

## THE FREQUENCY OF SEMEN COLLECTION ON SEMEN CHARACTERISTICS AND FERTILITY IN INTERGENERIC CROSS BETWEEN MUSCOVY DRAKES AND COMMON DUCKS

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### Abstract

Generally very little information is available on artificial insemination in Muscovy ducks. Semen dosages, site of insemination and interval between inseminations have not been studied sufficiently. Similarly the effect of frequency of semen collection on the yield of semen and fertility over certain period of time has not been investigated. Frequency of semen collection from once to three times per week did not affect the average semen volume, concentration, and motility. However, increasing the frequency of semen collection from once to three times per week affected total volume of semen per period per 5 birds. This means that more ducks can be inseminated. It has been demonstrated that by using artificial vagina (AV) technique for semen collection, three times collection of semen over a 16-week period is neither harmful to the drake nor to the quality and quantity of the output. Fertility rate after insemination with sperm collected once a week significantly higher than that of twice and three times per week collections. In conclusion, good quality of semen can be obtained by collecting sperm up to three times per week and good fertility can be obtained by inseminating ducks every four days.

Key words: Muscovy, Frequency, Semen collection, Sperm quality, Fertility

### Introduction

Muscovy (*Cairiana Moschata*) and common duck (*Anas Plathyrynchos var. domestica*) are two different genus. Both species have the same number of chromosomes (20 macrochromosomes and 60 microchromosomes) and very similar karyotypes except differences involving three pairs and the sexual chromosome Z (Deanjean et.al., 1997). Crossing between common duck male with Muscovy female by natural mating occurs more spontaneously where both species are raised together in the villages. Since the pioneer work of Watanabe (1961) artificial insemination in duck has been intensively used, and improved fertility by 10 – 15 % compared to natural mating (Huang and Chou, 1973). Basically there are three methods of semen

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collection, massage or manual manipulation, electrical stimulation and artificial vagina (Setioko, 1981). Tan (1980) described a method for semen collection by artificial vagina in Muscovy drake. He used a glass tube measuring 3.5 cm diameter and 10 cm long as an artificial vagina. By using this technique, semen could be collected relatively frequently without diminution of spermatozoa output. Setioko and Hetzel (1984) compared the three methods of semen collection on semen characteristics and fertility and found that artificial vagina produced the highest semen volume, concentration and number of sperm per ejaculate compared to manual manipulation or electrical stimulation techniques.

One of the important factors affecting the quality of sperm is the frequency with which semen is collected from individual drakes. Tan (1980) collected sperm from Muscovy drakes at frequencies of collection of twice daily, once daily, once every two days, and once every three days for a period of 24 days. It was concluded that once daily is the most efficient for production of spermatozoa of Muscovy drakes over a period of 24 days.

The objective of this study was to determine the yield and characteristics of semen collected from Muscovy drakes using artificial vagina over a six-week period.

### **Materials and Methods**

15 Muscovy drakes aged 32 week were used in this study. They were selected from 25 available drakes based on the homogeneity, good sperm quality and trained. They were divided randomly into three equal groups and subjected to three semen collection frequencies week at three different period, with interval of two weeks for each period. Data were analyzed using a latin square design as described by Steel and Torrie (1995), and the average values were further analyzed using Tukey test. The collection frequencies were once per week (Monday), twice per week (Sunday and Friday) and three times per week (Sunday, Wednesday and Friday as described in Table 1.

Table 1. Time table of semen collection in Muscovy drakes, once, twice and three times per week collection.

Period	Week	Day	Treatment			
			A	B	C	
I	1	Sunday	Ω	Ω	-	
		Wednesday	-	Ω	Ω	
		Friday	Ω	Ω	-	
	2	Sunday	Ω	Ω	-	
		Wednesday	-	Ω	Ω	
		Friday	Ω	Ω	-	
II	3	Sunday	-	Ω	Ω	
		Wednesday	Ω	-	Ω	
		Friday	-	Ω	Ω	
	4	Sunday	-	Ω	Ω	
		Wednesday	Ω	-	Ω	
		Friday	-	Ω	Ω	
	III	5	Sunday	Ω	-	Ω
			Wednesday	Ω	Ω	-
			Friday	Ω	-	Ω
6		Sunday	Ω	-	Ω	
		Wednesday	Ω	Ω	-	
		Friday	Ω	-	Ω	

Note : I - III = Semen collection period I - III  
 1 - 6 = Week 1 - 6  
 Ω = Semen collection  
 . - = No semen collection

Semen was collected by the AV method (Tan, 1980). Samples collected from each treatment were pooled to minimize variation between individual drakes, however, semen characteristics were measured individually after collection.

- a. *Semen volume*: The volume was measured with a 1 ml. tuberculin syringe attached to a 10 – 15 cm inseminating straw.
- b. *Total Volume of semen per period per 5 birds (TVSPB)*: The total volume of semen per period per 5 birds was calculated from the total volume of semen obtained from individual treatment (5 birds) for a period of one week. Consequently, the birds that were collected three times per week produced more semen compared to the birds collected once per week.
- c. *Spermatozoa concentration*: The concentration of spermatozoa is expressed as number of sperm per ml was determined using a direct account (haemocytometer) as described by Bearden and Fuquay (2000).

- d. *The sperm progressive motility*: Motility of sample semen is expressed as the percentage of cells that are motile under their own power live spermatozoa that move progressively, to all directions, circle, forward and backward (Sorensen, 1982 in Mopun, 1999). The motility is calculated based on judgment of the operator and varied from 0 – 100 percent.
- e. *Spermatozoa viability*: The viability of spermatozoa was expressed as the percentage of dead spermatozoa over total number of spermatozoa counted. The technique used is staining using 1 % eosin plus 5 % nigrosin between 100 – 200 total cells were counted, and the partially or totally stained sperm representing the number of dead sperm in the sample. The unstained sperm represent those that were alive in the sample.
- f. *Abnormal morphology*: The morphology of sperm cell was expressed as the percentage of abnormal spermatozoa over total number of spermatozoa counted. The same staining technique as viability of spermatozoa was used in this method. The abnormal spermatozoa that have been observed include broken neck, enlarged head, broken head, and broken tail.
- g. *Percentage of fertility*: Twenty Alabio ducks, aged 32 weeks were used to determine fertility. They were divided randomly into three equal groups and subjected to insemination with semen collected at three different frequencies. Each duck was inseminated at the uterovaginal junction with 0.25 ml of semen containing 150 million live spermatozoa, diluted with normal saline. Fertile eggs were determined by candling at day 7 incubation period. The percentage of fertility was calculated from the number of fertile eggs over the total number of eggs produced. The fertility rate was calculated from the second day after insemination until the last fertile egg was laid, because eggs lay the first day after inseminations are always infertile. The reason is that all eggs laid by 7 am during the experiment, and since the ducks were inseminated after they laid, ovulation probably occurs very early in the morning of the day, so the eggs produced on the first day after insemination are not fertilized.

## Results and Discussion

### a. Average volume of semen per ejaculate

The ejaculate volume was not significantly different between semen collection once, twice and three times per week. The average semen volume per ejaculate in all treatment is presented in Table 2. The results of this experiment show clearly that Muscovy drakes are capable of producing semen frequently and over extended period without adversely affecting the volume of semen. These results also concur with the preliminary results of Onishi *et.al.* (1955) that twice daily collections were possible over the relatively short period of 12 days.

**b. Total Volume of semen per period per 5 birds (TVSPB)**

The TVSPB increased significantly ( $P \leq 0.01$ ) as the frequency of collection increased from once to three times per week. Tan (1980) found that in Muscovy drakes, once daily collections yielded more total semen volume per bird than did collections once or twice per week. One of the major goals of an artificial insemination program is to extend the influence of the best sires as widely as possible. We can now confirm that three times per week collections not only achieve this goal by producing more semen than do less frequent collection, but they do not have long-term detrimental effects on the birds.

Table 2. Effect of frequency of semen collection on volume of semen per ejaculation and sperm concentration in Muscovy drakes (Values are means of 5 birds over a 6-week period)

Frequency of semen collection	Average sperm volume per ejaculate (ml.)	Total volume of semen per period per 5 birds (ml)	Average sperm concentration ( $\times 10^9$ / ml)
Once per week	1.016 $\pm$ 0.178	13.463 <sup>a</sup> $\pm$ 6.753	1.472 $\pm$ 0.363
Twice per week	1.015 $\pm$ 0.073	20.296 <sup>b</sup> $\pm$ 1.449	1.214 $\pm$ 0.042
Three times per week	0.966 $\pm$ 0.129	28.988 <sup>c</sup> $\pm$ 4.912	1.384 $\pm$ 0.035
<b>Average</b>	<b>0.999 <math>\pm</math> 0.129</b>	<b>20.915 <math>\pm</math> 7.960</b>	<b>1.357 <math>\pm</math> 0.216</b>
<b>Tukey test</b>	<b>n.s.</b>	<b>P <math>\leq</math> 0.01</b>	<b>n.s.</b>

Note : n.s. = Non significant

Means with the same superscript for each column (a, b, c) are not significantly different

**c. Average spermatozoa concentration**

The sperm density did not vary consistently with the frequency of semen collection. The average concentration of sperm for all treatment is presented in Table 2. The important favorable aspect of frequent collection of semen for ultimate use in AI programs is that the concentration is unaffected by frequency of collection. This means that neither dilution procedure nor volume of inseminate need to be altered if drakes are used more frequently to meet the demands for semen at peak period.

**d. Spermatozoa viability and mobility**

The sperm viability did not vary consistently with the frequency of semen collection. The average viability of spermatozoa for all treatment is presented in Table 3. The results indicate that semen collection up to three times per week can be conducted to produce the same viability of sperm. Similar mobility of sperm was not different between treatments. A study in turkey by Noirault and Brillard (1999) indicated that viability of the sperm after semen collection of once, twice and three times per week were 86.88%, 87.41% and 88.15 % respectively. These results demonstrated that both viability and mobility of spermatozoa were not significantly

different although the Muscovy semen was collected up to three times per week over a period of 6 weeks.

Table 3. The effect of frequency of semen collection on viability and abnormal morphology in Muscovy spermatozoa (Values are means of 5 birds over a 6-week period)

Frequency of semen collection	Viability of spermatozoa (%)	Mobility of spermatozoa
Once per week	90.333 ± 0.577	++++
Twice per week	89.667 ± 4.558	++++
Three times per week	88.389 ± 1.494	++++
Average	89.463 ± 2.562	
Tukey test	n.s.	

### e. Abnormal morphology

Every ejaculate of semen always contains some morphological abnormal spermatozoa. The expected range of 8% to 10 % has no adverse effect on fertility (Bearden and Fuquay, 2000). If the total abnormal spermatozoa exceed 25 % of the total in the ejaculate, reduced fertility can be anticipated. The average percentage of spermatozoa abnormal morphology in all treatment is presented in Table 4.

Table 4. The effect of frequency of semen collection on abnormal morphology in Muscovy spermatozoa (Values are means of 5 birds over a 6-week period)

Spermatozoa morphology	Semen collection		
	1 x per week (%)	2 x per week (%)	3 x per week (%)
Normal	81.46 ± 2.53	83.08 ± 5.86	81.07 ± 2.64
Enlarged head	12.30 ± 2.76	9.86 ± 0.18	11.89 ± 3.17
Broken head	4.50 ± 0.21	4.40 ± 0.30	4.41 ± 0.27
No tail	-	0.33 ± 0.01	0.16 ± 0.08
Broken tail	1.73 ± 0.38	2.36 ± 0.40	1.76 ± 0.21

It is shown clearly that morphology of Muscovy spermatozoa collected once, twice and three times per week was considered as a normal sperm, since the percentage of abnormal spermatozoa was less than 20 %. These results were in accordance with the results reported by Toelihere (1979) that abnormal spermatozoa varied from 5 – 20 percent.

### f. Percentage of fertility

The frequency of semen collection had no effect on the percentage of fertility, but the percentage of fertility decreased with the time after single insemination (Table 5. and Figure 1.). The reduction of fertility with longer period after insemination was probably due to the reduction of the number of sperm stored in the

uterovaginal junction gland. In birds, spermatozoa are stored in the uterovaginal glands after insemination or copulation (Lake, 1967)

Table 5. Decline in fertility of Alabio duck eggs after a single insemination with Muscovy drake semen collected at once twice and three times per week

Treatment	Days after single insemination									
	1	2	3	4	5	6	7	8	9	10
1 x per week	65.21	79.16	85.71	58.82	31.57	19.04	34.78	7.40	0	3.70
2 x per week	41.17	58.33	53.33	42.85	7.14	6.66	11.11	0	0	0
3 x per week	40.00	30.76	30.76	18.18	11.11	0	0	0	0	0

Insemination with sperm collected once, twice and three times per week progressively decreased the fertility rate. Since the dose of insemination was the same between the three treatments, this was probably due to the quality of sperm. Also, the reduction of fertility after insemination was due to rapid exhaustion of the sperm stored in the uterovaginal gland or high dilution rate (Setioko, 1981).

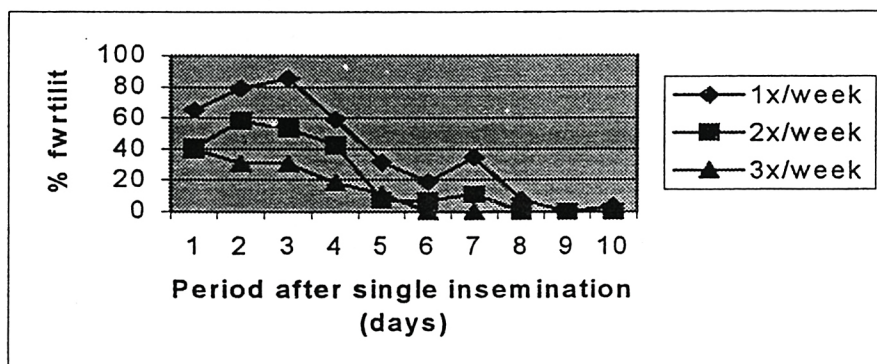


Figure 1. The average percentage of fertility after single insemination with Muscovy sperm collected at different frequencies

### Conclusion

1. Increasing the frequency of semen collection from once to three times per week did not significantly affect the average semen volume per ejaculate, total semen volume per period per 5 birds and semen concentration. This result of the experiment show clearly that Muscovy drakes are capable of producing semen frequently and over relatively extended period of 6 weeks without adversely affecting the sperm characteristics.

2. Sperm viability and mobility did not vary consistently with the frequency of semen collection. This result indicates that semen collection in Muscovy drakes up to three times per week can be conducted to produce the same quality.
3. The frequency of semen collection had no effect on the percentage of fertility, but the percentage of fertility decreased with the time after single insemination. The reduction of fertility with longer period after insemination was probably due to the reduction of the number of sperm stored in the uterovaginal junction gland.

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