J. Fac. Fish. Anim. Husb.
Hiroshima Univ. (1968), 7: 209~215

Studies on Deep-Freezing Preservation of Fowl Semen

II. On the Progeny Test by Making Use of Freezing Fowl Semen

Moriyuki WATANABE, Kazumasa IRIE and Takashi SAKABE

Department of Animal Husbandry, Faculty of Fisheries and Animal Husbandry, Hiroshima University, Fukuyama

(Table 1; Plate 1)

Since the possibility of long-term storage of fowl spermatozoa at low temperature was reported by SHAFFNER *et al.* (1941)[1], many investigations have been made by different authors[2)-14)], but never did they reach better results than with the bull semen. Therefore, the freezing preservation of fowl semen seems still to be a long way off from practical use.

Researches in the past were mainly carried out on how to increase the viability of fowl spermatozoa after freezing and thawing. Consequently, the greater part of them were carried out on whether glycerol removal was right or wrong[6),7),10),11)], and whether two rates of freezing which were referred to as "slow" and "fast" were good or not[9),11),14)]. Thus, it is no exaggeration to say that reports of the progeny test concerning fowls, produced by deep-freezing semen are not yet to be found.

The present experiments examine the general properties of semen produced by a cock, which was obtained from deep-freezing semen stored for three months at $-79^{\circ}C$. Moreover, the cock was mated with four White Leghorn hens, to make the progeny test and to observe the further fertility, hatcherbility and growing conditions of the progenies.

MATERIALS AND METHODS

The cock used in the present experiment was hatched normally from the egg laid by a hen inseminated with semen samples stored for 3 months (Pl. 1., Fig. 1). The collection of semen was made by the Hiroshima Method by YAMANE *et al.* (1962)[15]. The general properties of semen were examined in relation to the motility of sperms, the volume of semen, pH, sperm concentration per 1 mm^3, total number of sperms per ejaculate and the percentages of abnormalities of sperms.

The semen was collected in a pyrex glass funnel. The whole content was about 2.0 m*l*. The collected semen was measured by 1 m*l* pipette. Determination of sperm concentration was performed as usual with a Thoma-zeiss haemocytometer. For determination of the pH-values in the semen, brom-thymol-blue test paper was used. The percentages of abnormalities in the sperms have been counted, by taking first about 500 normal chick sperms and comparing them with those from the deep-

freezing preservation. Black-ink staining was used for this purpose. As for the progeny test, the test cock was mated with four White Leghorn hens (Pl. 1., Fig. 2) successively, and was examined about fertility and hatcherbility during two weeks after mating, moreover the abnormalities, sex ratio and live weight of one day old chicks were examined too.

RESULTS AND DISCUSSIONS

General properties of semen

The general properties of semen obtained from the deep-freezing preservation of fowl semen are shown in Table 1.

Table 1. General properties of cock semen hatched from the deep-freezing semen stored for three months at $-79°C$.

Date of collection	Volume per ejaculate (m*l*)	Sperm concentration per mm^3 (million)	Total number of sperms per ejaculate (billion)	pH
5. 15	0. 10	3. 89	0. 389	7. 4
5. 19	0. 20	3. 86	0. 772	7. 4
6. 3	0. 17	2. 65	0. 451	7. 0
6. 19	0. 10	1. 83	0. 183	7. 4
6. 27	0. 08	4. 90	0. 392	7. 2
6. 30	0. 10	4. 36	0. 436	7. 4
7. 4	0. 25	5. 03	1. 258	7. 0
8. 4	0. 09	5. 00	0. 450	7. 0
8. 14	0. 10	5. 56	0. 556	7. 0
Average	0. 13±0. 06	4. 12±1. 22	0. 543±0. 310	7. 18±0. 19

Namely, the amount of semen at one operation was 0.13 ± 0.06 m*l* on the average, ranging from 0.08 to 0.25 m*l*. The motility of semen just after collection was very active and did not show great differences compared to that of the control cock. This average volume of semen is little compared to that of 0.36 m*l* reported by PARKER *et al.* (1942)[16]. The reason seemed to be immaturity of the cock. Sperm concentration per 1 mm^3 was 4.12 ± 1.22 millions on the average, ranging from 1.83 to 5.56 millions. This is but a small difference compared with the range of 2.00 to 3.99 millions by PARKER *et al.* (1942)[16] or with that of 3.2 millions by Van DRIMMELEN (1951)[17]. The total number of sperms per ejaculate was 0.543 ± 0.310 billions on the average, ranging from 0.183 to 1.258 billions. This average number of sperms per ejaculate was lower than 3.25 billions on the average by PARKER *et al.* (1942)[16]. The pH-value of the semen was 7.18 ± 0.19 on the average, ranging from 7.0 to 7.4. This pH-value don't differ from that of 7.27 reported by PARKER *et al.* (1942)[16].

In the present experiment the percentages of abnormal sperms in test cock

semen was 11.0±6.3 on the average, minimum from 4.2 to maximum 21.3 in 8 samples. While, that of the controlled cock was 10.1±3.9 on the average ranging from 5.7 to 16.0. Therefore, no remarkable difference appears in the above two cocks' abnormalities, they are also lower compared with the range from 3.5 to 39 percent given by PARKER *et al.* (1942)[16]. According to PARKER *et al.*, where 20 percent or more of the sperms were abnormal, the fertility resulting from such semen insemination fell to 23.8 percent. Moreover, they reported that the negative coefficient of correlation between the percentages of abnormalities and that of fertility was highly significant ($r=-0.434$). Thus, the percentages of abnormal sperms in present cock semen seems to be normal.

Progeny test

The fertility rate during one and two weeks after crossing between the cock hatched from the deep-freezing semen stored for three months and the four White Leghorn hens was 81.25 percent and 76.47 percent respectively. One of the four hens had to be excluded from the calculation of fertility and hatchability because she did not lay eggs during the whole time of the experiment. The fertility rate in the present experiment was rather low compared to that of normal White Leghorn hens. This low fertility rate in the present experiment was due probably to the hot-summer conditions at the time, since the temperature in the room had gone up to 30°C～32°C. The average two-week hatchability was 53.9 percent and the result was not satisfying. The cause of this low pitch seems to lay in the bad circumstances as described above. Cock and hens showed traces of fatigue. Moreover the incubated eggs has encountered interruption of electric current four times, including for the longest four hours, during the incubation periods. The live weight of a day old chick was 43.03±1.82 g on the average, ranging from 39.1 to 44.9 g in 14 samples. According to the reports by ETO and ONISHI (1960)[18], the live weight of a day old White Leghorn chick is 37.0 g and 38.0 g on the average in female and male respectively, therefore, the average live weight of male and female is 37.5 g. Thus, the live weight of a day old chick in the present experiment is about 6 g more than that of ETO and ONISHI (1960)[18]. We have no idea that either the cells were influenced specially in the process of the deep-freezing preservation of fowl semen and it caused an abnormalities in cytoplasma or it has any other causes. At any rate, it is an interested matter to increase about 16 percent in live weight of a day old chick in the near future.

Behavior

One day old chickens bred by crossing between a cock hatched from deep-freezing semen stored for three months and four White Leghorn hens have shown a very active behavior and showed no abnormality compared to chicks hatched from normal nest eggs.

Secondary sex ratio

BYERLY and JULL (1935)[19] found the proportion of males among 28,015 chickens of various breeds alive at 10 weeks of age to be 50.1 percent. HAYS (1945)[20] also found the sex ratio to be 432 ♂♂ : 438♀♀ or, 49.7 percent male. This is as close to a 1:1 ratio as could be expected in general. This may allow to presume that the sex ratio in chicks is nearly close to a 1:1 ratio. The secondary sex ratio in the present experiment showed 8 ♂♂ : 6 ♀♀, 57.1 percent males among 14 chicks. According to the above described data, male chick seem to be slightly higher. But this phenomenon seems to be due to insufficiency in the samples used in this experiment. Therefore, the conclusion about the sex ratio must be confirmed by further examination.

The present authors did not reach any conclusion about the fact that the abnormalities in cytoplasma might have been caused by a particular influence on the cells during the process of deep-freezing preservation of the fowl semen, or had some other causes. Any way, the 16 percent in live weight increase of a one day chicken is a satisfying progress in science.

SUMMARY

The present experiments have examined the general properties of semen produced by the cock hatched from the deep-freezing semen stored for three months at $-79°C$. The cock was mated with four White Leghorn hens to examine the fertility, hatchability of the eggs produced by the hens. Further the abnormalities, sex ratio and growing conditions of chicks hatched from their fertilized eggs were studied in order to inquire into the progeny test. The results can be summarized as follows;

1. The amount of semen at one operation was 0.13 ± 0.06 m*l* average.
2. Semen concentration per 1 mm^3 was 4.12 ± 1.22 millions average and the total number of spermatozoa per ejaculation was 0.543 ± 0.310 billions average.
3. pH-value of the semen was 7.18 ± 0.19 on the average.
4. The percentages of abnormal spermatozoa in the semen was 11.0 ± 6.3 on the average.
5. The fertility and hatchability of the eggs produced by crossing between the cock hatched from the deep-freezing semen stored for three months at $-79°C$ and the four White Leghorn hens were respectively 78.8 percent and 53.9 percent on the average during two weeks after mating.
6. Sex ratio in the present experiments showed 8 ♂♂ : 6 ♀♀, or emerged with 57.1 percent males among 14 chicks. Male seems to be slightly in evidence in the ratio of sexes.
7. Live weight of a-day-old chicks was 43.03 ± 1.82 g on the average and about 16 percent heavier than that of a normal one day old White Leghorn chick before.
8. The progenies gained by the present experiments showed a very active behavior and no abnormality compared to chicks hatched from normal nest eggs.

REFERENCES

1) SHAFFNER, C.S., E.W. HENDERSON and C.G. CARD. Viability of spermatozoa of the chicken under various environmental conditions. Poult. Sci., **20**: 259-265. 1941.
2) SHAFFNER, C. S. Longevity of fowl spermatozoa in frozen condition. Science, **96**: 337. 1942.
3) POLGE, C., A. U. SMITH and A. S. PARKES. Revival of spermatozoa after vitrification and dehydration at low temperature. Nature, **164**: 666. 1949.
4) SMITH, A. U. and C. POLGE. Survival of spermatozoa at low temperatures. ibid., **166**: 668. 1950.
5) PARKES, A. S. Storage of mammalian spermatozoa at low temperatures. Proc. Soc. Stud. Fertil. (Camb.) **2**: 12-15. 1950.
6) POLGE, C. Functional survival of fowl spermatozoa after freezing at −79°C. Nature, **167**: 949. 1951.
7) ALLEN, T. E. and L. W. BOBR. The fertility of fowl spermatozoa in glycerol diluents after intrauterine insemination. Poult. Sci., **34**: 1167-1169. 1955.
8) ALLEN, T. E. The storage of fowl semen at low temperatures. Proc. Austr. Soc. Anim. Prod., **2**: 118-119. 1958.
9) CLARK, C. E. and C. S. SHAFFNER. The fertilizing capacity of frozen chicken sperm and the influence of related *in vitro* processes. Poult. Sci., **39**: 1213-1220. 1960.
10) BROWN J. E. and G. C. HARRIS. The influence of glycerol equilibration time on the metabolism motility and fertility of frozen chicken spermatozoa. ibid., **42**: 377-380. 1963.
11) SHAFFNER, C. S. Observation on freezing chicken semen. Proceed. 5th Inter. Congs. Anim. Reprod. A. I., **4**: 426-429. 1964.
12) TANAKA, K., T. Tomita and S. Okamoto. Studies on deep-freezing of fowl semen. Jap. J. Zootech. Sci., **37**: 134-138. 1966.
13) WATANABE, M. Fundamental studies on deep-freezing perservation of chicken semen. Ann. Rep. Individual Junior (Agri.) Minist. Educ., P. 247. 1966.
14) WATANABE, M. Studies on deep-freezing preservation of chicken semen. J. Fac. Fish. Anim. Husb. Hiroshima Univ., **7**: 9-23. 1967.
15) YAMANE, J., S. TSUKUNAGA and T. TAKAHASHI. A new model of bird holder and semen receptacle for collecting semen from cock. Bull. Hiroshima Agr. Coll., **2**: 17-24. 1962.
16) PARKER, J.E., M. MCKENZIE and H.L. KEMPSTER. Fertility in the male domestic fowl. Res. Bull. Misso. Agric. Exp. Sta., **347**: 1-50. 1942.
17) VAN DRIMMELEN, G. C. Artificial insemination of birds by the intraperitoneal route. Onderstep. J. Vet. Res., **1**: 1-194. 1951.
18) ETO, S. and N. ONISHI. New practical techniques in poultry farming. (in Japanese), lst edi., Asakura-shoten. 1960.
19) BYERLY, T. C. and M. A. JULL. Sex ratio and embryonic motality in the domestic fowl. Poult. Sci., **14**: 217-220.
20) HAYS, F. A. The primary sex ratio in domestic chickens. Am. Naturalist, **79**: 184-186. 1945.

鶏精液の凍結保存に関する研究

II. 凍結精液による後代検定

渡辺守之・入江和正・坂部鷹司

−79°Cで3カ月間凍結保存した精液を用いて人工受精し，得られた白レグ雄鶏の精液の一般性状を調べ，更にこの雄鶏を白レグ雌4羽に交配してその受精率，ふ化率，ふ化雛の性比および生時体重等を調べ，引きつづき雛の発育状態を観察した結果は次の如くである．

1. 凍結精液よりふ化した雄鶏の一射精時当りの平均射精量は 0.13±0.06 ml. であった．

2. 精子濃度は 1 mm^3 当り平均 4.12±1.22百万で一射精当りの総精子数は 0.543±0.310十億であった.

3. 採取精液の pH は平均 7.18±0.19 であった.

4. 採取精液中の畸形精子の割合は平均 11.0±6.3% であった,

5. −79°C で 3 カ月間凍結保存した精液からふ化した雄鶏を白レグ雌 4 羽に交配した場合, 交配後 2 週間の平均受精率およびふ化率はそれぞれ 78.8%, 53.9% であった.

6. 上述の交配試験の結果得られた雛14羽の性比は 8 ♂♂ : 6 ♀♀で雄鶏がやや多いように思われる.

7. ふ化雛の初生体重は平均 43.03±1.82 g で従来の白レグ雛の平均初生体重 37.5 g にくらべて約16%増重の雛が得られた.

8. 得られた雛はいずれも極めて活潑で, 普通の卵からふ化した雛とくらべて何等異なるところがなく順調な発育をつづけている.

EXPLANATION OF PLATE

Fig. 1. A cock hatched from the deep-freezing semen stored for three months at −79°C; About six months old.

Fig. 2. Four White Leghorn hens mated by the above described cock for examining the progeny test; About fourteen months old.

Fig. 3. A day old chicks hatched from the eggs during first one week after crossing between the cock and the four hens; Average live weight was 43.9 g.

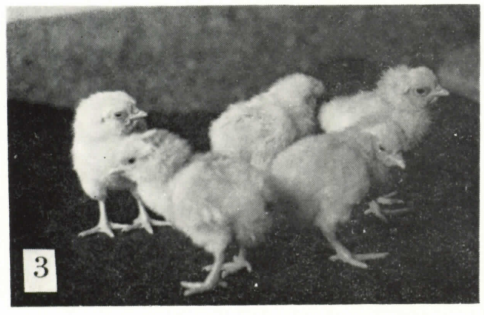
Fig. 4. A day old chicks hatched from the eggs during second one week after crossing between the cock and the four hens; Average live weight was 42.7 g.

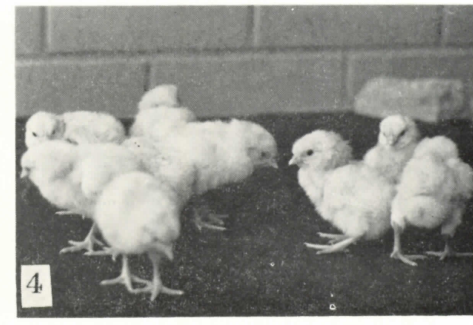
Fig. 5. Male progenies gained from above described crossing; About five months of old.

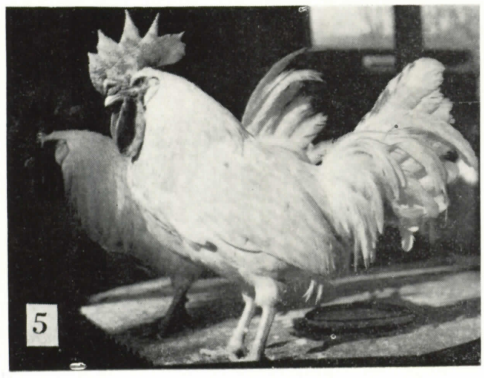
Fig. 6. Female progenies gained from above described crossing; About five months of old.

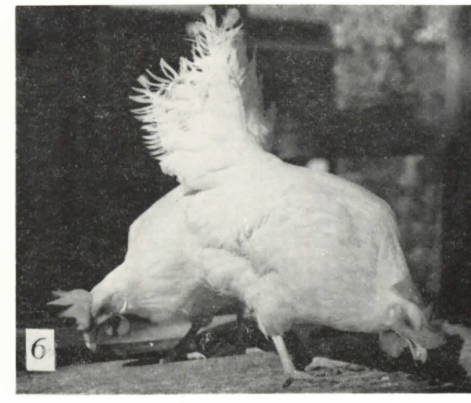

1


2


3


4


5


6