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Fertility of Muscovy Semen Frozen for about Three Years

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(Tables 1–3)

Attempts to inseminate liquid semen to ducks for the purpose of Mule-duck production have been carried out by several investigators such as Onish et al. (1955)[1], Watanabe (1959, 1961)[2),3)], Huang et al. (1974)[4)] etc. Yet it seems that very little research has been done until today on artificial insemination with frozen Muscovy semen since the fertility level for Mule-duck production does not give satisfaction. Hence, the present authors have been performing the experiments with frozen fowl,[5)–10)] drake,[11),12)] and turkey semen.[13)–15)]

The present paper reports the results of experiments for Mule-duck production which were carried out using frozen Muscovy semen stored for about three years.

MATERIALS AND METHODS

Semen ejaculates were collected from Muscovy drakes of 12 months of age as shown in Table 1. The experimental semen was processed and frozen by the procedures for fowls that Watanabe et al. (1970)[6)] used. The composition of the diluent for the frozen semen was follows: 5.5% C_6 H_{12} O_6 solution 85 plus fresh egg yolk 15. The Muscovy spermatozoa in the diluents gave a final concentration of 7% glycerol. The Muscovy semen was collected by intercepted method. The collected semen was diluted to four fold with the above diluent and kept at 5°C for 5 minutes immediately after collection. Afterwards about 0.8 ml of the diluted semen was transfered into 1 ml straw ampule and sealed. Following equilibration for 5 minutes, the samples were subjected to pre-freezing in evaporated vapour of liquid nitrogen (about −110°C to −120°C) for 3 minutes and then stored in the liquid nitrogen. This was done in 1978. After 3 years storage, each sample was thawed in May 1981, and the motility of the spermatozoa and the percentage of abnormal spermatozoa including the neck-bending ones were examined microscopically. The motility was scored subjectively with five representing optimum motilities (+++, ++, +, ±, −). The smear preparation of each smaple 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 the neck-bendding ones, approximately 500 spermatozoa were investigated under the light microscope. The fertility of frozen semen stored for about three years (1032 to 1060 days) at −196°C in

the method explained above was examined by artificial insemination on 23 Osaka ducks, 12 months of age which had been selected according to the laying records during one month prior to the test. The volume of semen sample for insemination was 0.2 ml per head and it was deposited into the uterus.

RESULTS AND DISCUSSION

Table 1 indicates the general properties of the semen stored for about three years. The mean percent of motile spermatozoa in thawed semen after storage for 1032 to 1060 days was 68.8% (over ++), ranging from 60 to 75%. Therefore the influence of storage at −196°C upon the motility of frozen Muscovy semen was not very striking. The differences of the motility of spermatozoa after storage above mentioned seem to be due to the initial quality of each sample collected firstly. Nevertheless the motility of this thawed semen after 3 years storage seems to be partially lesser than that of our frozen fowl semen.[10]

Table 1. The motility and abnormality of the Muscovy spermatozoa used in the experiments

Drakes No.		Storing period (days)	Motility of raw semen (%)	Motility after thawing (%)	Abnormality of spermatozoa(%)	Injected ducks No.
Trial 1	5	1032	95	75	16.8	416, 468, 471 481
	7	1032	95	75	20.2	418, 420, 421 422, 424, 430 433, 435, 437 451, 480
Trial 2	6	1060	95	65	7.6	434, 443, 447 474, 486
	7	1034	95	60	9.3	427, 438, 445 450, 462, 475 491
Mean			95	68.8	13.5	

The abnormalities in the present experiment were 13.5% on the average, in fact ranging from 7.6 to 20.2%, as shown in Table 1. This rate is rather high compared to that of our previous fowl semen.[10]

The results of single insemination with the semen samples stored for 1032 to 1060 days, are shown in Table 2 and Table 3.

The "first week fertility" viz., percentage of fertile eggs produced during the first week reckoned from the second day following insemination was 25.0% (18/72), the "second week fertility" viz., percentage of fertile eggs produced during the second week reckoned from the ninth day following insemination was 22.4% (15/67) and that of 1–2 week fertility was 23.7% (33/139) in Trial 1. The percentage of the first, second and 1–2 week fertilities were respectively 28.6% (10/35), 17.4% (4/24) and 23.7% (14/59) in Trial 2. The fertilities in Trial 1 and 2 are not so differend as those shown in Table 2 and

Table 2. Fertility of the drake spermatozoa stored for 1032 days (Trial 1)

	Ducks No. \ Date	First week							Second week							Number of eggs laid	Number of fertilized eggs
		May 17	18	19	20	21	22	23	24	25	26	27	28	29	30		
Trial 1	416	×			×			×				×			×	5	0
	418	○	×		×	×	×	×	×		×	×	×		×	11	1
	420	×	×	×	○	×	×	×	○	×	○	×	×	×	×	14	3
	421		×	○	×		×	×	×			○		×		8	2
	422	×	×	×		○	×	×	○	○		×	×	×		11	3
	424	○	○			×				×	×	○	×		×	8	3
	430	×	×		×		○		×	×			×			7	1
	433	×	×		×	×			×	×		×	×	×		9	0
	435		×		×			○	×			×	×			6	1
	437	×	○		×	×	○	×	×	×		×	×	×	×	12	2
	451	×	×	×	○	×	○	○	○	○	○				○	11	7
	468		×		×	○		○	○	○	×	×	×	×	○	11	5
	471		×	○		○	×		×	○			×		○	8	4
	480	×	×	×	×	×	×		×	×	×	×		×	×	12	0
	481		×	×	×			○		×	×					6	1
Number of eggs laid		10	14	7	12	10	9	10	12	11	7	11	10	7	9	139	33

Note: ○ Fertile egg; × Infertile egg
First week fertility 25.0% (18/72); Second week fertility 22.4% (15/67)
1–2 week fertility 23.7% (33/139)

Table 3. Fertility of the drake spermatozoa stored for 1034 to 1060 days (Trial 2)

	Ducks No. \ Date	First week							Second week							Number of eggs laid	Number of fertilized eggs
		June 1	2	3	4	5	6	7	8	9	10	11	12	13	14		
Trial 2	427	○		×	○	×	×		×		×					7	2
	434	×									×	×				3	0
	438	×						×	○	×	×	×	×			7	1
	443		×		×	○						○	○		×	6	3
	445		×		×		×			○				×		5	1
	447	×	×	×		×										4	0
	450		○				○		×			×				4	2
	462	○	○	×	×	×	×			×			×			8	2
	474				×		×		×		×		×			5	0
	475	×							×							2	0
	486	×	○			○								×		4	2
	491	○	×			×					×					4	1
Number of eggs laid		8	7	3	5	6	5	1	5	3	5	4	4	2	1	59	14

Note: ○ Fertile egg; × Infertile egg
First week fertility 28.6% (10/35); Second week fertility 17.4% (4/24)
1–2 week fertility 23.7% (14/59)

3, and those of 1–2 week fertility in Trial 1 and 2 gave quite the same resulting average of 23.7%. Yet this is rather low compared to those of Huang et al. (1974) which had

been performed with liquid semen by artificial insemination. The causes of these low fertilities are not always clearly known but it may depend upon the treatment skill during the frozen process of semen samples or upon the diluent used. If these lowering factors could be removed, the use of frozen Muscovy semen for artificial insemination opens certainly great possibilities in the near future.

SUMMARY

Muscovy semen diluted with 5.5% glucose egg yolk solution, which contained 7% glycerol in its final concentration, quickly frozen by means of liquid nitrogen was stored at −196°C during three years. After that storage period the motility, abnormality and fertility of the spermatozoa in the thawed semen were studied and showed the following results.

1. The percentage of motile spermatozoa in thawed semen stored for 1032 to 1060 days was 68.8% (over ++) on the average in Trial 1 and Trial 2.
2. The percentages of abnormal spermatozoa in thawed semen stored for the same period was 13.5% on the average in Trial 1 and Trial 2.
3. The fertility of the thawed semen was 26.8% in the first week, 20.0% in the second week and 23.7% in the 1–2 week on the average, in Trial 1 and Trial 2.

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約3年間凍結保存したマスコビーダック精液の受精率について

渡辺守之・松本安雄・竹下信子・寺田隆登

横取り法によって採取したマスコビーダック精液をグリセリンの最終濃度7％の5.5％ブドウ糖・卵黄希釈液で4倍に希釈し，急速法によって－196℃の液体窒素中に約3年間（1032～1060日間）凍結保存し，融解後の精子活力，畸形率および同精液使用による受精率を調べた結果は次の如く要約される。

1. 液体窒素中に1032～1060日間凍結保存したマスコビーダック精子のTrial 1，2 の融解後の活力は60～75％，平均68.8％（++以上）であった。
2. 又同期間凍結保存したTrial 1，2の融解後の畸形率は7.6～20.2％，平均13.5％であった。
3. 同期間凍結保存した精液使用によるTrial 1および2の1週目平均受精率は26.8％，2週目平均受精率は20.0％，又1－2週目平均受精率23.7％であった。