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FREEZING OF FOWL SEMEN (*Gallus domesticus*)

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(Table 1; plate 1)

Since Shaffner et al. (1941) proposed the possibility of long-term storage of fowl semen at low temperatures, a large number of studies have been undertaken on preservation by freezing of fowl semen. However, most of those studies brought only unsatisfactory results compared with those of frozen bull semen.

In 1977, from September 1st to October 12th, Watanabe had an opportunity to work with the staff of the Poultry Science Department in the Institute of Animal Husbandry, in Krakow, Poland, on deep freezing preservation of fowl semen. This was under the auspices of the Exchange Programme of Scientists between the Japan Society for the Promotion of Science and the Polish Academy of Sciences. He studied the freezing procedures outlined by Lake (1970) and modified by Lada-Gorzowska et al. (1975). In this method, the temperature of the semen samples was gradually reduced to −20°C before deep freezing in liquid nitrogen (−96°C). Glycerol was removed from the semen prior to intra-vaginal insemination. The fertility following single insemination was then determined.

MATERIALS AND METHODS

The semen of 11 Leghorn, New Hampshire and New Hampshire X Leghorn cocks was frozen and White Leghorn hens were used for the insemination test. The collected semen was diluted four times with Lake's solution# (1 part semen: 3 parts diluent). The freezing, glycerol removal and insemination were carried out according to the Lake's method (1970); i.e. the temperature of the semen samples was gradually reduced to −20°C before deep freezing in liquid nitrogen (−196°C). After thawing, the glycerol was removed by centrifuging at 2500 rpm at 700 g at 5°C, and then the Lake's non-glycerol diluent## was slowly added at the same temperature. The artificial insemination was intra-vaginal (about 4 cm depth) and estimated to be in the vicinity of the sperm-host glands. After storage

Composition of Lake's solution: glycerol 13.64 g, Na glutamate H_2O 1.92 g, Fructose 0.8 g, Mg acetate 4 H_2O 0.08 g, K acetate 0.5 g and Polyvinyl pyrrolidone (MW 10.000) 0.3 g, dissolved in 100 ml distilled water.

Non-glycerol diluent by Lake: Na glutamate H_2O 1.92 g, Fructose 0.6 g, Mg acetate $4H_2O$ 0.08 g, K citrate H_2O 0.128 g, and Na acetate 2 anhydrous 0.51 g, dissolved in 100 ml distilled water.

Table 1. Fertility after artificial insemination with semen gradually cooled to −20°C before deep freezing in liquid nitrogen (−196°C)

Trial	Number of hens	Number of eggs		Fertility (%)
		laid	fertile	
I (Sept. 7th, '77)	12	32	17	53.1
II (Sept. 13th, '77)	12	36	20	55.6
III (Sept. 21st, '77)	8	22	14	63.6
Total & average	32	90	51	56.7

of one to three days, about 0.25 ml of the diluted semen was inseminated into each hen. The freezing and inseminating appliances are shown in Plate 1.

RESULTS AND DISCUSSION

The mean percentage of motile spermatozoa in thawed semen after storage for one to three days ranged from 50 to 75 percent. The motility differences of spermatozoa after the above mentioned storage seems to be correlated to the initial quality of each sample. This estimation was made by Lada-Gorzowska and Watanabe. The fertility test was carried out more than three times. Each hen received 0.25 ml diluted semen. The fertility was examined on eggs laid from the 2nd to 5th day following insemination. The results are shown in Table 1. In trials I and II, 12 hens were inseminated, 8 hens in trial III. As shown in Table 1, the fertility in trials I, II and III was 53.1, 55.6 and 63.6 percent, respectively. The mean fertility of these three trials was 56.7 percent. These results seem to be fairly good as compared with those of Lada-Gorzowska et al. (1975) using Cornish and White Rock chickens.

As mentioned above, freezing, thawing and insemination procedures were carried out according to Lake's method (1970). In this case, particular attention was given to the following three points: 1) Gradual reduction of temperature to −20°C before deep freezing in liquid nitrogen (−196°C). 2) Glycerol removal from the thawed semen by centrifuging. 3) Intra-vaginal insemination in the vicinity of the sprm-host glands. The first two points are fairly troublesome and seem to require special techniques. Especially, the glycerol removal from the thawed semen and the addition of the non-glycerol diluent by Lake's method (1970) after the removal of the glycerol at 5°C require special technical skills and the beginner is often apt to fail. In fact these two points contain some practical problems solved in the future.

In a preliminary experiment, we planned to reexamine Lake's method in order to compare it with Watanabe's method (1976) which requires only 15 minutes from semen collection until the completion of freezing. But Watanabe's method which tried out in the Institute of Animal Husbandry, in Krakow, Poland during his stay did not work smoothly, meaning that the results in this paper have been obtained only from Lake's

method, modified by Lada-Gorzowska et al. (1975). For further improvement of deep freezing preservation of fowl spermatozoa, a open exchange between Japan and Poland of the data will be necessary.

SUMMARY

11 Leghorn, New Hampshire and New Hampshire X Legnorn cocks, aged 15 months, as well as 23 White Leghorn hens were used in the examination of deep freezing preservation of fowl spermatozoa. The semen was processed and frozen by Lake's method modified by Lada-Gorzowska et al. (1975). These procedures included three principal points:

(1) The temperature of semen samples was gradually reduced to −20°C before the deep freezing in liquid nitrogen (−196°C).

(2) Before insemination, the glycerol was removed from the semen by centrifuging at 700 gl at 5°C and a non-glycerol diluent by Lake was added.

(3) Artificial insemination was intra-vaginal (about 4 cm depth), estimated to be in the vicinity of the sperm-host glands.

The average percentage of fertile eggs following single inseminations in three trials was 56.7 percent, ranging from 53.1 to 63.6 percent.

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鶏精液の凍結保存

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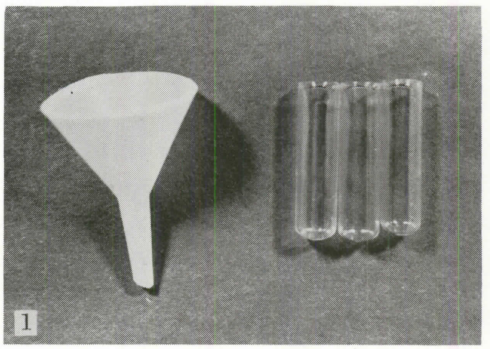
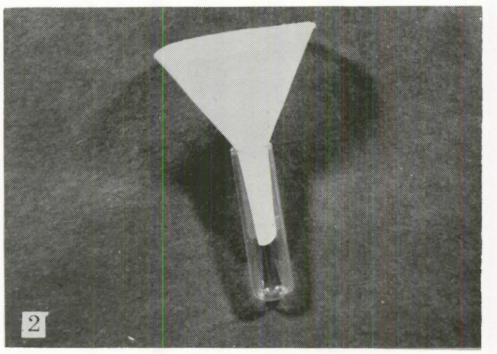
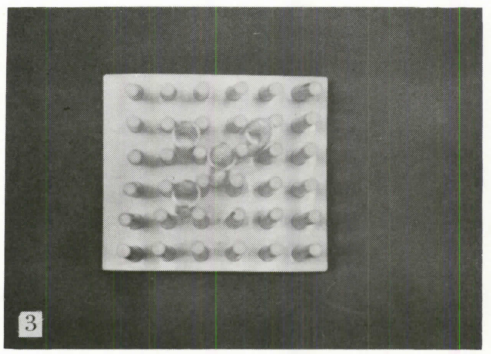
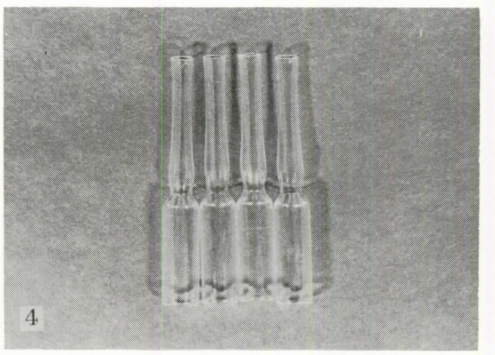
鶏精子の凍結保存試験に15カ月令のLeghorn種，New Hampshire種およびNH×Lの雄鶏11羽の精液を又凍結精液をもってする人工授精には21羽のWhite Leghorn種を使用した。凍結はポーランドのLada-Gorzowskaら（1975）の方法によって実施した。この方法の要点は次の3つにある。(1) 採取精液は−20℃までは除々に下げてゆきその後−196℃の液体窒素中に凍結する。(2) 凍結精液は融解後直ちにglycerolを除去しこれにLake氏液を数回に分けて添加して授精する。(3) 人工授精部位はcloacaより凡そ4㎝内部にあるsperm-host gland附近の腟内に注入する。

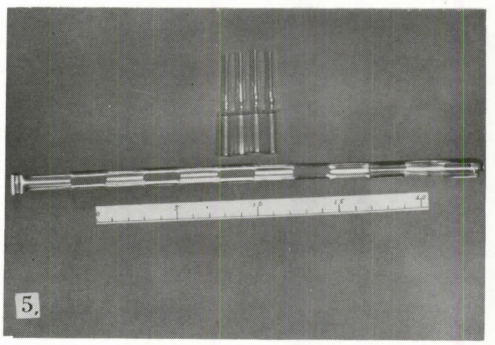
その結果受精率は53.1～63.6％，平均56.7％でかなり良好な結果を示した。











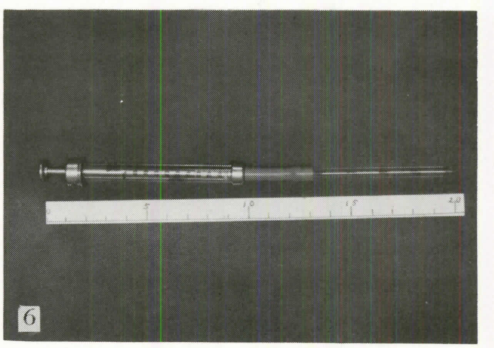


Fig. 1. Small funnel (diameter 3.5 cm; length 5 cm) and glass tube (diameter 9 mm; length 4 cm) for collecting semen
Fig. 2. The same as left Fig. 1
Fig. 3. Rack for collecting test tube
Fig. 4. Ampules only during the freezing process
Fig. 5. A holder for ampules
Fig. 6. An avian inseminator (total length 20 cm)