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Studies on Deep Freezing Preservation of Turkey Semen

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According to SMITH[1] (1968), it is said that the turkey industry has utilized artificial insemination to a much greater degree than any other in the poultry field in America. Much of the progress in breeding heavy broad-breasted turkeys has come about through extensive use of artificial insemination. Some of the larger and meatier strains can barely reproduce themselves naturally, but it is possible to have over 80 per cent of their fertile eggs following artificial insemination. He estimates that about 85 per cent of all turkeys presently hatched in the United States are the product of matings by artificial insemination.

Heretofore, attempts to store diluted turkey semen in liquid state have carried out by MORAVEC et al.[2] (1954), HARPER[3] (1955), CARTER et al.[4] (1957), WILCOX et al.[5] (1960), HARRIS et al.[6] (1963), BAJPAI et al.[7] (1963) and HARRIS[8] (1968) but it seems that very little research has been done on deep freezing preservation of turkey semen with the exception of RAJAMANNAN's report[9] (1968). The present experiment was conducted to apply our techniques[10] (1970) of frozen fowl semen to the artificial breeding in turkeys which will be expected to be a superior meat-producing bird in the future.

EXPERIMENTAL PROCEDURE

Semen was collected from 7 Bronze turkey males of 10 to 20 months of age. The collection of semen was made at 7.00 to 8.00 a. m. by abdominal massage of WATANABE et al.[11] (1967). Semen receptacle was used a pyrex funnel tube of 3.5 cm in diameter and 6.0 cm in depth devised by author [12] (1969). The semen collected were used for the experiments individually. The freezing and thawing methods employed in the present experiment were made by the same ones as our quick freezing and thawing methods in the fowl as shown in Fig. 1. Namely, the freezing method requires only 15 minutes from the collection of semen until the completion of freezing.

In the beginning of the experiment, 5 per cent of glucose solution was used for freezing extender as in the case of the fowl but when a microscopic examination was made on the semen, neck-bending spermatozoa were found in large numbers of spermatozoa. Thus, the freezing point depression of turkey semen was measured ($\Delta = -0.77°C$), and 5 per cent, 7 per cent and 9 per cent of glucose solutions were prepared for the diluent of the present experiments as shown in Table 1. The effect of

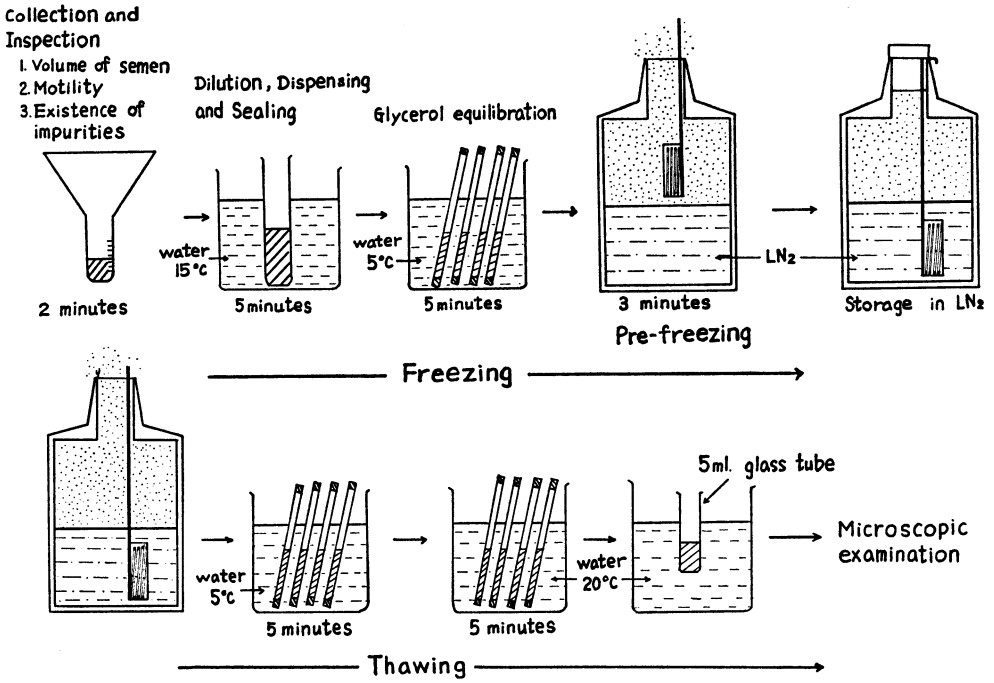

Fig. 1. Manipulations of freezing and thawing of turkey semen.

each concentration of glucose on the thawed semen after freezing was investigated. The semen was diluted to 4 times its original volume with above each glucose solution. All semen samples were frozen in liquid nitrogen vapour. After the storage, each semen sample was thawed and the motility of spermatozoa and the percentage of abnormal spermatozoa including neck-bending ones were examined microscopically. In each sample, approximately 500 spermatozoa were fixed with a routine procedure for carbor-fuchshin-eosin staining. The results were compared with each other.

Table 1. pH and depression of freezing point of undiluted turkey semen and diluents.

Item	pH	Depression of freezing point (−°C)
undiluted semen	7.80	0.77
5% $C_6H_{12}O_5$ solution*	6.30	0.59
7% $C_6H_{12}O_6$ solution*	6.18	0.82
9% $C_6H_{12}O_6$ solution*	6.28	1.07

* glucose solution 85 + fresh egg yolk 15

RESULTS AND DISCUSSION

As for the freezing point depression of Bronze turkey seminal fluid, BROWN[13]

(1959) already reported that it was −0.715°C on the average for four periods from January 23rd to April 18th. The freezing point depression of undiluted turkey semen in the present experiment was −0.77°C and closely resembles that of Brown stated above. The freezing point depression of 5 per cent, 7 per cent and 9 per cent of glucose solutions were −0.59°C, −0.82°C and −1.07°C respectively as shown in Table 1. The freezing point depression of 7 per cent of glucose solution gave the most approximate value compared with that of undiluted turkey semen.

The motility of spermatozoa in thawed semen diluted with 5 per cent glucose solution stored for 6 to 200 days after freezing was shown in Fig. 2 and Fig. 5. The

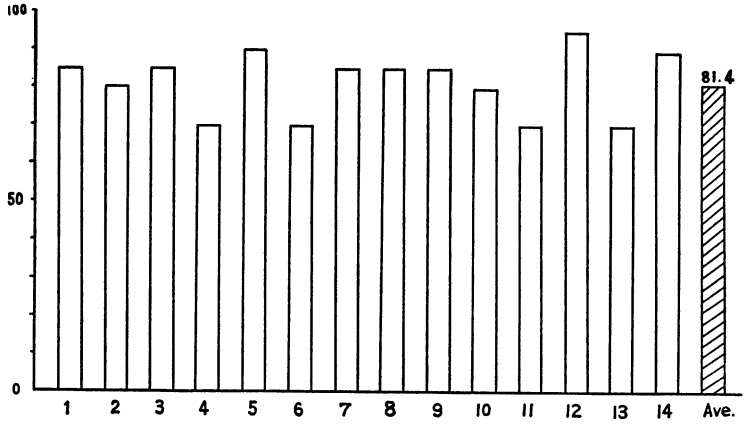

Fig. 2. The effect of 5 percent glucose solution on the percentage of motile sperm (over ++) after thawing.

percentage of motile sperm (over ++) was 81.5 per cent on the average per 14 samples. That of 7 per cent glucose solution stored for 6 to 173 days after freezing was shown in Fig. 3 and Fig. 5. The percentage of motile sperm (over ++) was

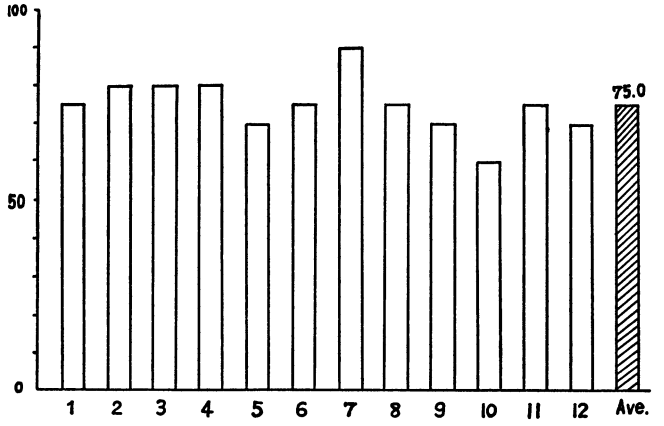

Fig. 3. The effect of 7 percent glucose solution on the percentage of motile sperm (over ++) after thawing.

75.0 per cent on the average per 12 samples. That of 9 per cent glucose solution stored for 6 to 163 days after freezing was shown in Fig. 4 and Fig. 5. The percentage of motile sperm (over ++) was 23.0 per cent on the average per 9 samples. There was no remarkable difference between the former two but the motility of spermatozoa in thawed semen diluted with 9 per cent glucose solution was markedly low compared with that of the former two solutions as shown in Fig. 5.

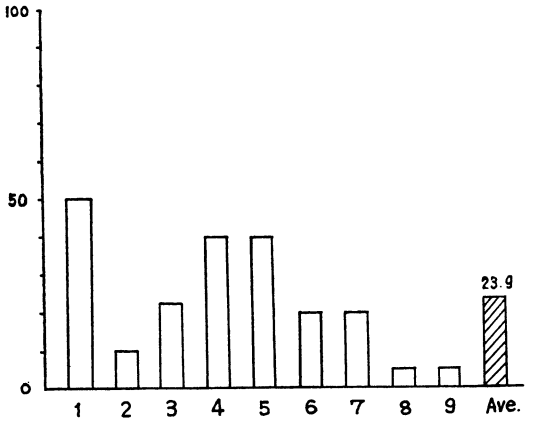

Fig. 4. The effect of 9 percent glucose solution on the percentage of motile sperm (over ++) after thawing.

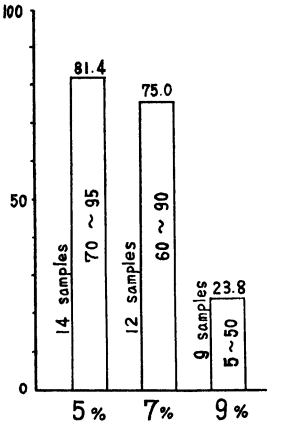

Fig. 5. The effect of various diluents on the percentage of motile sperm (over ++) after thawing.

The percentage of abnormal spermatozoa in the undiluted turkey semen calculated as the control was 6.1 per cent on the average. The percentage of abnormal spermatozoa including neck-bending ones in the frozen semen which was diluted with 5 per cent glucose solution was 31.5 per cent on the average and that of 7 per cent and 9 per cent glucose solutions were 14.3 per cent and 53.7 per cent as shown in Fig. 6. From the view point of an appearance of abnormal spermatozoa incluc-

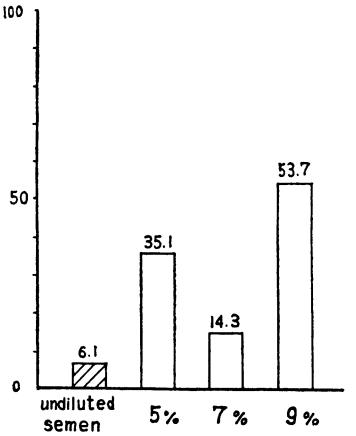

Fig. 6. The effect of various diluents on the deformity percent appeared in the turkey semen after thawing.

ing neck-bending spermatozoa, 7 per cent glucose solution seems to be the most suitable for the turkey semen dilutor in freezing preservation. The percentage of abnormal spermatozoa in thawed semen diluted with 5 per cent and 9 per cent glucose solutions were too high as compared with that of undiluted turkey semen and these are far apart from that of the category of so-called normal semen previously stated by author (1958). These results seem to be concerned with the freezing point depression of turkey semen fluid. After that the experiment of fertility test by the turkey semen which were frozen by the use of liquid nitrogen may be promoted with 7 per cent glucose solution as a diluent.

SUMMARY

The effect of three different levels of glucose solution as a component of deep freezing diluent on the motility, per cent neck-bending spermatozoa of turkey semen was studied.

The percentages of motile spermatozoa (over ++) in thawed semen diluted with 5 per cent, 7 per cent and 9 per cent glucose solutions were on an average of 81.5 per cent, 75.0 per cent and 23.9 per cent respectively. The percentages of abnormal spermatozoa including neck-bending ones in thawed semen diluted with 5 per cent, 7 per cent and 9 per cent glucose solutions were on an average of 31.5 per cent, 14.3 per cent and 53.7 per cent. From the two view points of motility after freezing and the appearance of abnormal spermatozoa including neck-bending ones, 7 per cent glucose solution seems to be more suitable for the freezing diluent of turkey semen than 5 and 9 per cent glucose solutions.

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七面鳥精液の凍結保存に関する研究

渡辺　守之・加藤　秀一

5％，7％および9％ブドウ糖液を希釈液とした場合の七面鳥精子の融解後の活力および頸曲り異常精子の出現率について調べた結果は次の如くである．

1.　5％，7％および9％ブドウ糖液で4倍に希釈し急速凍結法によって凍結後融解した精子の活力割合（＋以上）はそれぞれ81.5％，75％および23.9％で5％，7％ブドウ糖液を使用した精液の間にはそれほど著明な差異は認められなかったが，9％ブドウ糖液を使用した場合には前二者の場合にくらべて融解後の精子活力は著しく低下した。

2.　上記5％，7％および9％ブドウ糖液使用による融解後の頸曲り異常精子の出現率は対照の原精液の6.1％に比較しそれぞれ31.5％，14.3％，53.7％で七面鳥精液の凍結用希釈液としては7％ブドウ糖液が好適のように思われる．

3.　この凍結融解後の精子活力および頸曲り異常精子出現率から7％ブドウ糖液が七面鳥精液の凍結用希釈液としてその受精率を高める上に役立つものと思われる．