparent fluid collected by abdominal massage.

b) *Origin of the fluid.*

The problem whether the accessory reproductive fluid (transparent fluid) originated in blood or as a secretion may be clarified by intravenous injection of isotope ^{32}P. If the fluid originates from blood or blood serum, the radioactivity of the fluid just after injection will show the highest activity and thereafter the activity will decrease continuously with time, parallel with the decrease of radioactivity of the blood serum. And if the fluid originates as a secretion, the activity should show a very low value just after injection, increase gradually with time to a peak, and thereafter decrease gradually.

The changes in counts of the transparent fluid and blood plasma of each bird with time after intravenous injection of ^{32}P were shown in Fig. 3. From Fig. 3, it is apparent that the counts of transparent fluid just after injection are very high and rapid decreases

* Cf. Chalaza-like substance (Nishiyama, 1955, 1954). This substance was assumed to be fibrin.

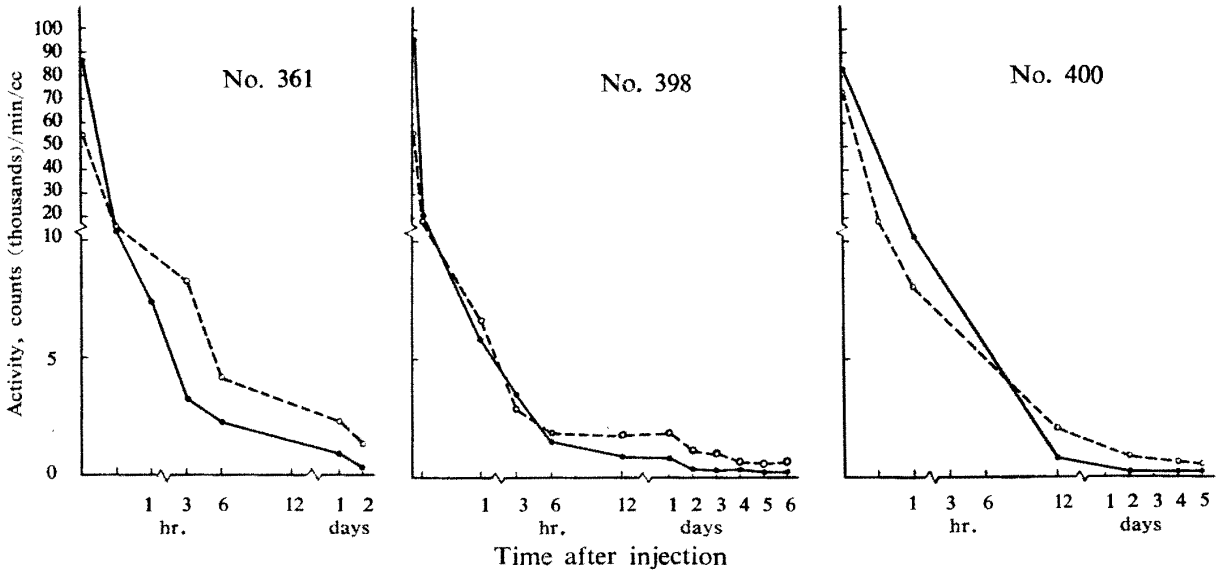


Fig. 3. Change of activity of blood plasma and transparent fluid with time after intravenous injection of ^{32}P.
●——● transparent fluid. ○- - -○ blood plasma.
No. 361 did not mate on and after 3rd day after injection. No. 400 did not mate at 3 h., 6 h. and on 1 day after injection.

ensue for 1 to 3 hours. Thereafter the decrease in count is at a slower rate, and the counts from 2nd to 6th day are similar for each day. This pattern of the change of radioactivity of the transparent fluid with time, i. e., the very high activity just after injection and thereafter a steady decrease with no peak, clearly demonstrates that the fluid is of blood origin.

The pattern of the radioactivity of the blood serum was similar to that of the transparent fluid. However, comparing the counts of transparent fluid and blood serum, the former was larger just after injection in all of three cases. On the other hand, the values of the transparent fluid at and after 12 hours, became smaller than those of blood serum. These differences might be due to the different levels of protein in the transparent fluid and blood serum, about 0.4 per cent in the former and 4 to 5 per cent in the latter. According to Nishiyama and Ogawa (1960), the radioactivity of the supernatant fraction of the blood serum was much higher than that of the protein fraction just after injection of ^{32}P, but 12 hours after injection, the activity of the supernatant became 2–3 times lower. Thus, if a fluid is separated from blood serum and the protein level of this fluid is much lower than that of blood serum, as is the case with the transparent fluid in this experiment, the activity of the fluid would be higher than that of the serum right after injection but it would be lower than the serum 12 hours after injection.

(3) **Aldose test of the transparent fluid, blood plasma, vas deferens semen and ejaculated semen.**

The transparent fluid collected from the vasectomized cock with a semen collector

gave a positive test for aldose, and all of the scores of aldose tests of the fluid were the same as those of blood serum, i. e., score 3. This result means that the transparent fluid contains blood gulcose at the same level as blood serum, and this evidence supports the conclusion that the transparent fluid is of blood origin.

To the contrary, the aldose test of the semen from the vas deferens was negative in all of 6 samples, as had been reported by Lake (1957b), and the chromatograph showed rather white in color in contrast to the intact part of paper which was slightly colored with aniline phtarate (Table 5, Fig. 4).

The aldose tests of the ejaculated semen just after ejaculation were, contrary to the result of Lake (1957b), positive in most cases, though a few were negative; 54 of 64 samples obtained from 20 cocks revealed positive chromatographic patterns and the other 10 samples were negative. The intensity of coloration of the chromatograph was rather weak and very variable, as is shown in the large standard error, and seemed to be related to

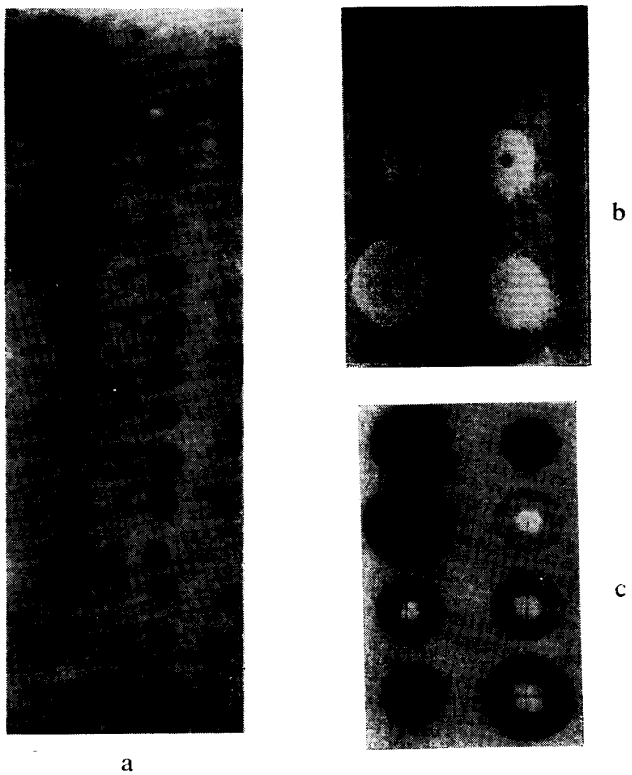


Fig. 4. Aldose test of semen and transparent fluid.

a. Some of the transparent fluid collected from four vasectomized cocks, showing positive reaction of score 3. The samples were stored in frozen state and tested simultaneously. P=blood plasma, S=blood serum.

b. Vas deferens semen (semen obtained from the vas deferens). Showing negative reaction. B=blood plasma, VD=vas deferens semen, AVD=vas deferens semen in ampulla ductus deferentis. Black dot in the center of chromatograph is a mass of spermatozoa, SS=sperm serum of vas deferens semen.

c. Ejaculated semen just after collection. Chromatographs showed positive reactions of various degree. S=blood serum. White spot in the center of chromatograph is a mass of spermatozoa.

Table 5. Aldose test of vas deferens semen, transparent fluid and ejaculated semen*

	No. of cocks	No. of samples	Score of aldose test	Sperm concentration (10^4)
Vas deferens semen	6	6	0	957±93.6
Transparent fluid	4	44	3	0
Ejaculated semen 1	20	64	1.4±0.10	—**
Ejaculated semen 2	6	22	1.6±0.17	328±29.0***

* Vas deferens semen was obtained by squeezing the vas deferens at autopsy. Transparent fluid and ejected semen was collected with a semen collector from vasectomized cocks and normal cocks respectively.

** Semen density was not determined.

*** The correlation coefficient between the score of aldose test and sperm density within individual was -0.599 and it was significant.

the densities of semen samples. Generally speaking, there was a tendency for the higher semen densities to be associated with the lower aldose and vice versa. To clarify this tendency, the sperm number and aldose score were determined on 22 semen samples from 6 different cocks. The correlation coefficient between the score of the reaction and the semen density within individual was −0.599, and was significant at 5% level of confidence (Table 5).

Since an aldose, presumably blood glucose, is present in transparent fluid whereas no detectable amount is present in vas deferens semen, the score of aldose test, i. e., the glucose level of an ejaculate will be influenced by the proportion of the transparent fluid in the ejaculate. The tendency for higher aldose scores to be associated with less dense ejaculates apparently reflects the increased proportions of transparent fluid.

The ejaculated volume of the vas deferens semen may vary among individuals and with collections within individual. Also, as shown in a previous section of this report, the amount of the transparent fluid ejected may vary greatly with collections and in a few cases, especially in cases of instantaneous copulation, there may be no ejection of the fluid at all. This explains why the aldose reactions of the ejaculates were weaker than those of the transparent fluid, varied markedly, and in a few cases lacked the aldose at all.

The proportion of the transparent fluid to the ejaculate was presumed to be 66 per cent on the average, because the average density of the semen contained in the vas deferens and ejaculate was 9.57 and 3.28 millions respectively*.

(4) The aldose level of the succeeding ejaculates in successive collections.

According to Nishiyama (1955), the amount of the vas deferens semen ejected in suc-

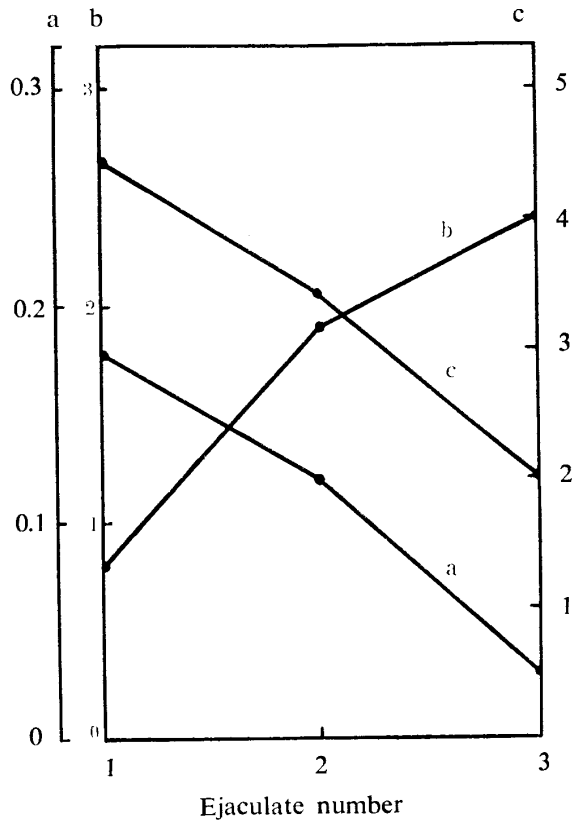
* 1. The vas deferens of the cock adds a secretion to the semen (Nishiyama, 1951), hence it is considered that the density of the semen in the lower part of the vas deferens or the semen ejaculated from the vasa deferentia is slightly lower than that of the semen in the vas deferens. Hence, the proportion of the transparent fluid may be lower than 66 per cent. 2. This estimation is not so accurate, owing to small number of samples.

cessive collections was very large at 1st ejaculation and thereafter underwent marked reduction, while the amount of the transparent fluid suffered little reduction even if the semen collections were successively performed. Then, the proportions of the transparent fluid increased with succeeding ejaculations, and the ejaculates had a tendency to become dilute. Hence, to ascertain the assumption that the aldose level of an ejaculate is influenced by the proportion of the transparent fluid, the aldose scores of the ejaculates in successive collections were determined.

Table 6. Volume, density and aldose score of the semen obtained by successive collection.

Ejaculate no.	Aldose score			Volume of semen			Density of semen		
	No. of birds	No. of samples	Mean*	No. of birds	No. of samples	Mean* (ml)	No. of birds	No. of samples	Mean* (million)
1	3	13	0.8	3	13	0.18	3	12	444
2	3	13	1.9	3	13	0.12	3	11	344
3	3	11	2.4	3	10	0.03	3	8	201

* Mean: the average value of 3 cocks.



The volume, density and aldose score of the semen which was collected 2 to 3 times in a period of 5 minutes, using a semen collector, are shown in Fig. 5 and Table 6. There was a tendency for aldose score to increase and for density and volume of semen to decrease in the succeeding ejaculates. The relationship between the aldose score and semen density in the succeeding ejaculates reveals that the aldose level is determined by the proportion of the transparent fluid in the ejaculate.

Fig. 5. Volume, density and aldose score of the semen obtained by successive collections.
a; volume of semen in ml.
b; aldose score
c; density of semen; number of sperm per cubic millimeter (million).

General discussion

From the 1st, 2nd and 3rd phase of study, it was clearly determined that the transparent fluid was ejected during normal copulation, and that it was a normal constituent of the ejaculate of the cock.

Now, it may perhaps be appropriate to discuss Lake's view (1957b) on the nature and origin of transparent fluid in the light of the results of this experiment.

The grounds for his argument that the transparent fluid is not a constituent of semen and that it is only obtained as a result of forcibly compressing the engorged copulatory organ, are presumably based on the following four of his observations or assumptions.

First is his observation of the ejaculatory phenomenon by the massage method; in cases in which the cloaca of a cock was not compressed forcibly for the collection procedure and the ejaculation completed extremely rapidly (a fraction of a second), a very little of the transparent fluid was expelled into the semen (Lake, 1957b).

It is in the nature of case that when the ejaculation is completed rapidly, the transparent fluid that is expelled into the semen is small in amount, since the beginning of ejection of the transparent fluid is delayed to a little after the ejection of the vas deferens semen (Lake, 1957b, Nishiyama, unpublished data). In this experiment, it was also observed that when the copulation was completed nearly instantaneously, little of the fluid was ejected. In addition, in cases where a large amount of the dense semen is ejected from the vasa deferentia, it is rather impossible to observe the addition of the transparent fluid to the semen giving the appearance that only the dense semen is expelled (Nishiyama, 1955)*. Hence, it may be said that the Lake's observation is correct, but his assumption that the transparent fluid is obtained as a result of forcibly compressing the copulatory organ, is not sustained since an adequate explanation is simply that the amount of the fluid ejected is small in cases in which the ejaculation has been completed extremely rapidly. Actually, the transparent fluid was ejected in most of the cases in this experiment during natural copulation and without any compressing of the copulatory organ.

Lake's 2nd argument is that the density of the spermatozoa of his so-called "true semen" corresponds closely to the density of vas deferens semen (Lake, 1957b) and that the concentrations of some electrolytes of seminal plasma of the cock differ from those of blood plasma (Lake et al. 1958).

In sampling for analysis, Lake (1957b) selected the birds which ejaculated rapidly after massage and collected the semen samples at three day intervals. Further, as far as possible, samples of a semen which was not contained the transparent fluid were withdrawn (Lake, 1957b). As a result of this method of sampling, his samples were special ones which contained little of the transparent fluid, and corresponded to the semen which was collected directly from the ejaculatory ducts, as he described (Lake et al. 1958). Consequently, it is natural that the concentration of spermatozoa of these samples closely corresponded to that in vas deferens semen. And a comparison of the electrolytes of these samples with those of blood plasma does not mean a comparison between those of ejaculates and blood plasma, but is strictly a comparison between those of vas deferens semen and blood plasma. Recently, Takeda (1959) studied the concentration of some electrolytes

* Cf. Nishiyama, 1955, P. 281, 1st ejection of semen.

(Na, K and Ca) of seminal and blood plasmas of the cock and reported that both plasmas resembled each other, and especially that the seminal plasma of dilute ejaculates was quite like blood plasma in concentration of the electrolytes. Tashiro who studied the seminal plasma of the cock immunologically, stated that the seminal plasma was just the same as blood serum in protein fractions contained in them. These findings support the conclusion of Nishiyama (1955) and the results of this experiment, i. e., the transparent fluid originates from blood and the more dilute semen contains much of the fluid.

The density of the ejaculated semen obtained by the semen collector method, which is considered as a most resembled method to natural copulation, was 2.3 millions according to Parker et al. (1940) and 3.28 millions in this experiment. These values clearly differ from those of the semen contained in the vas deferens, 7.8 millions by Lake (1957 b), 5.5 to 7.4 millions by Nishiyama (1951) and 9.6 millions in this experiment. The densities of the semen obtained by the massage method, according to most authors (Cf. Nishiyama, 1961), are about 2 to 3.5 millions on the average, similar to the concentration of the semen obtained with a semen collector, which therefore can not be regarded as semen of abnormally low density.

Lake's 3rd argument that the transparent fluid is not a normal constituent of semen is based on his experimental results that no detectable amount of aldose is present in the ejaculate (Lake, 1957b).

In this experiment, contrary to his result, aldose was present in most of the ejaculates, although the level as scored on the aldose test varied considerably among semen samples (Table 5). The evidence that aldose is present in ejaculates, indicates that the transparent fluid is contained in the normal ejaculate of the cock, since the vas deferens semen lacks the aldose (Lake, 1957 b, and in this experiment), whereas the transparent fluid contains it as shown in this experiment.

Since the transparent fluid is of blood origin and considered to be the fluid which is mainly composed of lymph (Nishiyama, 1955, and in this experiment), the aldose present in the fluid and ejaculates of the cock must be blood glucose. However, there may be a possibility that the cock semen contains fructose, scanty as it is, because Lorenz (1958) suggested that the cock spermatozoa had the ability to convert glucose to fructose. Mann and Hancock (1952) described cock semen as cantaining no or only a negligible amount of fluctose but as containing a certain amount (20–100 mg./100ml.) of glucose. And Mann (1954) also stated that in six individual specimens of cock semen, he found 7.7 to 81 mg/100 ml. glucose but never more than 4 mg / 100 ml. fructose. De Muelenaere and Quicke (1958) reported similar results and concluded that "total reducing sugars" were in a range of 25–55 mg per 100 ml. whole semen (av. 35 mg), and of the so-called "total reducing sugars" yeast fermentable sugars accounted for 92.1%, of which glucose represented 81.6% and fructose (probably) 10.5 %.

As for the disagreement between the result of this experiment and Lake's, one cause may be the difference in methods of sampling the ejaculated semen. In this experiment, semen was collected daily with a semen collector and the semen was obtained in a clean state. On the other hand, Lake (1957b) had obtained the semen from hens immediately after natural copulation by evertion of vaginae using cocks which had rested for 2 days before copulation. After 2 days sexual rest, the amount of the semen ejaculated from vasa

deferentia might be considerably larger and the proportion of transparent fluid lower, resulting in indistinct reactions for aldose. The intensity of sexual activity of the cock was studied by several authors, who reported that the number of matings reached 35 times during 1 to 1.5 hours (Williams and McGibbon, 1957), and 25 to 53 times a day (Heuser, 1916, Philips, 1918, Parker et al., 1942, Skard, 1937). Hence, there is no reason to assure that only the semen ejaculated after 2 days sexual rest is normal, and the semen ejaculated one or more times a day is abnormal. On the contrary, the semen obtained after 2 days sexual rest may be regarded as specially dense semen. The semen collection technique used in his experiment, i. e., the collection from the everted vagina is an older method that has long been out of favor, because the semen becomes dilute with secretions from the cloacal and vaginal region of the hen and is often laden with bacteria. The same procedure as used by Lake (1957b) was tested in a preliminary experiment in which 20 semen samples were obtained from hens mated with 6 different cocks. The collected semen was always found to be diluted with cloacal secretions and often contaminated with urine or feces. The results of the chromatograph of the semen are shown in Table 7, but one can not to say from these results that aldose is present or lacking, because the semen was diluted with vaginal and cloacal secretions.

Table 7. Aldose score of the semen collected from the everted vagina after copulation.

Bird No.	No. of semen samples	Degree of aldose score					
		0	0.5	1	1.5	2	2.5
274	7	2	1	1	1	2	
249	5	1		1	3		
380	5	1		1		2	1
248	1					1	
236	1				1		
136	1			1			

The 4th of Lake's arguments that the transparent fluid is not a normal constituent of the semen is based on his opinion that the erection of the copulatory organ is brought about by engorgement with blood, as in mammals, and the blood plasma transudate (which is corresponds to the transparent fluid) is only obtained as a result of forcibly compressing the engorged copulatory organ.

It appears likely that this opinion of Lake's is not based on experimental data. From the anatomical and histological studies on the copulatory organ (Nishiyama, 1955), as well as from the evidence that the fluid inflowing into the copulatory organ at erection is not blood but a clear watery fluid (Nishiyama, 1950), it is obvious that the erection in the cock is induced by inflow of lymph into the lymph sinuses of the copulatory organ, as in the drake. As it has been shown previously, the transparent fluid is ejected even during natural copulation or when the copulatory organ is not compressed forcibly. Lake (1957b)

stated in his paper that "after much handling for massage, some cocks will, by association of ideas, erect and actually emit a little semen as they are taken out of the cage. With this type of cock the copulatory organ becomes very much engorged after massage and the operator, if not careful, will milk out much of fractions 7 and 8 (transparent fluid) immediately following fractions 1 and 2 (vas deferens semen)". Although not specifically stated, it is implied that in some birds, the transparent fluid is ejected without forcible compression of copulatory organ. In our experience, this is often found in the collection of semen by massage method from sexually active cocks. It was also ascertained that the transparent fluid was not expelled by the engorgement of the copulatory organ and lymph-folds (Nishiyama and Ogawa, 1961).

From these discussions, it may be concluded that the transparent fluid is a normal constituent of the ejaculate of the cock, then that the semen collected by abdominal massage (Burrows and Quinn, 1937) is normal.

Lake (1957b) reported that a clear mucinous fluid from epithelial secretions was a normal constituent of semen. On the other hand, Nishiyama (1955) who studied the origin of the transparent fluid using radioisotope ^{32}P reported that the pure accessory reproductive fluid, i. e., the transparent fluid obtained from vasectomized cocks by the massage method, was not a secretion but of blood origin, or lymph. The result of the experiments reported here support his conclusion by showing that the transparent fluid ejected in a state of natural copulation is also of blood origin. Further, Nishiyama (1957) reported that the electrophoretic pattern of the transparent fluid was quite similar to that of blood serum and there was no sign of contamination of secretion. On the other hand, secretory cells have been found in some areas of the cloacal epithelium (Nishiyama, 1955, Nishiyama and Ogawa, 1961, Lake, 1957a), hence, there is a possibility that the secretion from these cells are added to the transparent fluid. But from all evidences it is probable that such secretions are minute and even if contained in the fluid, have no influence on the quality of the ejaculated semen in most cases.

Summary and Conclusion

Present study was performed to ascertain whether the accessory reproductive fluid of the cock or the so-called transparent fluid was ejected during natural mating or whether the fluid was a normal constituent of the ejaculate. The study was divided into three phases and each phase was composed of one or more experiments.

The results obtained were as follows;

1. The accessory reproductive fluid of the cock, i. e., the so-called transparent fluid could be collected from the vasectomized cocks in most cases using a semen collector or in a state of natural copulation. The volume of the fluid obtained was quite variable and ranged from 0.01 to 0.33 ml.

2. The mean value of the viscosity, per cent of protein and pH of the fluid was 1.16, 0.41 and 8.6 respectively, and a fragment of dilute gelatinous substance developed frequently in this fluid, within 10 to 20 minutes after collection.

3. The origin of this fluid was investigated using isotope ^{32}P, and it was revealed that the fluid was of blood origin.

4. These characteristics of this fluid were quite similar to those of the transparent fluid which had been obtained by abdominal massage and described in a previous paper, though the pH of the former was rather higher than that of the latter.

5. Aldose, presumably blood glucose, was present in the transparent fluid at a similar level to blood serum.

6. Aldose was also present in most of the ejaculates of the cock, though the level varied considerably among semen samples. The level of the aldose was related to the densities of semen samples; there was a tendency for higher semen densities to be associated with the lower aldose and vice versa.

7. From these evidences, it was concluded that the transparent fluid was also ejected during natural copulation, and that the fluid was a normal constituent of the ejaculated semen.

8. The causes of disagreement between the results of this experiment and Lake's one were discussed in the general discussion.

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