J. Fac. Fish. Anim. Husb.,
Hiroshima Univ. (1977), **16**: 59 ~ 64

A Diluent for Deep Freezing Preservation of Fowl Spermatozoa

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Received March 22, 1977

(Figure 1; Tables 1 – 3 and Plate 1)

Since the time that the possibility of long-term storage of fowl spermatozoa at low temperature was reported by SHAFFNER *et al.*, [1] numerous attempts have been made on this subject. However, non of them provided satisfying results in the case of bull semen.

Hitherto, we had used in the experiment of frozen fowl semen from the depression of freezing point (-°C) of view, a diluent* which contained 7% glycerol in its final concentration. The semen was stored by means of the quick freezing method[2] which was completed within 10 to 17 minutes. The fertilities by the long-term storage for 2 to 5 years showed a 50 to 60 percent level.

Recently, we has adjusted the new diluent to promote the fertility furthermore, and met with a better result than that obtained by utilizing previous diluent.

EXPERIMENTAL PROCEDURE

Pooled semen was obtained from a group of White Leghorn cockerels by the abdominal massage technique of BURROWS and QUINN[3]. The pooled samples of semen were kept at a 5°C temperature for 15 minutes immediately after collection, and then diluted 1 to 3 with the diluent indicated above in the control group and with a non-yolk 5.7% glucose solution newly adjusted in the experimental group, both diluents contained 7% glycerol in their final concentration. Approximately 0.2 ml of semen were dispensed into a 1 ml straw ampule and sealed. After 15 minutes glycerol equilibration, the samples were subjected to pre-freezing into the evaporating vapour of liquid nitrogen (about -110°C to -120°C) for 2 minutes, and subsequently stored in liquid nitrogen. Semen samples frozen as described above were kept in storage during periods running from 20 hours to 24 days. For the thawing process the frozen samples were placed for 5 minutes in a beaker containing water at 5°C first, and then transferred to another one containing water at 20°C, then were held at this temperature (Fig. 1). Following the thawing process, the motility was scored subjectively with five representing optimum motilities (+++, ++, +, ±, -). The smear preparation of each sample was fixed in formalin vapour and stained with carbol-fuchsin-eosin by the routine staining procedure of our labor-

*The constituent is composed of 5% $C_6H_{12}O_6$ solution 85, plus fresh egg yolk 15 by the volume.

atory. In order to examine the percentage of deformed spermatozoa including the neck-bending ones, approximately 500 spermatozoa were investigated under the light microscope.

The results obtained with the present new diluent were compared with those in a previous diluent reported elsewhere. The fertility of the frozen semen stored for 14 days and 73 days by the above two diluents was examined by the artificial insemination administerd to 12 and 7 White Leghorn layers for the former diluent; 9 and 6 ones for the later one.

RESULTS AND DISCUSSION

In our experiments[2), 4), 5)]on the fowl semen frozen by means of previous diluent, the fertility was not more than 65 percent level (Table 1 and Plate 1). Moreover, it was observed by WILCOX[6)] that additions of egg yolk to the diluent, or substitution of the LAKE'S solution[7)],as a diluent for storing chicken semen resulted in a marked lowering of the fertility. In accordance with these findings, we have newly adjusted a diluent which contained non-yolk in order to promote the fertility furthermore.

As shown in Table 2, the semen was chracterized at intervals of storage up to 24 days. In the case of our new diluent, the mean percentage of motile spermatozoa in thawed semen after storage for 20, 25, 30, 48, 60, 72 hours, 14 and 24 days was 85.6% (over ++), ranging from 70 to 95%, while that obtained by the previous diluent was 73.9% (over ++), ranging from 60 to 80%.

Furthermore, the percentage of deformed spermatozoa including neck-bending ones in the frozen semen for the same period in the former was 8.4% on the average, ranging from 3.8 to 11.2%, while that of the latter was 10.1% on the average, ranging from 6.7 to 12.3%. Thus, we obtained a clear significant difference in the percentage of motile spermatozoa in thawed semen but a no significant difference in that of deformed spermatozoa for the above two groups. Furthermore, as the percentages of motile and deformed spermatozoa in the undiluted semen were 91.7% and 7.0% respectively, those in our new diluent are better than those obtained by the previous one.

The results of single insemination using semen samples stored for 14 and 73 days in experimental and control groups, are shown in Table 3 and Plate 1. "The first week fertility" viz., percentage of fertile eggs produced during the first week as reckoned from the second day after the insemination were 75% and 73% in the experimental group and 58.0% and 47.8% for the control group. "The second week fertility" viz., percentage of fertile eggs produced during the second week as reckoned from the ninth day after the insemination were 51.8% and 60.0% in the experimental and 22.2% and 43.3% for the control group. Thus, there were clear difference in fertility between the two groups of Trial 1 and Trial 2. In the experimental group, the duration of fertile eggs produced from the second day following insemination to the 14th day showed an average of 12.3 days in Trial 1 and Trial 2, and for the control group an average of 11.8 days. Moreover, in the third week, the fertile eggs were gained on the 19th and 18th day following in-

Table 1. Comparison of the motility, defomity and fertility of fowl spermatozoa after freezing during different periods

Freezing period (years)	Motility (%)	Deformity (%)	Fertility (%)	
			1st week	2nd week
1/4	88.3	14.9	60.0	5.4
			32.7*	
2	88.3		62.7	42.4
			52.6*	
5	84.0	10.8	53.1	45.9
			50.0*	

* Average two-week fertility.

Table 2. Comparison with the motility and deformity of fowl spermatozoa stored by two diluents

Freezing period (days)	Undiluted semen		Egg-yolk solution		Glucose solution	
	Motility (%)	Deformity (%)	Motility after Thawing(%)	Deformity (%)	Motility after thawing(%)	Deformity (%)
5/6	95	8.4	80	12.2	90	11.2
1	95	9.0	80	11.1	95	9.1
1-1/24	95	5.4	70	6.7	90	7.5
1-1/4	95	7.3	80	11.2	90	10.2
2	85	5.4	60	9.6	70	8.1
2-1/2	85	4.0	70	10.2	80	3.8
3	85	6.0	75	7.6	75	7.2
14	95	8.0	70	12.3	90	8.7
24	95	9.3	80	10.4	90	10.0
Mean± S.D.	91.7± 4.7	7.0± 1.7	73.9*± 6.6	10.1**± 1.8	85.6*± 8.0	8.4**± 2.0

* ($p > 0.01$), ** ($p < 0.05$)

Table 3. Comparison of the fertility of spermatozoa stored by two diluents

	Glucose solution		Egg-yolk glucose solution		Difference favouring glucose solution	
	Fertility (%)		Fertility (%)			
	1st week	2nd week	1st week	2nd week	1st week	2nd week
Trial 1	75.0	51.8	58.0	22.2	+17.0	+29.6
Trial 2	73.0	60.0	47.8	43.3	+25.2	+16.7
Mean	74.0	55.9	52.9	32.8	+21.1	+23.1

semination in the case of the experimental group of Trial 1 and Trial 2, but no further more in the control group. These results obtained with the experimental group may be considered as a remarkable progress over our previous results.[2),4),5)] The mechanism of non-yolk diluent should be superior to the previous one, remains an open question. At any rate, if it becomes possible to get a constant of 75% or more fertility in fowl insemination by making use of our new diluent, it might follow that the application of frozen fowl semen in the field of artificial insemination becomes a success.

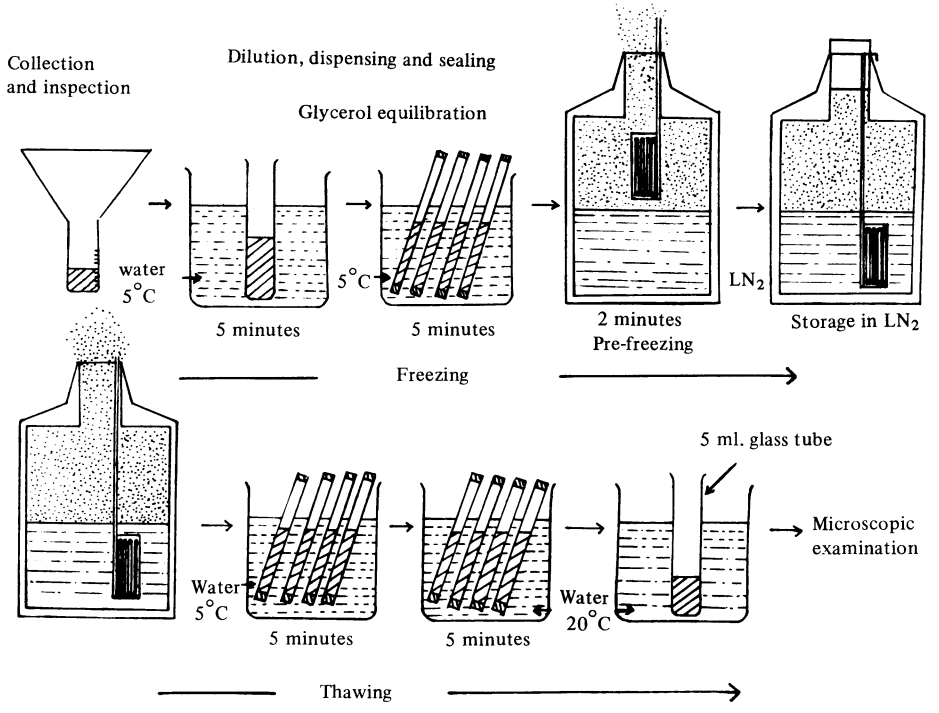

Fig. 1. Manipulations of freezing and thawing of fowl semen.

SUMMARY

The motility, deformity and fertility of the frozen semen which were diluted 1 to 3 with a non-yolk 5.7% glucose solution and the previous diluent containing 7% glycerol in its final concentration were compared mutually. The results are as follows:

The motility and the deformity percent of thawed semen treated by a non-yolk glucose solution was 85.6 ± 8.0 percent and 8.4 ± 2.0 percent on the average. The Motility was higher and the deformed sperm average lower than those in the previous diluent. The fertility of semen diluted by the present new diluent were 74.0 and 55.9 percent in the first and second week respectively. These were fairly higher than those in the previous diluent.

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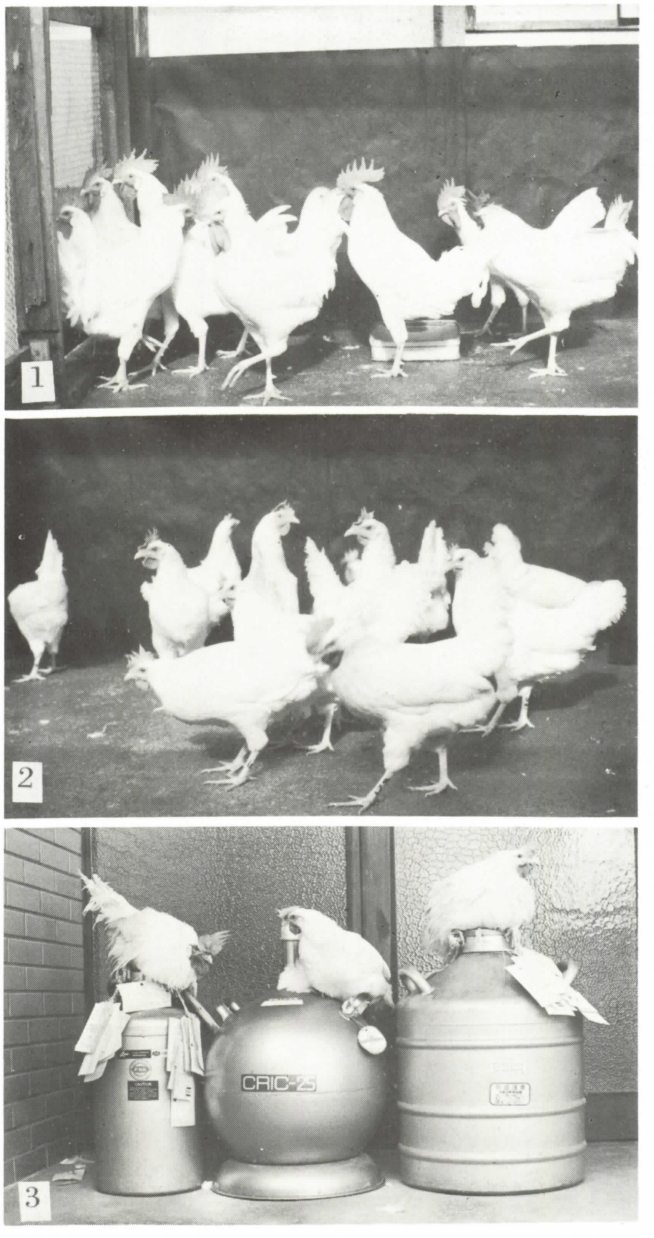

EXPLANATION OF PLATE

1. White Leghorn cocks gained from the eggs laid by hens which had been inseminated with semen stored for two years; 132 days old.

2. White Leghorn hens gained from the eggs laid by hens which had been inseminated with semen stored for two years; 132 days old.

3. White Leghorn cock and hens gained from the eggs laid by hens which had been inseminated with semen stored for five years; 180 old.

鶏精子の凍結保存用希釈液について

渡辺守之・寺田隆登・白河義久

著者らはこれまでの実験で，グリセリンの最終濃度が7％になるよう調整した卵黄ブドウ糖希釈液を用いて鶏精液を4倍に希釈して凍結保存を実施してきたが，その受精率は50～60％の水準にとどまった。今回この従来の希釈液を対照区とし，新たにnon－yolk 5.7％ブドウ糖液を試験区としてそれぞれ4倍希釈で凍結保存を実施し，融解後の精子活力，奇形率および受精率について比較検討した結果，融解後の精子活力については両区間に明らかな有意差が認められた（$P>0.01$）が奇形率においては有意差は認められなかった（$P<0.05$）。試験区の1週目受精率は平均74.0％，2週目受精率は平均55.9％で対照区の1週目平均受精率52.9％，2週目平均受精率32.8％に比らべかなり良好な結果を示した。

以上の結果，鶏精子の凍結保存においてもnon－yolkブドウ糖液は好結果を与えるもののようである。