On the Quality and Quantity of the Cock Semen Obtained by Different Collection Methods

Hisayoshi NISHIYAMA

(Laboratory of Zootechnical Science)

The quantity and quality of ejaculate of the cock is primarily influenced by the spermatogenetic activity of testes or the volume and density of the semen ejaculated from the vas deferens (the semen is called vas deferens semen in following sentence). However, it must be born in mind that the quantity and quality are secondarily influenced by the amount of the "transparent fluid" added to the vas deferens semen, since the ejaculate of the cock is the mixture of both the vas deferens semen and transparent fluid[1].

1. Relation between the amount of the transparent fluid ejected and the quantity and quality of ejaculate.

The accessory reproductive fluid or the so-called transparent fluid is of blood origin, and it contains aldose, presumably blood glucose, at approximately the same level as blood serum[2]. The vas deferens semen lacks the aldose and has a lower pH than the transparent fluid[1]. Hence, if a larger volume of the transparent fluid is ejected, the ejaculate will increase in volume, pH, and glucose content, and decrease in density, if it is assumed that the volume and density of the semen ejected from the vasa deferentia are constant. On the other hand, in the case where the volume of the transparent fluid ejected is constant, more ejection of the vas deferens semen will result in a larger volume of ejaculate, higher sperm concentration, lower level of glucose, and a pH close to neutrality.

In brief, the quantity and quality of ejaculate are influenced by three variables; the amount of the transparent fluid ejected, the quality and quantity of the semen ejaculated from the vas deferens. For example, assuming that the density of the vas deferens semen is 8 millions, the quality and quantity of ejaculate have to change in accordance with the amounts of the transparent fluid and the vas deferens semen ejected, as shown in Table 1.

Thus, volume, density, and glucose level of ejaculate are expressed as follows;

$$V_e = V_v + V_t$$

$$D_e = \frac{D_v \times V_v}{V_v + V_t}$$

$$G_e = \frac{2V_t}{V_v + V_t}$$

where

V_e=Volume of ejaculate in ml.

V_v=Volume of semen ejected from the vas deferens in ml.

V_t = Volume of transparent fluid ejected in ml.

D_e = Density of ejaculate, in number of sperm per cubic millimeter.

D_v = Density of semen ejected from the vasa deferentia in number of sperm per cubic millimeter.

G = Glucose level of ejaculate in mg. per ml.

In this formula, the glucose content of transparent fluid is assumed as 2 mg. per ml., because the aldose reaction of transparent fluid is similar to blood serum (score 3)[2] and the level of glucose in blood serum is considered as about 2 mg.* To be exact, it is "glucose content in mg. per ml. of the transparent fluid" in place of 2.

The example and the formula help clarify why the ejaculates have a tendency to become more dilute, when large amounts of semen are ejaculated, although a larger ejaculate is not necessarily a dilute semen[1]. They also help explain the tendency for a dilute semen to be higher in pH and glucose content[1,2].

It has been reported by some authors[1,2,4,5,6,7] that when successive collections were performed, the density of ejaculate tended to decrease with succeeding ejaculations. The cause of this tendency is presumably the increase in proportion of the transparent fluid in the succeeding ejaculates. Because, according to Nishiyama (1955)[1], the amount of the vas deferens semen ejected was very large at 1st ejaculation and thereafter underwent marked reduction, while the amount of transparent fluid suffered a little reduction even if the semen collections were successively performed. Hence, the proportion of the transparent fluid increased with succeeding ejaculations.

This assumption may also account for the tendencies for glucose level to be higher in the succeeding ejaculates[2], and for the pH of semen to increase with the frequency of collection[8,9], since the increase in proportion of the transparent fluid is accompanied by an increase of pH and glucose content of the ejaculate.

2. Differences in volume and density among the semens ejaculated during natural copulation, abdominal massage and electrical stimulation.

Nishiyama (1955)[1] summarized the mechanism of ejection of the transparent fluid as follows: When the cock is sexually excited, lymph is generated in the tissue of the vascular body and flows into the lymph-folds and copulatory organ through the lymph sinuses, causing erection of the copulatory organ and temporary swelling of the lymph-folds. The lymph is in turn expelled from the lymph-folds and this expelled lymph composes the main part of the transparent fluid or the accessory reproductive fluid which is added to the semen ejaculated from the vasa deferentia. When ejaclation is completed, the lymph remaining in the copulatory organ and lymph-folds returns through the same passages to the vascular bodies and finally runs into the lymphatic vessels. The copulatory organ, then, retracts to its original condition and the ejection of the transparent fluid ceases. Hence, the ejection of the transparent fluid is always accompanied with the erection of

* Glucose level of blood serum was reviewed by Sturkie (1954)[3] as 2.8 mg. (Horvath) and 1.7 mg. (Hermann) per 1 ml. of serum. For convenience, it was assumed arbitrarily that gulcose level of serum was 2 mg. per ml.

Table 1.

Volume of semen from vas deferens (ml)	Volume of transparent fluid (ml)	Ejaculated semen					Reference	Example
		Volume (ml)	Sperm per mm³ * (million)	Proportion of transparent fluid (%)	Glucose level** (mg/ml)	pH***		
0.05	0	0.05	8	0	0	Rather low (close to neutrality)	Special and very dense semen with no transparent fluid	The semen which may be obtained in a case of incomplete copulation.
0.3	0.01	0.31	7.7	3.2	0.06	Rather low	Very dense semen	The semen which may be obtained by the massage method without milking.
0.1	0.003	0.103	7.7	3.2	0.06	〃	Small amount of very dense semen	
0.3	0.3	0.6	4	50	1	Moderate	Large amount of semen of moderate density	An ordinary kind of cock's semen which may be ejaculated during copulation or massage.
0.1	0.1	0.2	4	50	1	〃	Small amount of semen of moderate density	
0.05	0.05	0.1	4	50	1	〃	A little amount of semen of moderate density	
0.3	0.6	0.9	2.7	67	1.3	〃	Very large amount of semen of moderate density	The semen which may be obtained in successive milkings or collections.
0.1	0.6	0.7	1.1	86	1.7	High	Very large amount of dilute semen	
0.05	0.6	0.65	0.62	92	1.8	〃	Very dilute semen	
0.01	0.2	0.21	0.38	95	1.9	〃	Small amount of very dilute semen	The semen which may be obtained from the cock of low gonadal activity. In this case, the density of the semen ejaculated from the vas deferens may be also reduced.

* When the density of the semen ejected from the vas deferens differs from 8 millions, the density of ejaculate will change according to it.

** The level was calculated by assuming the glucose content of the transparent fluid as 2 mg/ml.

*** When the semen is contaminated with urine or feces, the pH of the semen may be decreased, since the pH of chicken urine ranges from 6.22 to 6.7 (Hester, 1940) and pH of the content in rectum, according to most authors, is acid (cited from Sturkie[3]).

the copulatory organ and lasts as long as the erection continues. The intensity of the ejection of transparent fluid or its amount which will be ejected in a unit of time, may be proportional to the degree of pressure of lymph in the lymph sinuses of the copulatory organ and lymph-folds, i. e., to the degree of erection.

In the massage method, both sides of cloaca are pressed for milking the semen contained in the ampulla ductus deferentis. By this milking process, the vascular bodies are also pressed automatically, because the ampulla ductus deferentis adjoins inside the vascular body (Fig. 1). When the vascular bodies are pressed by the milking process, the lymph generated in them by sexual excitement will be forced into the copulatory organ and lymph-folds, resulting in higher pressure of lymph in these organs, so a greater erection and more rapid ejection of the transparent fluid are caused. Besides, the pressure on the vascular bodies by the milking process may block the return of lymph at retraction in some degree, persisting the erection and ejection of the transparent fluid longer. Consequently, it may be said that the milking process used in the massage method increases the degree and duration of erection of the copulatory organ and also increases the volume of the transparent fluid ejected. On the other hand, the milking process may also increase the amount of the semen ejected from the vasa deferentia, since the semen stored in the ampulla ductus deferentis are milked out by this process. Thus, a larger amount of moderate density semen may be obtained by the massage method.

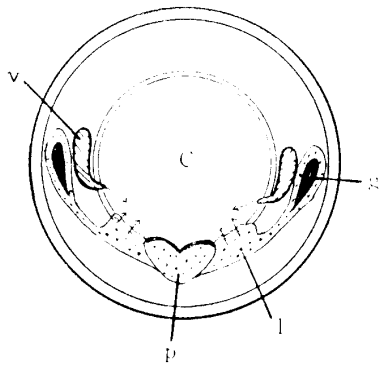

Fig. 1. Diagram to illustrate the ejection of the transparent fluid and vas deferens semen. c, cloaca; g, vascular body; l, swelled lymph-fold which is ejecting the transparent fluid; p, erected copulatory organ; v, ampulla ductus deferentis; ↑, ↑, denotes the ejection of the transparent fluid and vas deferens semen.
(From Nishiyama 1955[1], modified)

Successive collections were often performed in the massage method and the total amount of the semen obtained is regarded as an ejaculate of a cock. In this case, a very large amount of low density semen may be obtained as reported by Nishiyama (1955)[1], since the density of ejaculate tends to decrease with succeeding ejaculations as described before. Sexually active cocks can eject the transparent fluid 4 to 10 times or more in succession[1], so the quality and quantity of the semen obtained will be strongly influenced by the number of collections.

The collection of semen without milking[10] or with only slight milking[11,12] may result

in a lower degree and instantaneous erection and ejection of a smaller amount of the transparent fluid. Hence, a small amount of dense semen may be obtained.

Under natural copulation, there is no milking process but sexual excitement may be greater than abdominal massage, then the semen ejaculated by natural copulation or obtained with a semen collector may be similar to that obtained by the massage method, but the volume may be smaller.

The semen collected by electrical stimulation will be affected by the number of stimulations, since the vas deferens semen was ejected only in the first twice or thrice stimulations and the transparent fluid could be ejected 10 times or more by successive electrical stimulations[13].

Thus, the volume and density of ejaculates differ with the different methods of semen collection, and a similar evidence has also been recognized in animals. For example, the quantity of the semen obtained by electrical stimulation from a bull is much greater than that obtained with an artificial vagina, but the density is usually reduced[14].

The above mentioned facts are a genenal tendency, but in all of collection methods,

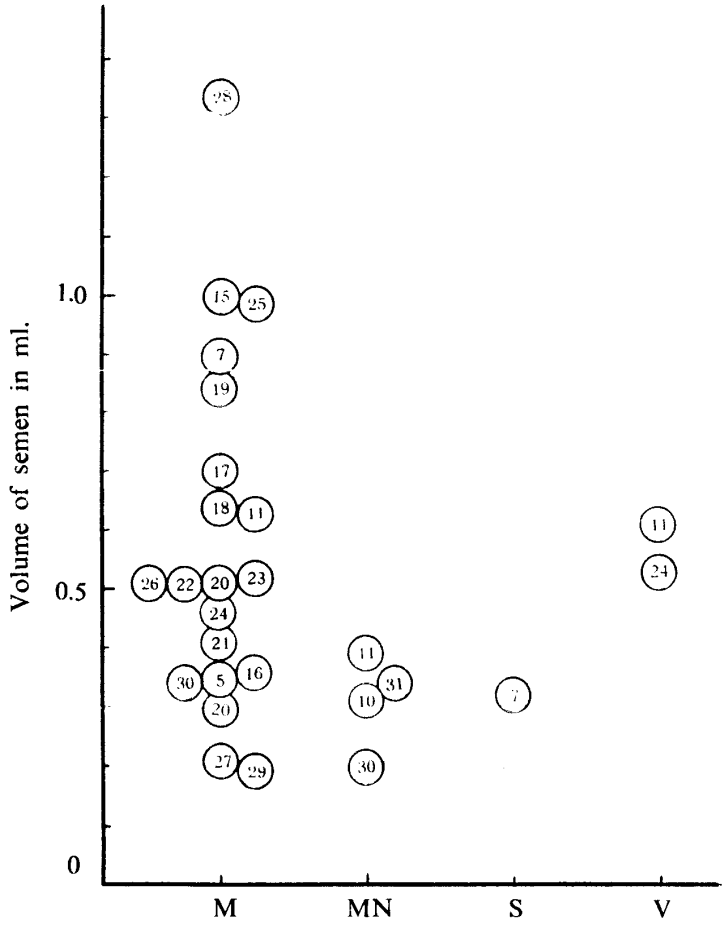

Fig. 2. Scatter diagram of mean volumes of semen. (Numbers refer to authors in the list of references) M- Massage. MN- Massage, no milking. S- Semen collector. V- Total volume of semen contained in the vas deferens. (10, 16, 17, 18, 19, 22, 25, 26, 27, 30, 31···the average of data).

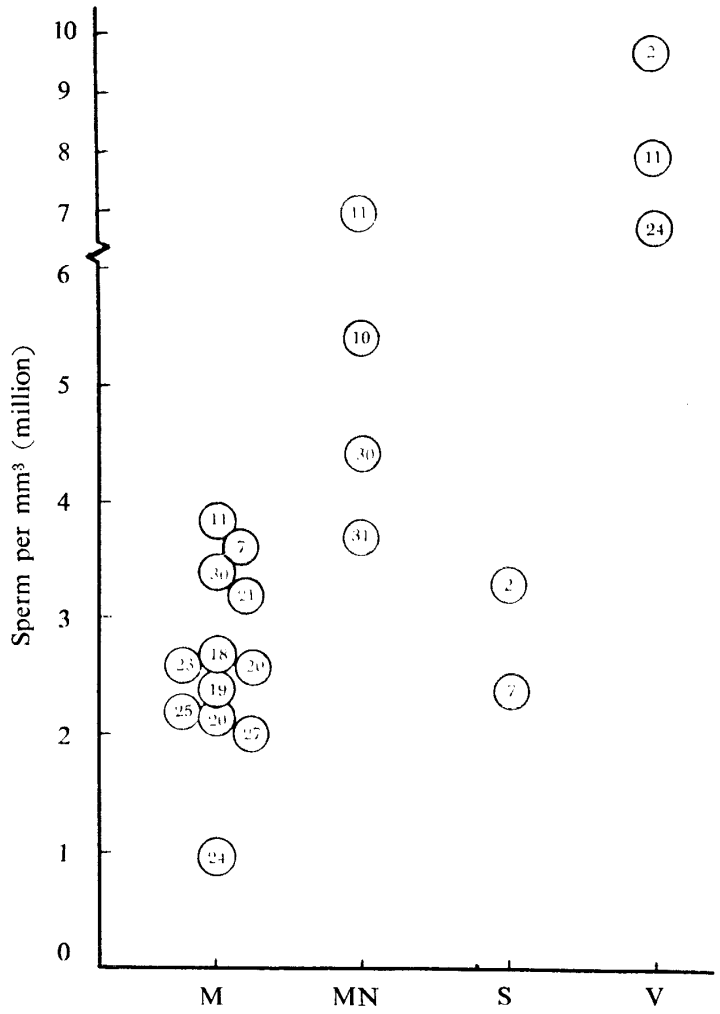

Fig. 3. Scatter diagram of mean densities of semen (Numbers refer to authors in the list of references). M- Massage. MN- Massage, no milking. S- Semen collector. V- Vas deferens semen. (10, 18, 19, 25, 26, 31…the average of data)

there are great variations in volumes of the transparent fluid and the vas deferens semen ejected, hence, in the quality and quantity of the semen obtained, among individuals and collections in the same individual. Some birds will eject large amount of vas deferens semen and others eject only small. A very excitable bird responds easily by a slight stimulation and generates larger amounts of lymph in vascular bodies, hence will result in a high and easy erection and ejection of a larger amount of the transparent fluid. Nishiyama (1955)[1] and Nishiyama and Fujishima (1961)[2] found such a variation during massage and natural copulation. It has been also recognized that the volume and density of ejaculate differ with different breeds, lines and seasons.

The average volumes and densities of the semens reported by several authors are shown in Figs. 2, 3 and Table 2. From these figures and the table, one can see the general tendency and great variation. One cause of extreme variation in volume and density of the semen obtained by abdominal massage may be due to the number of collections successively performed.

Table 2. Average volume and density of semen obtained by simple average of data diagrammatized in Figs. 2 and 3.

Method of collection	Volume per ejaculate (ml)	Sperm per mm^3 (million)
Massage	0.59	2.63
Massage, no milking	0.31	5.14
Semen collector	0.32	2.34
(Vas deferens semen)	0.57	8.00

3. The proportion of the transparent fluid in an ejaculate.

Nishiyama (1955)[1] reported that the ejaculated semen obtained by the massage method (four or more successive collections), contained 14 to 78 per cent transparent fluid, and Takeda (1959)[32] stated that the vas deferens semen was diluted with the transparent fluid about 10-fold in the successively collected semen. It was also presumed that the semen ejaculated during natural copulation contained the transparent fluid about 66 per cent on the average[2].

Calculating the proportion of the transparent fluid in ejaculates from the data of Mann (1954)[33] who reported the glucose level of ejaculates as 7.7 to 81 mg. per 100 ml., it may be presumed to be 4 to 50 per cent. Because, no detectable amount of aldose was present in the vas deferens semen and the aldose level of the transparent fluid was assumed to be about 200 mg. per 100 ml. as described previously.

From these reports and the difference between the densities of ejaculate and vas deferens semen (Table 2), it is considered that the vas deferens semen may be diluted with the transparent fluid 2- to 3-fold, in an ordinary cock's semen of about 3 millions in density.

4. Semen suitable for use in artificial insemination.

It is well known that the accessory reproductive fluid of animals has an adverse effect on the survival of spermatozoa when the fluid is added too much and the spermatozoa in the dilute semen have the shorter survival time than those in the dense semen. In some cases, separate collection of dense semen part in an ejaculation or removal of the accessory reproductive fluid by centrifugation is resorted to. The same may be true for cock's semen. When adequate amounts of the transparent fluid are added to the semen from the vas deferens, the fluid may exert favorable effects, such as increasing the volume, activating the sperm and supplying a nutrient (glucose) to the sperm. On the other hand, if added too much, the fluid exerts deleterious effects such as severe clumping of sperm and shortening of sperm longevity[1,34]. Hence, more dense semen may be suitable for artificial insemination and especially for semen storage.

Acknowledgement: The author wishes to express his sincere gratitudes to Dr. H. S. Weiss of Rutgers University, for reading the original manuscript.

References

(1) NISHIYAMA, H.: *J. Fac. Agric. Kyushu Univ.*, **10**, 277 (1955).
(2) NISHIYAMA, H. and T. FUJISHIMA: *Mem. Fac. Agric. Kagoshima Univ.*, **5**, 27 (1961).
(3) STURKIE, P. D.: *Avian Physiology*, New York (1954).
(4) PENQUITE, R., W. A. CRAFT and R. B. THOMPSON: *Poult. Sci.*, **9**, 247 (1930).
(5) SHIBATA, S., Y. FUJIOKA, A. MURATA, K. NANBA, and T. TOMONORI: *Rept. Imp. Zootech. Exp. Stat.*, **35**, 1 (1938).
(6) HUTT, F. B.: *Proc. Roy. Soc. Edin.*, **49**, 102 (1939).
(7) PARKER, J. E., F. F. MCKENZIE and H. L. KEMPSTER: *Poult. Sci.*, **19**, 191 (1940).
(8) ZAGAMI, V.: *Arch. Sci. Biol.*, **25**, 208 (1939). (Cited from *F. W. Wilcox, Poult. Sci.*, **37**, 444).
(9) PARKER, J. E., F. F. MCKENZIE and H. L. Kempster: *Missouri Agric. Expt. Sta. Research Bull.*, **347**, 1 (1942). (〃).
(10) KAMAR, G. A. R.: *Poult. Sci.*, **37**, 1382 (1958).
(11) LAKE, P. E.: *J. Agric. Sci.*, **49**, 120 (1957).
(12) LAKE, P. E., E. J. BUTLER, J. W. MCCALLUM and I. J. MACINTYRE: *Quart. J. Exp. Physiol.*, **43**, 309 (1958).
(13) NISHIYAMA, H. and K. OGAWA: *Jap. J. Zootech. Sci.*, in press.
(14) ROWSON, L. E.: Reproduction in domestic animals, Edited by Cole and Cupps, New York and London, Vol. **2**, 102 (1959).
(15) BURROWS, W. H. and H. W. TITUS: *Poult. Sci.*, **18**, 8 (1939).
(16) JONES, D. G. and W. F. LAMOREUX: *ibid.*, **21**, 173 (1942).
(17) WARREN, D. C. and C. L. GISH: *ibid.*, **22**, 108 (1943).
(18) PARKER, J. E. and B. J. MCSPADDEN: *ibid.*, **22**, 142 (1943).
(19) PARKER, J. E. and B. J. MCSPADDEN: *ibid.*, **22**, 170 (1943).
(20) SEARCY, G. L. and F. N. ANDREWS: *ibid.*, **22**, 235 (1943).
(21) WHEELER, N. C. and F. N. ANDREWS: *ibid.*, **22**, 361 (1943).
(22) SHAFFNER, C. S. and F. N. ANDREWS: *ibid.*, **27**, 91 (1948).
(23) HUSTON, T. M. and R. S. WHEELER: *ibid.*, **28**, 262 (1949).
(24) NISHIYAMA, H.: Sci. Bull. *Fac. Agric. Kyushu Univ.*, **13**, 377 (1951).
(25) ALLEN, C. J. and L. R. CHAMPION: *Poult. Sci.*, **34**, 1332 (1955).
(26) WILLIAMS, C. and W. H. MCGIBBON: *ibid.*, **35**, 617 (1956).
(27) KATSURAGI, T. and Y. SAEKI: *Jap. J. Zootech. Sci.*, **29**, 21 (1958).
(28) PARKER, J. E. and W. H. MCCLUSKEY: *Poult. Sci.*, **38**, 858 (1959).
(29) MCDANIEL, G. R. and J. V. CRAIG: *ibid.*, **38**, 1005 (1959).
(30) NAKANO, K. and K. YAMADA: *Rept. Shiga Shukeijo, Showa* **34**, 15 (1960).
(31) KAMAR, G. A. R. and A. L. BADRELDIN: *Poult. Sci.*, **38**, 301 (1959).
(32) TAKEDA, A.: *Research Bull. Fac. Textile & Sericul. Shinshu Univ.*, **9**, 55 (1959).
(33) MANN, T.: The biochemistry of semen, London and New York, p. 33, (1954).
(34) LAKE, P. E.: III Int. Congr. Anim. Repr. (Sect. 3), 104 (1956).