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Fertility of Frozen Fowl Semen Stored for Long Term (9 Years)

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(Text-fig. 1; Tables 1–3)

Since the Possibility of deep freezing preservation of bull spermatozoa was reported by Polge *et al.* in 1952[1], artificial insemination with frozen semen in cattle has developed throughout the world. During the past 28 years fundamental and practical studies on frozen semen have been repeated and numerous attempts of long-term storage in cattle and other animal species have been made. Mixner and Wiggin (1964)[2] reported on the effects of ageing on the motility and fertility of frozen bull semen. Mixner (1968)[3] also reported the results on the fertility of bull semen frozen for twelve years. Moreover, Iritani *et al.* (1980)[4] reported about conception with frozen bull semen stored for 16 years. However, similar studies on frozen semen in poultry are few compared with those on bull semen. Recently, however, these studies augmented gradually. The present authors have reported about the fertility of fowl semen frozen for 3 months in 1970[5] for two years in 1972[6] and for five years in 1975[7]. The authors (1973)[8] also reported the fertility of turkey spermatozoa frozen for two years.

The object of the research described in this paper was to investigate the effects of ageing on motility, abnormality and fertility with frozen fowl semen stored for nine years.

MATERIALS AND METHODS

The semen was collected from the White Leghorn cockerel (No. 7), 9 month of age 9 years ago (May, 1971) by abdominal massage as explaned by Yamane *et al*[9]. This collected semen was diluted to four fold with a diluent composed of 5% $C_6H_{12}O_6$ solution 85 plus fresh egg yolk 15 immediately after collection. It contained 7% glycerol in its final concentration. It was kept in 5°C ice water for 5 minutes. Afterwards, each 0.8 ml diluted semen was dispensed into 1 ml straw ampule under similar conditions and sealed respectively. After 5 minutes equilibration, the samples were subjected to prefreezing in the evaporated vapour of liquid nitrogen (about at −110°C to −120°C) for 3 minutes and then stored in the liquid nitrogen.

After the storage for 9 years, each sample was thawed and mixed. The motility of the semen sample was scored subjectively, with five representing optimum motility (+++, ++, +, ±, −) under light microscope at 37°C. The smear preparation of semen sample was fixed in formalin vapour and stained with carbol-fuchsin-eosin by the routine staining

procedure of our laboratory. In order to examine the percentage of abnormal spermatozoa including neck-bending ones, approximately 500 spermatozoa were examined under a light microscope.

The fertility test was performed by the artificial insemination using 10 White Leghorn layers which had been selected according to the laying record during one month prior to the test. The dosage of the semen sample used for artificial insemination was 0.18 ml per hen. Insemination was performed into the posterior regions of the uterus by means of a syringe connected to a glass tube (length: about 11 cm). The fertility potential was examined during a period of two weeks from the second day to the fifteen day following insemination. These results were compared with those of our previous works[5–7].

RESULTS AND DISCUSSION

The motility of the undiluted semen used in this experiment was 90% (++ over) at the time of semen collection in 1971. The percentage of motile spermatozoa in thawed semen after storage for 9 years was 75% (++ over) and that of abnormal spermatozoa including neck-bending ones frozen for the same period was 8.3% respectively. The results set these results against the previous ones[5–7] concerning to the percentages of the motility and abnormal spermatozoa after storing for 1/4, 2 and 5 years are summerized in Table 1.

Table 1. Comparison of the motility and abnormality of fowl spermatozoa stored for 1/4, 2, 5 and 9 years

Freezing period (years)	Date of execution	Motility after thawing (%)	Abnormality after thawing (%)
1/4	1970	88.3	14.9
2	1972	88.3	–
5	1975	84.0	10.8
9	1980	75.0	8.3

The motility of the thawed semen stored for 9 years is slightly low as compared with that of our previous ones. The reason must have been that the former was the result of only one sample while the latter was an average of several samples. As the motility of this thawed semen comes within the category of that of the previous works, it seems that the result is not always to show a striking decrease of sperm motility.

The abnormality in the present experiment was 8.3% on the average as shown in Table 1, this is rather low as compared with that of our previous works[5–7]. These results show that the abnormality of the stored spermatozoa do not increase with the lapse of time.

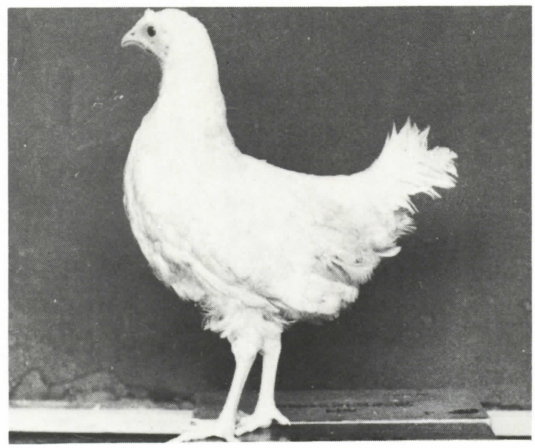
The results of the fertility tests are shown in Table 2 and Text-fig. 1.

The "first week fertility" viz., percentage of fertile eggs produced during the first week reckoned from the second day following insemination was 46.8% (29/62) and the

Table 2. Fertility of the fowl spermatozoa stored for 9 years

Date / No. of hens	First week							Second week						
	May 16	17	18	19	20	21	22	23	24	25	26	27	28	29
2	×		○	○	○	○	○		○	×	○			×
3	○	×		○		○		○	×	○	○			○
4	×	×	○	×	×	×	×	×	○	○		○	○	○
5	×	×	○	×	○	×	○	○	○	○	○	○	○	○
6	○		×	○	○	○	○		×		○	○	○	○
7	×	×	○	○	×	×		○	○	○	○	○		×
8	○	○	×	×	×	○	○	○		○	○	○	○	○
9	×	×	×	×	×	×	×	○	○	×	○			×
11	○	○	○	○	×		○	○	○	○	○	○	○	○
12	×	○		○	○	○	○		○	○		○	○	○

Note: × . . . . Fertile egg, ○ . . . . Infertile egg; 1st week fertility 46.8% (29/62)
2nd week fertility 14.3% (8/56)



Text-fig. 1.
Normal White Leghorn hen of seventy days old secured from the egg laid by hen which was inseminated with semen stored for 9 years.

"second week fertility" viz., percentage of fertile eggs produced during the second week reckoned from the ninth day following insemination was 14.3% (8/56). The results set the present fertility against the previous ones by making use of the semen samples stored for 1/4, 2 and 5 years are summerized in Table 3.

Text-fig. 1 shows the normal hen of seventy days old secured from the egg laid by a hen that had been inseminated with semen stored for 9 years.

Table 3. Comparison of the fertility of fowl spermatozoa stored for 1/4, 2, 5 and 9 years

Freezing period (years)	Fertility (%)	
	Fitst week fertility	Second week fertility
1/4	60.0	5.4
	32.7*	
2	62.7	42.4
	52.6*	
5	53.1	45.9
	50.0*	
9	46.8	14.3
	31.4*	

Note: *Average two-week fertility

The first week fertility decreased in some degree as compared with the previous three results[5-7]. The average 2-week fertility was 31.4%. This is lower than that of storing for 2 and 5 years but is rather close to that of 1/4 year. The cause of the lowering of this fertility is not always clear but it may depend upon the taking in and out of the semen samples contained in the canister. An other possible reason is that the number of semen samples used in this experiment were limited in number. Anyhow the fertility in this experiment lowered in comparison with that of the previous works.

SUMMARY

Fowl semen diluted with 5% glucose egg-yolk solution which contained 7% glycerol in its final concentration and quickly frozen by liquid nitrogen was stored at −196°C for 9 years. The motility, abnormality and fertility of the spermatozoa in thawed semen were studied and showed the following results.

1. The percentage of motile spermatozoa in thawed semen stored for 9 years was 75% on the average. The spermatozoa motility was slightly low as compared with that of the previous ones which had been stored for 1/4, 2 and 5 years.
2. The percentage of abnormal spermatozoa in thawed semen stored for 9 years was 8.3% on the average. This spermatozoa abnormality is rather low as compared with that of our previous works and did not seem to be adversely affected by the long storage time.
3. The fertility of the thawed semen was 46.8% in the first week and 14.3% in the second week. The first week fertility decreased in some degree as compared with the previous three results and the second week fertility decreased more than that of 2 and 5 years storage with the exception of 1/4 year.

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長期間（９年）凍結保存した鶏精液の受精結果について

抄　　録

グリセリンの最終濃度７％の５％ブドウ糖・卵黄液で４倍に希釈した鶏精液を急速凍結法によって液体窒素中に９年間保存し，融解後の精子の活力，奇形率を調べ受精試験を行なった結果は次の如くである。

1. ９年間凍結保存した鶏精子の融解後の平均活力は75％（＋以上）で，これまでの３か月，２年および５年間凍結保存した精子の活力にくらべやや低下を示した。
2. ９年間凍結保存した鶏精子の平均奇形率は8.3％で，３か月，２年，５年間凍結保存した精子のそれにくらべむしろ低く，凍結保存期間が長くなるにつれ奇形率が増加する傾向は認められなかった。
3. １週目受精率は46.8％，２週目受精率は14.3％で，１週目受精率は３か月，２年，５年間凍結保存精子のそれにくらべ幾分低下したが，２週目受精率は３か月凍結保存精子による受精率より高かったが，２年，５年間凍結保存精子による受精率にくらべるとさらに低下した。