J. Fac. Fish. Anim. Husb.
Hiroshima Univ. (1967), 7: 9~23

Studies on Deep-Freezing Preservation of Chicken Semen

Moriyuki WATANADE

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

(Text-figs. 1-5; Diagram 1; Tables 1-8; Plate 1)

Since it was discovered by POLGE *et al.* (1949)[1] that spermatozoa could be frozen without loss of motility if glycerol was added in their suspending medium, the deep-freezing preservation of spermatozoa has been held an important position in the fields of artificial insemination in place of storage for liquid semen up to now employed.

SHAFFNER *et al.* (1941)[2] reported that the chicken semen was "quick frozen" to a solid state at -6°C, stored for 30 minutes and thawed at 42° to 45°C with no apparent damage to either motility or cell structure. They secured one chick from an egg fertilized by frozen and thawed spermatozoa as described. In so far as we are aware this is the first chick hatched from an egg fertilized by frozen and thawed spermatozoa. The next year SHAFFNER (1942)[3] reported that spermatozoa have been maintained at a temperature of dry ice (-79°C) for 14 months and little if any difference could be noted in the percentage of cells that regained motility between samples thawed immediately after freezing or those thawed after 14 months storage. POLGE *et al.* (1949)[1] maintained motile fowl sperm up to 10 weeks by adding 10 percent to 15 percent glycerol to the semen and freezing at -79°C. But SMITH & POLGE (1950)[4] observed that the fertilization was nil with either frozen or unfrozen glycerolized semen when the semen was deposited in the vagina. Moreover POLGE (1951)[5] reported that 2 percent was the maximum glycerol concentration compatible with retention of full fertilization power of fowl semen and levels above 5 percent destroyed all fertilizing power. He stated that when glycerol was added to fowl semen at levels above 5 percent and the semen then frozen to -79°C, thawed and then dialyzed for 2 hours, it produced some fertility in 51 out of a total of 83 hens into which it was inseminated. ALLEN & BOBR (1955)[6] reported a decrease in the harmful effects of high levels of glycerol on the fertilizing capacity of fowl sperm if intrauterine insemination were made. When this intrauterine insemination technique was used with glycerolated semen that had been frozen, 25 percent fertility resulted but the majority of the embryos were dead after 48 hours of incubation (ALLEN 1958).[7] BROWN & HARRIS (1963)[8] reported that the removal of glycerol was apparently unnecessary since the intrauterine insemination of glycerolized chicken spermatozoa that were equilibrated prior to freezing gave normal embryonic development. In contrast, CLARK & SHAFFNER (1960)[9] obtained 40 percent fertility with intrauterine insemination of semen that had been frozen and the glycerol removed after thawing by dilution and centrifugation.

The present investigation was carried out to established the techniques on the deep-freezing preservation of the chicken spermatozoa. Various *in vitro* treatment for chicken spermatozoa were studied in an effort to approach this objective including differences in diluents, dilution rates, freezing and thawing methods, glycerol concentration, equilibration temperature, holding temperature after thawing, equilibration time, motility and freezing preservation periods and fertility test.

MATERIALS AND METHODS

The three cocks used for present experiments were of 3-year old Single Comb White Leghorn. For collecting semen the "Hiroshima" pattern bird holder deviced by YAMANE *et al.* (1962)[10] was used. The semen samples were collected at 7.00 to 7.30 a. m. on every three days or on every four days by abdominal massage method. The semen collected were used for the experiments individually. Four diluents prepared for present experiments, Yamane's[11] and Locke-Ringer's[9] solutions were used for freezing diluents. The composition, pH and depression of freezing point of diluents used are shown in Table 1. The semen collected was diluted four or eight times with the above various diluents at 37°C and the semen samples were frozen and thawed in the next four methods respectively as shown in Diagram 1.

Table 1. The composition, pH and depression of freezing point of diluents used for this experiments

Diluents	pH	Depression of freezing point (− °C)
A*	6.2	0.58
B**	6.3	0.52
C***	6.3	1.11
D****	6.5	1.08
Yamane's solution	7.0	1.08
Locke-Ringer solution	7.1	0.61

Composition:
*: 5% $C_6H_{12}O_6$ solution 85 plus fresh egg yolk 15
**: 〃 95 〃 5
***: 9% $C_6H_{12}O_6$ solution 85 plus fresh egg yolk 15
****: 〃 95 〃 5
Yamane's solution: One volume 6.5% $Na_3C_6H_5O_7 \cdot 2H_2O$ solution plus one volume fresh egg yolk plus two volume 9.0% $C_6H_{12}O_6$ solution
Locke-Ringer solution: NaCl 9.0g, KCl 0.42g, $CaCl_2$ 0.24g, dextose 0.5g, $MgCl_2$ 0.2g and $NaHCO_3$ 0.5g per distilled water 1,000 ml.

Diagram 1. Temperature change in freezing and thawing of the chicken semen

A) Four freezing methods of the chicken semen
1) 37°C (diluted with diluent, addition of glycerol) ⋯→ 20°C (glycerol equilibration) → ——→ -79°C
2) 37°C (diluted with diluent) ⋯→ 20°C (glycerol addition and equilibration) ⟶ -79°C
3) 37°C (diluted with diluent) ⋯→ 20°C (glycerol addition and equilibration) ⋯→ 0°C → ——→ -79°C

4) 37°C (diluted with diluent) ·····→ 5°C (glycerol addition and equilibration) ···→ 0°C →
——————→ -79°C

B) Four thawing methods of the chicken semen

a) -79°C ——————————————————————→ 37°C
b) -79°C → 5°C (keeping gently for 5 mins.) ——————————→ 37°C
c) -79°C ——————————————————————→ 20°C
d) -79°C → 5°C (keeping gently for 5 mins.) ——————————→ 20°C

Remarks:

·····→ shows the falling of temperature 1°C/minute
——→ shows the quick rising and falling of temperature

The concentration of glycerol varied with the design of each experiment. In Trial I and II, the concentration of glycerol ranged from 7 to 50 percent, whereas, in Trial III and IV, that of glycerol raged from 5 to 12 percent. The glycerol equilibration temperature and the holding temperature of semen samples after thawing were studied in 20°C, 10°C and 5°C in the former and in 20°C, 30°C and 37°C in the latter respectively. The glycerol equilibration time varied with the design of each experiment. In Trial I and II, the equilibration time ranged from 30 to 90 minutes and in Trial III and IV, that time ranged from 60 to 180 minutes and from 60 to 90 minutes respectively.

As for the relationships between the various freezing periods of chicken semen and the motility of semen after thawing were examined with the motility of semen thawed after 1, 7, 14, 30 and 90 days' storage.

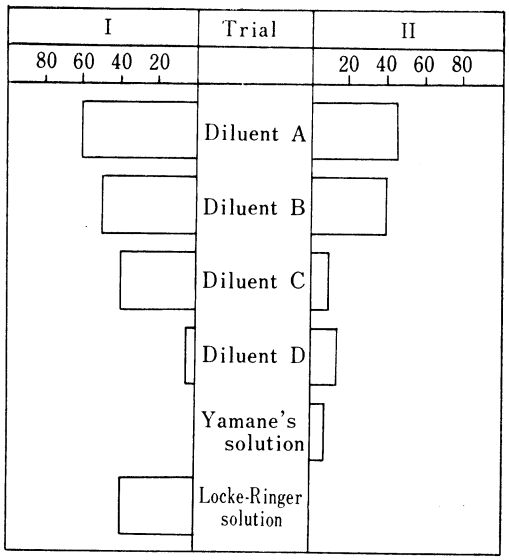
Fertility test was practised with the semen stored at -79°C for 30, 90 and 100 days. In these test, each freshly collected semen was diluted four or eight times with the Diluent A prepared by present author at 37°C and the semen samples were cooled to 5°C at 1°C per minute and 7 percent glycerol were added to it in the tube at the same temperature and both were mixed gently. The semen sample was equilibrated at 5°C for 60 minutes. Following equilibration the sample was cooled from 5°C to 0°C at 1°C per minute and then frozen fastly from 0°C to -79°C by fast freezing method. Regarding to the thawing temperature, the semen sample was thawed in a water bath at 5°C for five minutes by keeping gently and transfered in a water bath at 20°C and then the semen sample was used in the fertility test. The time needed from the thawing of semen to the injection of it was within thirty minutes.

RESULTS AND DISCUSSION

Diluent

The effect of each diluent on freezing-preservation of the chicken semen was shown in Text-fig. 1.

In Trial I, Diluent A, B, C, D, and Locke-Ringer solution were used and in Trial II, Diluent A, B, C, D and Yamane's solution were used and examined by comparison. In these experiments the concentration of glycerol and the glycerol



Text-fig. 1. The effect of various diluents on the freezing preservation of chicken spermatozoa

equilibration time are 7 percent and 60 minutes respectively. Concerning to the motility of spermatozoa after freezing-preservation, Diluent A was the most active of the above five diluents in both experiments. Diluent B was the most active in motility after Diluent A and Diluent D and Yamane's solution were by far the worst in comparison with others in Trial I and II respectively. The motility of spermatozoa diluted with Locke-Ringer solution was fairly active just after thawing but the movement was not normal and behave wildly and later lost the motility rapidly. Yamane's solution seems to be unfitted for diluent of freezing preservation of the chicken spermatozoa. The reason is not clear yet but it is suposed that the damage* on freezing results largely from the concentration of the electrolytes within the cell.

Moreover, the effects of Diluent A and B on deep-freezing preservation of the chicken semen were examined by comparison. The results of Trial I, II, III and IV are summerized in Table 2. As it is clear in the results of Trial I, II, III, and IV, Diluent A is the most effective for freezing-preservation of the chicken semen and it was used in the whole experiments after this.

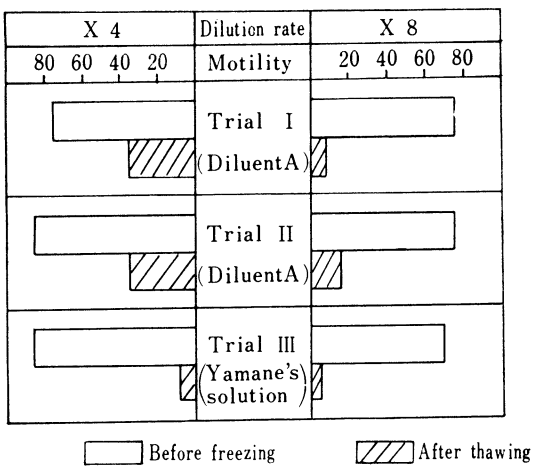
Dilution rate

As for the dilution rate on deep freezing of the chicken semen, the semen collected was diluted four or eight times with Diluent A and Yamane's solution and examined by comparison. The results are sohwn in Text-fig. 2. In each Trials (I–III), it seems to be better for deep-freezing preservation not to promote the dilution rate.

* The damage was said so-called "The destructive action of strong salt solutions by Lovelock[12] (1953)".

Table 2. The comparison of diluents A and B on deep-freezing preservation of chicken spermatozoa

Trial	Diluent	Cocks used	Preservation period (days)	Motility after dilution	Motility after thawing (Hours)													
					0	½	1	2	3	4	5	6	7	8	9	10	11	12
I	A	No. 1	1	95	50	50	50	48	48	—	40	35	—	35	—	35	—	35
	B			95	50	50	50	45	45	35	40	20	—	25	—	8	—	30
II	A	No. 1	14	95	35	30	30	35	—	—	—	30	—	25	—	2	—	—
	B			95	30	30	30	30	—	—	20	15	—	25	—	5	—	—
III	A	No. 1	7	95	60	55	50	55	55	—	50	—	—	25	—	15	—	—
	B			95	40	35	45	50	45	—	30	—	—	15	—	6	—	—
IV	A	No. 3	7	95	55	50	40	40	40	—	25	—	—	10	—	7	—	—
	B			95	50	50	30	45	30	—	15	—	—	5	—	5	—	—



Text-fig. 2. The effect of dilution rate on the freezing preservation of chicken spermatozoa

Freezing and Thawing Methods

In the freezing and thawing experiments, Method 1 to 4 and Method a) to d) were used respectively, but from the results of preliminary freezing experiments of the chicken semen Method 1 was omitted in the present experiments because the result was inferior to others. Thus, the results of motility of spermatozoa thawed after storing at −79°C by various freezing methods are shown in Table 3.

As it is clear in the results of Table 3, the result of Method 4 is better than that of the other two methods.

The results of motility of spermatozoa thawed by various thawing methods after storing at −79°C are shown in Table 4. In view of the results of next Table 4, Method d) is the most effective of the four. Therefore, Method 4 and Method d) were used in freezing and thawing experiments after this.

Table 3. The motility of spermatozoa stored at -79°C by various freezing methods

Freezing method	Diluent	Cocks used	Motility after dilution	Motility after thawing (Hours)											
				0	½	1	2	3	4	5	6	7	8	9	10
2	A	No. 1	95	50	45	30	20	20	10	—	5~8	—	0		
3	A	No. 1	95	25	40	25	25	20	20	—	20	—	10	—	5
		No. 2	95	50	45	30	20	15	15	—	5~8	—	3	—	1~2
4	A	No. 1	95	60	50	50	40	40	40	—	30	—	30	—	25
		No. 2	95	40	35	40	50	50	50	—	40	—	25	—	25

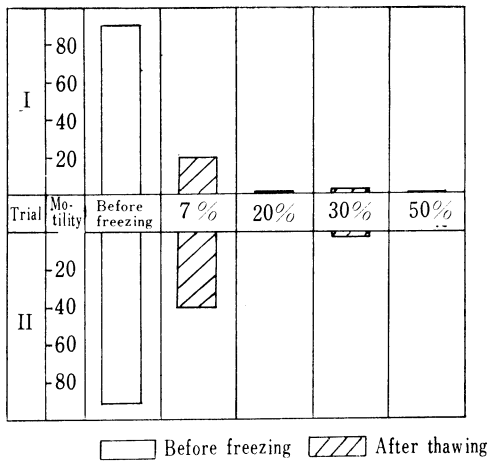
Table 4. The motility of spermatozoa thawed by various methods after storing at -79°C

Thawing method	Diluent	Cocks used	Preservation period (days)	Motility after dilution	Motility after thawing (Hours)											
					0	¼	½	1	2	3	4	5	6	7	8	9	10
a	A	No. 1	3	90	40	0											
		No. 2	7	90	15	0											
b	A	No. 1	7	90	15	0											
		No. 1	7	90	30~35	8											
c	A	No. 3	1	90	55	—	50	45	45	45	45	—	40	—	15~20	—	10
		No. 1	7	90	—	—	25	25	25	—	3	—	0				
d	A	No. 3	1	90	60	—	60	60	60	60	60	—	50	—	25	—	10~15
		No. 1	7	95	60	—	55	50	55	55	50	—	—	—	25	—	15~20

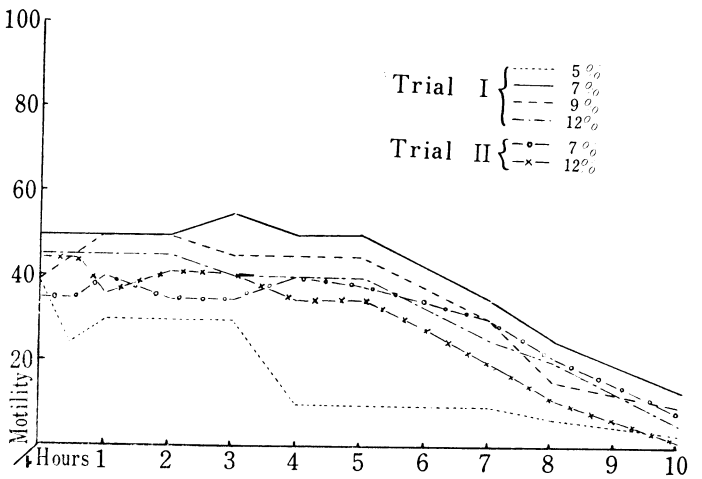
Concentration of Glycerol

The results of glycerol concentration on freezing preservation of the chicken semen are shown in Text-fig. 3 and 4. In the first experiments the concentration of glycerol was practised with in the limits from 7 to 50 percent (Text-fig. 3). In Trial I, the concentration was practised by comparison in 7, 20, 30 and 50 percent and in Trial II that was practised in 7 and 30 percent respectively. As it is clear in view of the results of above described Fig. 3, 7 percent glycerol was the most effective in both trials. So, in the next experiment, the concentration of glycerol was promoted with 7 percent as the central figure and the limits from 5 to 12 percent (Text-fig. 4). In Trial I the effective rank of the concentration of glycerol was arranged in 7, 9, 12, 5 and in Trial II, that was arranged in 7, 12. Therefore, throughout the first and second experiments, the most effective concentration of glycerol was 7 percent.

As for the concentration of glycerol on freezing preservation of the chicken spermatozoa a pretty number of reports has been described by many investigators. POLGE *et al.* (1949)[1] maintained motile fowl spermatozoa up to 10 weeks by adding 10 to 15 percent glycerol to the semen and freezing at -79°C. POLGE (1951)[5] also



Text-fig. 3. The effect of glycerol concentration on the freezing preservation of chicken spermatozoa



Text-fig. 4. The effect of glycerol concentration on the freezing preservation of chicken spermatozoa

reported that 2 percent glycerol was the maximum glycerol concentration compatible with the retention of full fertilizing power of fowl semen and that levels more than 5 percent glycerol destroyed all fertilizing power. Later CLARK & SHAFFNER (1960)[9] reported that the glycerol levels of 7 to 8 percent was the optimum glycerol level in the final volume. BROWN & HARRIS (1960)[8] also observed that the semen samples, equilibrated with 8 percent glycerol at 2°C for 90 minutes and frozen for one hour at −79°C gave highest average motility and fertility. Recently SHAFFNER (1964)[13] found that the 8 percent glycerol is the maximum amount that can be used to successfully protect against the harmful effects of freezing of fowl semen. As above stated, the glycerol concentration of 7 percent was the most effective to protect

the survival of fowl spermatozoa thawed out after freezing in the present experiment.

Equilibration Temperature

The effects of equilibration temperature on the freezing-preservation of chicken spermatozoa are summarized in Table 5. As it is clear from the results in Table 5, the semen samples equilibrated at 5°C was superior to 10°C and 20°C in maintaining the motility. But it is unknown that the motility of the semen samples equilibrated at 10°C was much lower than 5°C and 20°C. Since equilibration data for chicken semen were quite limited except for the experiment that BROWN & HNARRIS (1963)[8] equilibrated the semen samples at 2°C in their experiment, this study was initiated to determine the influence of this variable on the fertilizing capacity of frozen spermatozoa.

Table 5. The effect of equilibration temperature on deep freezing preservation of chicken spermatozoa

Equilibration temperature	Diluent	Cocks used	Preservation period (day)	Stored temperature after thawing	Motility after dilution	Motility after thawing (Hours)										
						0	½	1	2	3	4	5	6	7	8	9	10
20°C	A	No. 1	1	20°C	95	25	40	25	25	20	20	—	15	—	10	—	5
		No. 2	1	20°C	95	50	45	30	20	15	15	—	5~8	—	3	—	1~2
		No. 1	1	37°C	95	5~8	0										
10°C	A	No. 2	1	20°C	95	15	25	10	7	5	5	—	2~3	—	5	—	3
5°C	A	No. 1	1	20°C	95	60	50	50	40	40	40	—	30	—	30	—	25
		No. 2	1	20°C	95	40	35	40	50	50	50	—	35	—	25	—	25
		No. 1	1	37°C	95	35	30	0									

Holding Temperature after Thawing

The results of motility of spermatozoa held at 20°C, 30°C and 37°C after thawing are shown in Table 6. The results of Table 6 shows that the semen samples held at 20°C after thawing had significantly higher motility when compare to that of 30°C and 37°C. Concerning those problems, there are some reports. SMITH & POLGE (1950)[4] and CLARK & SHAFFNER (1960)[9] thawed the semen samples at 40°C holding them there until completely liquid. BROWN & HARRIS (1963)[8] also thawed the frozen semen in a thermos bottle containing water with the temperature adjusted at 20°C and the samples were held at this temperature until completely liquid. Later SHAFFNER (1964)[13] reported that the best results were secured when thes emen samples were held at 10°C to 15°C after thawing. The results in the present experiments agree with that of above described BROWN & HARRIS (1963)[8].

Equilibration Time

The effects of various equilibration times on freezing-preservation of the chicken

Table 6. The motility of spermatozoa held at various temperatures after thawing

Temperature after thawing	Diluent	Cocks used	Preservation period (days)	Motility after dilution	Motility after thawing (Hours)											
					0	½	1	2	3	4	5	6	7	8	9	10
20° C	A	No. 1	14	85	45	40~45	45	40	40	40~50	35	40~45	—	45	—	40
		No. 3	7	95	40	40	40	45	43	35	35	30	—	40	—	15~20
30° C	A	No. 1	14	85	45	40~43	40~45	35	40	30	20	25	—	10		
		No. 3	7	95	40	30	20	45	25	5~10	2	0				
37° C	A	No. 1	7	95	30~35	8	0									
		No. 1	3	85	35~40	0										

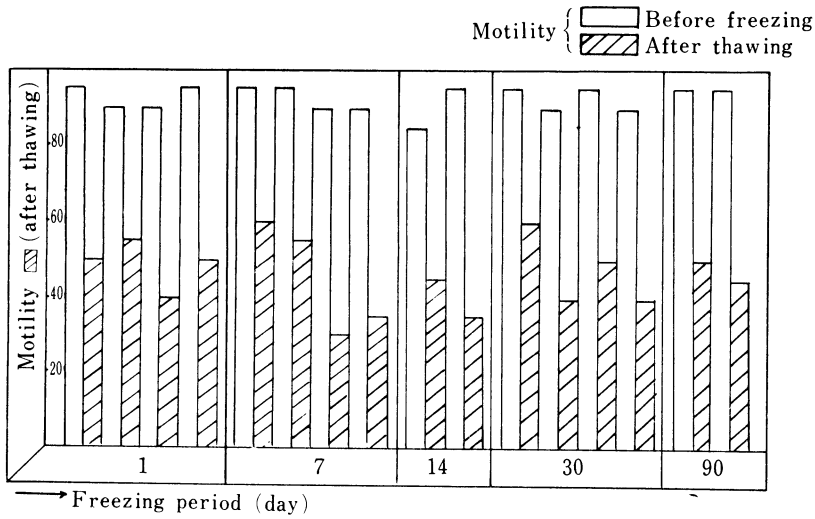
spermatozoa are summarized in Table 7. Through Trial I, II, III, and IV the best results were secured when the samples equilibrated at 5°C for 60 minutes. The present author reported in preliminary experiment (1966)[14] that the 15 minute equilibration time showed slightly higher motility when compared to that of 30, 60 and 180 minute equilibration times but when it exceeded the limits of 180 minute equilibration times, the motility was reduced quickly and became zero when the equilibration time was over 360 minutes. BROWN & HARRIS (1963)[8] also reported that the semen samples equilibrated at 2°C for 90 minutes and frozen for one hour at -79°C had highest motility and slightly higher fertility when compared to 30 and 60 minute equilibration periods. Judging from the result of above such experiments in the fowl, the most adequate equilibration time seems to be within the limits of 60 to 90 minutes.

Table 7. The effect of equilibration time on deep-freezing preservation of chicken spermatozoa

Trial	Diluent	Cocks used	Preservation period (day)	Motility after dilution	Motility after thawing — Equilibration time			
					30 min.	60 min.	90 min.	180 min.
I	A	No. 1	1	75	5	35	7	—
II	A	No. 3	1	90	15~20	25~30	3	—
III	A	No. 1	1	85	—	25	—	3~5
IV	A	No. 1	1	95	—	35	0~2	—

Motility and Freezing preservation Periods

The semen samples diluted with Diluent A and equilibrated at 5°C for 60 minutes and frozen for 1, 7, 14, 30 and 90 days respectively were examined by comparison. The resumptions of motility of the chicken spermatozoa after above designed days' storage at -79°C were shown in Fig. 5. The difference among 1, 7, 14, 30 and 90 days of storage in maintaining the motility and percentage of motile sperm was not significant as shown in Text-fig. 5.



Text-fig. 5. The motility of the chicken spermatozoa by various storing periods

The study of this kind was practiced by some investigators. According to SHAFFNER *et al.* (1941)[2] BOETZ in 1938 estimates that in a storage temperature of −252°C a cell would age only about a minute in 10,000 years. With this same thought in mine, JOHNEL (1938)[15] also found that a few human spermatozoa recovered motility after being frozen and stored at −79°C, for 40 days, at −196°C for 52 hours and at −269.5°C for 3 hours. SHAFFNER (1942)[3] indicated that time is not an important factor in the retention of motility within the first year, when fowl semen is held constantly at the temperature of solid CO_2. He also reported that the spermatozoa have been maintained at a temperature of dry ice (−79°C) for 14 months and little if any difference could be noted in the percentage of cells that regained motility between samples thawed immediately after freezing or those thawed after 14 month storage. GRAHAM *et al.* (1957)[16] found that there were no differences between one, two and three weeks of storage prior to use in bull semen.

Considering from the results of present experiment inclusive of each report above, it seems to be observed that no significant difference in motility after storage for long times when fowl semen is held constantly at the temperature of dry ice (−79°C).

Fertility Test

The results of insemination making use of the semen samples stored for 30, 90 and 100 days by the various designed methods above described are shown in Table 8.

The fertility of 30 day storage in Trial I was 29.1 percent in the first week and 8.6 percent in the second week after insemination respectively. That of 90 day storage in Trial II was 25.0 percent in the first week and zero in the second week. This non-fertility in the second week in Trial II was due to no further laying occurred from the 8th on because the hens used in this experiment molted seriously

Table 8. The motility and fertilizing capacity of semen stored at -79°C

Trial	Cocks used	Date of freezing	Date of thawing	Deep freezing periods (days)	Dilution rate	Motility of sperm			Fertility (%)	
						Just after collection	After dilution	After thawing	1st week	2nd week
I	No. 3	42 7, 13	42 10, 13	90	4	95	95	50	25.0 *(1/4)	—
II	No. 3	42 8, 13	42 11, 23	100	8	90	90	30	14.3 *(1/7)	25.0 *(2/8)
III	No. 2	42 10, 22	42 11, 22	30	4	95	95	75	29.1 *(7/24)	8.6 *(2/23)

* The parenthesized number indicates the ratio of the number of fertile eggs to the number of eggs laid within the period.

and stopped laying in this season. That of 100 day storage in Trial III was 14.3 percent in the first week and 25.0 percent in the second week. Throughout this experiment, the result of fertility was poor in comparison with the motility of the semen after thawing.

BROWN & HARRIS (1963)[8] reported that the removal of glycerol was apparently unnecessary, since the intrauterine insemination of glycerolized chicken spermatozoa that were equilibrated prior to freezing gave normal embryonic development. ALLEN & BOBR (1955)[6] also reported that almost all birds so inseminated with semen containing 15 percent glycerol have laid fertile eggs.

In present experiment, 25 percent fertility was secured with semen containing 7 percent glycerol that had been frozen for 90 days as shown in Trial I and the fertile egg hatched on schedule into an apparently normal chick and the chick is full grown and still living (Plate 1).

On the other hand, some investigators such as CLARK & SHAFFNER (1960)[9] and SHAFFNER (1964)[13] stated that glycerol is effective in protecting chicken spermatozoa from the rigors of freezing but is harmful to the fertilizing process and apparently must be either removed prior to insemination or a more complex insemination technique employed.

In order to rise the fertility on freezing-preservation of the chicken spermatozoa in future, it will be necessary to examine the opinion of the latter fully.

SUMMARY

The effect of various diluents, dilution rates, freezing and thawing methods, glycerol concentrations, glycerol equilibration temperatures, holding temperatures after thawing, equilibration times and freezing preservation periods on the viability of chicken spermatozoa were examined and the fertility test was carried out successively. The results were summarized as follows.

1. Diluent A was the most effective in maintaining motility and percentage of motile sperm and Diluent B was the most effective after Diluent A. The effect of

Diluent C, D, Locke-Ringer solution and Yamane's solution were clearly inferior to that of Diluent A and B.

2. As for the dilution rates on deep freezing of the chicken semen the buffer diluted for four times was superior to the buffer diluted for eight times and it seems to be necessary for freezing preservation of chicken spermatozoa not to rise the dilution rate.

3. In the case of freezing the chicken spermatozoa, Method 4 was the most effective in maintaining the motility and percentage of motile sperm of the three and Method d) was the most effective of the four in the case of thawing the chicken spermatozoa after freezing.

4. The glycerol concentration of 7 percent was the most effective to protect the survival of chicken spermatozoa thawed out after freezing in the present experiment.

5. The semen samples equilibrated at 5°C was the most superior in maintaining the motility and percentage of motile sperm as compared with that of 10°C and 20°C.

6. The holding temperature of 20°C after thawing was the most effective in maintaining the viability of chicken spermatozoa and 30°C and 37°C ranked to it in order.

7. The semen samples equilibrated at 5°C for 60 minutes gave higher motility and percentage of motile sperm than those of 30, 90 and 180 minutes.

8. There were no remarkable differences between the resumptions of motility of chicken spermatozoa and storage periods when the semen samples stored for 1, 7, 14, 30 and 90 days.

9. The striking differences among fertility when the semen samples stord for 30, 90 and 100 days respectively at −79°C were not observed.

ACKNOWLEDGEMENTS

The author is indebted to Assistant Prof. T. Takahashi of Hiroshima Agricultural College for technical assistance with artificial insemination. Thanks are also due to Mr. K. Hamanaka and Mr. M. Yasui for assisting in much of the experimental work.

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鶏精液の凍結保存に関する研究

渡　辺　守　之

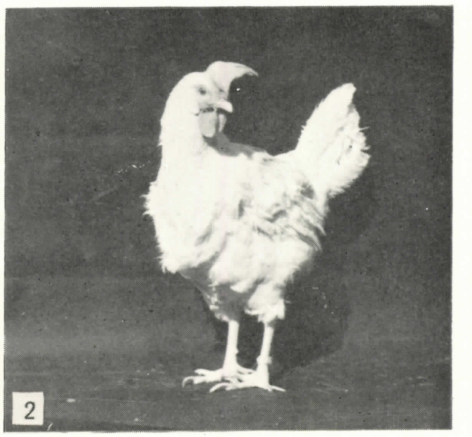
鶏精液の凍結保存に関する基礎的研究として凍結用各種希釈液の調整を行ない，融解後の精子活力に及ぼすグリセリン濃度，グリセリン平衡温度および平衡時間，各種凍結法および融解法の影響について検討し，更に引きつづき授精試験を行なった結果は次のごとくである．

1. 凍結用希釈液としては A 液が精子の活力の維持および生存精子割合に最も効果的であった．
2. 鶏精液の凍結保存においては希釈倍率は低い方が良好であった．
3. 鶏精液の凍結方法については 37°C で精液を希釈し，5°C まで毎分 1°C ずつ下げ，5°C でグリセリンの添加平衡を行ない，更にグリセリン平衡後 0°C まで毎分 1°C ずつ下げ，0°C から -79°C に急速凍結した第 4 の方法が，また融解においては -79°C から 5°C に 5 分間静置して融解しその後 20°C に保持する d) の方法が精子の活力維持および生存精子割合に最も効果的であった．
4. 上記の凍結ならびに融解法でグリセリン濃度 7 %，グリセリン平衡温度 5°C で60分間平衡したものが凍結融解後の精子の活力の維持および生存精子割合に最も効果的であった．
5. 鶏精子の融解後の活力と凍結期間との間には何等著明な差異は認められなかった．
6. 鶏精液の凍結保存期間と受精率との間には著明な差異は認められなかった．

EXPLANATION OF PLATE 1

Fig· 1. A White Leghorn cock (NO. 3) used for collecting the semen.
Fig. 2. A White Leghorn hen (NO. 2) artifiially inseminated with the semen stored for 90 days at -79°C.
Fig. 3. A day old male chick secured from frozen sperm (No. 3 ♂ X NO. 2 ♀), the weight was 38.5g.
Fig. 4. The same as Fig. 3., it was 21 days old and weighed 132g.
Fig. 5. The same as Fig. 3., it was 60 days old and weighed 650g.
Fig. 6. The same as Fig. 3., it was 162 days old and weighed 1,600g.


1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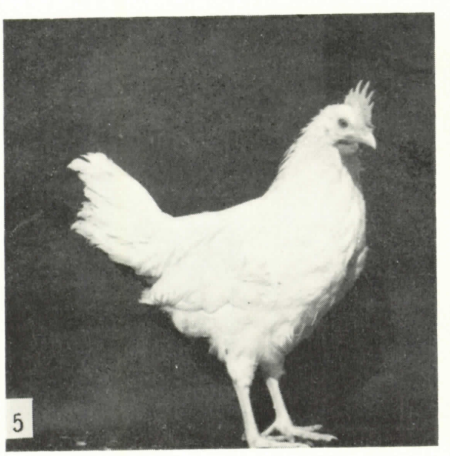
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